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REVIEW ARTICLE

Can vitamins improve periodontal wound healing/regeneration?

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1 | INTRODUCTION

The mature periodontium, anchoring and supporting the tooth in its alveolar bone, is a multifaceted vital construct. Anatomically, it is responsible for the dento-alveolar homeostasis and function and is comprised of four tissues of divergent embryological origins, namely the alveolar bone, the cementum, the gingiva, and the periodontal ligament.[1] An intricate attachment of these tissues results from a sequence of synchronized compound interactions between neural crest ecto-mesenchymal and epithelial cellular components during embryogenesis.[2] Divergent cellular populations reside in the mature functional periodontium, comprising cementoblasts, endothelial cells, epithelial cells, fibroblasts, nerve cells, osteoblasts, in addition to a minor population of stem/progenitor cells.[3–9]

Periodontitis is a chronic complex multifactorial inflammatory disorder of the tooth supporting and investing structures, associated with microbial dysbiosis, and hall-marked by a phasic destruction with irreversible damage of the periodontal tissues.[10] A multitude of investigations associated periodontitis with major systemic conditions, including unfavorable pregnancy outcomes,[11] cardiovascular disorders,[12] diabetes mellitus,[13,14] gestational diabetes[15] and rheumatoid arthritis.[16] Surgical as well as nonsurgical periodontal treatment approaches, employing a multitude of biomaterials, and biological mediators, were proposed to re-establish the destroyed periodontal tissues and prevent further disease progression, thereby enhancing teeth prognosis and quality of life.[17–22]

Initiation of periodontitis requires a challenging of the periodontal tissues via the dysbiotic subgingival oral microbial biofilm's virulence factors, spurring an immune response, which principally dictates the course of the disease and associated tissue destruction.[23,24] In the primary phase, polymorphonuclear lymphocytes (PMNs) liberate lysozymes, proteinases, and reactive oxygen species (ROS),[25–29] in an attempt to eradicate the subgingival tissue-invading periodontal pathogens of the subgingival dysbiotic plaque. The resultant oxidative stress is believed to amplify the subsequent periodontal tissue damage.[30] This amplified dysregulated periodontal chronic inflammation is characterized by a disproportionate phagocytic recruitment and activation, especially of M1 macrophages, massively liberating inflammatory cytokines, comprising interleukin-1 beta (IL-1β), interleukin-4 (IL-4), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interferon gamma (IFN-γ),[23,31] accompanied by an excessive release of proteinases and additional ROS, depleting the body's own antioxidants,[32] and reducing its total antioxidant capacity (TAOC).[33] In this context it is generally believed that a physiological antioxidant status, with low ROS, could reduce the periodontitis-induced tissue damage and disease progression.[34] Yet, whether a pre-existent antioxidants depletion increases the patient's susceptibility to periodontal disease or such a depletion arises as a consequence of periodontal disease remains debatable.

In this context, a balanced nutrition or a supplementation/replenishment of essential antioxidant micronutrients, restoring the body's TAOC have been suggested as possible modifying factors with positive

effects on periodontal disease occurrence and progression, as well as periodontal wound healing.[35,36] It was hypothesized that the outcome of periodontal treatment could be enhanced through a synchronized supplementation of these micronutrients with surgical as well non-surgical periodontal therapy, relying on their antioxidant properties as well as their abilities to govern and direct pivotal cellular repair/regenerative events during periodontal wound healing.[37] Thus, the aim of the present article was to review the current evidence on the possible effects of vitamins on periodontal wound healing and regeneration.

2 | THE ROLE OF MICRONUTRIENTS IN PERIODONTAL HEALTH, DISEASE, AND PERIODONTAL WOUND HEALING/REGENERATION

A multitude of investigations have explored the possible impact of nutritional adequacy or deficiency on periodontal health and disease.[35,38,39] Malnutrition is a broad term that encompasses deficiency, excess or imbalance in the intake of essential nutrients, including vitamins, minerals, proteins, carbohydrates and fats, that can be caused by reduced dietary intake, altered metabolic demands, malabsorption or increased nutrients loss.[40] An unbalanced nutrition could be branded by a lack of certain micronutrients, a tissue depletion of important antioxidants, an inverted helper-suppressor T-cell ratio, pronounced histaminemia, hormonal imbalance with increased free cortisol levels in the blood and saliva, defective mucosal integrity as well as impaired acute-phase protein cellular and cytokine response.[41,42] In addition, malnutrition has the potential to influence the prognosis of periodontal disease, through depletion of micronutrients essential for healing/regeneration, through disruption of cellular and immune functions, through a disturbance of the oral microbial ecology, as well as through altering the volume and the antimicrobial properties of saliva.[43]

Currently, it is widely accepted that a higher dietary intake of nutrients with anti-inflammatory and antioxidant properties in a balanced nutrition[44] could be strongly linked to lower odds of developing periodontitis with slower disease progression. Higher, vitamin A,[33] vitamin C,[33,45–49] vitamin E,[45,48] fruits and vegetables,[48] and omega-3 fatty acids (FAs)[50–53] intake were linked to populations with pronounced periodontal tissue health.[34,54] Certain modifiable lifestyle risk factors associated with periodontal disease, including smoking and alcohol consumption, have been further demonstrated to result in lowered serum levels of essential micronutrients,[55] including vitamin A,[56,57] vitamin C[58] and vitamin E[56,57] (typical serum vitamin levels are listed in Table 1). It appears plausible to assume that these vitamins may be pivotal in the initial periodontal disease stages to avert its onset rather than reversing its effects once the disease has been established.[59] Yet, due to the intricacy of biological systems, it is still not possible to accurately state whether the low dietary antioxidants intake or the periodontitis-associated high oxidative stress levels within the periodontal tissues (or a combination of both) contributes stronger to the periodontal disease occurrence and progression.[59] In this context, it is pivotal to note that the TAOC of an individual does not necessarily represent the arithmetic sum of each antioxidant's concentration. Thus, measuring each individual antioxidant quantity in the serum or tissues does not necessary give the complete biological picture. Moreover, it remains a possibility that further antioxidants exist, which till now have not been accounted for.[33] Furthermore, an antioxidant's serum or tissue level could act as biological marker of active pathological processes, and hence a higher supplementation would not have an impact on disease progression.[60] Finally, apart from the antioxidant activities, these micronutrients govern pivotal cellular events, affecting periodontal disease onset, progression as well as healing and regeneration.[36]

A multitude of studies have produced conflicting findings, making it ambiguous when micronutrients could be most effective in halting or preventing the periodontal pathological processes. Thus, the currently existing data should be interpreted with caution, as the majority of the available data stem primarily form in vitro, preclinical (animal), cross-sectional and cohort studies.

TABLE 1 Typical plasma/serum concentration ranges for vitamins.

Vitamin	Plasma/serum concentration ranges	References
Vitamin A (retinol)	0.7–2.8 μmol/L	[346]
Vitamin B1 (thiamine)	66–200 nmol/L	[347]
Vitamin B2 (riboflavin)	5.4–28.4 μmol/L	[348]
Vitamin B3 (niacin)	28–125 pmol/L	[349]
Vitamin B5 (pantothenic acid)	1.1–12 μmol/L	[350]
Vitamin B6 (pyridoxine)	4.1–43.7 ng/mL	[351]
Vitamin B7 (biotin)	0.49–1.33 nmol/L	[352]
Vitamin B9 (folic acid)	7–39.7 nmol/L	[353]
Vitamin B12 (cobalamin)	152–568 pmol/L	[354]
Vitamin C (ascorbic acid)	23–85 μmol/L	[355]
Vitamin D (25-hydroxyvitamin D)	75–120 nmol/L	[356]
Vitamin E (α-tocopherol)	7–40 μmol/L	[357]
Vitamin K (phylloquinone)	0.2–1 μg/L	[358]
Coenzyme Q_{10} (CoQ_{10})	0.5–1.5 μg/mL	[359]

3 | VITAMINS AS MICRONUTRIENTS IN PERIODONTAL WOUND HEALING/REGENERATION

3.1 | Vitamin A and carotenoids

3.1.1 | Sources, biological structure and function and roles in periodontal health/prophylaxis and disease

Vitamin A, a major antioxidant, is a lipid-soluble vitamin, represented in various isotypes, including retinol (A_1), 3,4-dehydroretinol (A_2), 3-hydroxyretinol (A_3) and retinal in addition to 50 carotenoids

(vitamin A precursors).[37] The various forms of vitamin A have diverse biological effects on cellular metabolism, growth, proliferation, differentiation, apoptosis, and pluripotency.[61,62]

Retinoic acid (the biologically active form of vitamin A) has been shown to be pivotal in preserving the mucosal integrity, for ensuring proper cellular differentiation and for apt immune cell functions.[37,63] Investigations reported a significant association between deficiencies in vitamin A and a number carotenoids, and the onset and severity of periodontal disease,[64–66] yet with the exception for retinol, where no association was noted between its deficiency and the severity of periodontitis.[59,67] In a survey conducted on almost 21 000 subjects in Alaska, Chile, Colombia, Ecuador, Ethiopia, Lebanon, Thailand and Vietnam, vitamin A deficiency tended to be associated with higher periodontal disease scores.[68] In the United States' third National Health and Nutrition Examination Survey (NHANES III) conducted on 11 480 male and female patients from various ethnic groups,[33] a significant correlation was evident between a low serum level of a number of carotenoids, particularly α-, β-carotene, β-cryptoxanthin, vitamin C, bilirubin and TAOC, and periodontitis, when the analysis included all periodontitis cases irrespective of their severity. Yet, focusing solely on severe periodontitis cases, with the presence of two or more mesiobuccal sites with clinical attachment level (CAL) loss of at least 5 mm and at least one mesiobuccal site with probing depth (PD) of 4 mm or higher, a significant association of periodontitis with uric acid, β-carotene, vitamin A, vitamin C, and bilirubin levels,[33] especially in never smokers, was evident. Similar observations on periodontitis prevalence were revealed in an investigation on 60–70 years old western men in Northern Ireland.[59] A further investigation demonstrated that a positive outcome following scaling and root planning (SRP), was demonstrated in patients showing higher intake of β-carotene, α-tocopherol, vitamin C, fruits, vegetables, docosahexaenoic acid, and eicosapentaenoic acid.[69] Yet, a 2-year follow-up study from Japan, estimating the intake of α- and β-carotenes, through a brief self-administered diet-history questionnaire, negated the reported positive effects of a higher β-carotene intake on periodontal disease severity.[48] Similarly, further studies found no association between an increase in periodontal index scores and vitamin A deficiency.[70,71]

Vitamin A and carotenoids express a variety of immunological and anabolic properties, which combined could augment biological events during periodontal healing/regeneration. Apart from their antioxidant roles, with their ability to reduce the levels of markers for systemic inflammation, including IL-6[72] and C-reactive protein (CRP),[60,73] vitamin A, and carotenoids exert immunomodulatory effects on B cells, dendritic cells, macrophages, stem/progenitor cells and T cells in a doses-dependent manner, inducing pro-inflammatory (to combat the periodontal pathogens), or tolerogenic (to endorse the anabolic tissue formation phases during healing/regeneration of the periodontium) responses.[59,74] Vitamin A can increase cellular viability and counteract the inflammatory reaction, following the activation of toll-like receptor (TLR)-4 through its ligand lipopolysaccharides (LPS).[75] The observed beneficial effects of vitamin A on the periodontium were further attributed to its bone anabolic[76–78] and osteoclastic inhibitory as well as apoptotic[79,80] effects. Furthermore, vitamin A is chemotactic for stem/progenitor cells, inducing their migration, amplifying the expression and activity of matrix metalloproteinases-2/-9, inhibiting caspase-3 enzyme activity,[81] and driving cellular pluripotency and differentiation,[82] all important events during periodontal healing/regeneration.

3.1.2 | Roles in periodontal therapy and biological wound healing/regeneration

In vitro studies results

Although no association between retinol adequacy/deficiency and severity of periodontitis was observed,[59,67] retinol at a concentration of 20 μmol/L has been shown to cause epigenetic demethylation of nuclear bases, driving the de-differentiation of adult cells into pluripotent ones[62] and to increase the colony-forming units (CFUs) ability of adult gingival mesenchymal stem/progenitor cells (G-MSCs).[83] These effects are thought to be primarily brought about through activation of the ten-eleven translocation (TET) demethylases, causing DNA demethylation with epigenetic reprogramming and heightened pluripotency.[62,84] Similar pluripotency inductive effects were further reported for retinoic acid (RA) with a potential for teratoma formation.[85] Such anabolic potential remains of importance to periodontal tissue engineering/regeneration approaches, where pluripotency inductive attributes could help formation of the three tooth supporting tissues of different embryological origin, namely alveolar bone, periodontal ligament and cement, pivotal for a complete periodontal regeneration, from in the defect-residing periodontal or gingival fibroblasts, even without the need for exogenous cellular transplantation.[83]

RA further demonstrated notable anabolic properties in vitro. RA enhanced epithelial growth and differentiation, induced directly through the epithelial nuclear RA receptors,[86] as well as indirectly through connective tissue fibroblasts' growth factors expression independent of RA concentration in the culture medium.[87,88] The keratinocytes growth factor (KGF), whose presence is important in the maintenance and development of oral epithelial tissues as well as during epithelial wound healing, was higher expressed in gingival and periodontal fibroblasts treated by RA in vitro.[89] A short-term RA stimulation of human dental pulp,[90] gingival and periodontal ligaments cells,[91] and adipose tissue-derived mesenchymal stem cells[92] was shown to enhance their osteogenic potential and tissue nonspecific alkaline phosphatase (TNSALP) expression. Similarly, RA treatment of periodontal ligament stem cells (PDLSCs) or stem cells from human-exfoliated deciduous teeth (SHED) for 21 days at concentrations of up to 2 μM inhibited their proliferation, yet significantly enhanced their osteogenic differentiation, alkaline phosphatase (ALP), *RUNX2* and *osteopontin* (OPN) *gene* expression as well

as calcified deposits formation compared to dexamethasone. This remarkable osteogenic inductive effect could be further boosted through additional insulin supplementation to RA in the culture medium. Remarkably, RA-induced PDLSCs demonstrated a higher proliferative and osteogenic potentials compared to RA-induced SHED[93] (Table 2).

TABLE 2 Effect of vitamin A—in vitro studies.

Author, year, country, study type	Study material (stem cells, fibroblasts, etc.), sample size	Form of vitamin application	Groups	Methods of evaluation (ELISA, etc.)
Chadipiralla et al. 2010[93] USA	PDLSCs and SHED *N* = 5	In culture medium	Groups: Serum-free medium either alone or supplemented with RA (0.5, 1, 2 μM) or Dexamethasone (Dex: 1, 10, 100 nM) for 21 days	Proliferation assay Enzyme assay Immunohistochemistry RT-PCR Western blot
Fawzy El-Sayed et al. 2019[83] Egypt	G-MSCs *N* = 5	In culture medium	Control group: basic medium Inflammatory group: basic medium with IL-1β (1 ng/mL), TNF-α (10 ng/mL), and IFN-γ (100 ng/mL), Retinol group: basic medium with retinol (20 μmol/L) Inflammatory/retinol group: retinol added to the inflammatory group.	ELISA Cell count Histochemistry RT-PCR.
Kautsky et al. 1995[87] USA	Oral epithelial keratinocytes from cornified or noncornified epithelium seeded on collagen lattices of: GM10 fibroblasts from human fetal dermal tissue, oral and dermal fibroblast	In culture medium	Control group: Serum retinoid concentration 5×10^{-7} mol/L Test groups: RA prepared under yellow light at concentrations 0, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7} mol/L.	Morphology Immunohistochemistry Immunoblotting
Mackenzie & Gao 2001[89] UK	Human gingival and periodontal fibroblasts *N* = 5 (tissue culture) *N* = 10 (sectioning)	In culture medium	Groups: RA at concentrations from 10^{-9} to 10^{-6} M	RT-PCR In situ hybridization with digoxigenin-labeled riboprobes
San Miguel et al. 1998[91] Japan	hPDL cells *N* = 3	In culture medium	Groups: RA at concentrations 0, 10^{-5}, 10^{-6}, 10^{-7} M	RT-PCR Enzyme assay
San Miguel et al. 1999[90] Japan	Human dental pulp cells *N* = 3	In culture medium	Groups: RA at concentrations 0, 10^{-5}, 10^{-6}, 10^{-7} M	RT-PCR Protein assay

Animal studies results

Although animal preclinical models can provide valuable insights on potential agents favoring periodontal wound healing/regeneration,[94–96] a limited number of animal studies explored the effects of vitamin A or carotenoids supplementation on periodontal healing/regeneration. In a Wistar rat animal experiment on

Evaluated parameters	Outcomes	Conclusion
Cell count ALP activity Mineralization *Col-I* *FSP* *PPARγ2* *ALP* *ONN* *OPN* *RUNX2*	• Proliferation of SHED and PDLSCs was significantly inhibited by RA and Dex. • RA significantly upregulated gene expression and activity of ALP in SHED and PDLSCs. • Positive Alizarin red and von Kossa staining of calcium deposition was seen on the RA-treated SHED and PDLSCs after 21 days. • Influences of RA on osteogenic differentiation of SHED and PDLSCs were significantly stronger than Dex. • Supplementation with insulin enhanced RA-induced osteogenic differentiation of SHED.	RA is an effective inducer of osteogenic differentiation of SHED and PDLSCs, whereas RA treatment in combination with insulin supplementation might be a better option for inducing osteogenic differentiation.
β-catenin levels Cellular proliferation Colony-forming Pluripotency genes Multilineage differentiation.	• Intracellular β-catenin significantly declined in the stimulated groups ($p < 0.001$). • Cellular proliferation at 72 h were most prominent in the control and inflammatory group followed by an upsurge in the retinol group. • At 14 days, the retinol group exhibited the highest CFUs. • Nanog was most expressed in inflammatory and retinol group. • Inflammation significantly upregulated Sox2 expression, while its expression was diminished in retinol and inflammatory/retinol group ($p < 0.001$). • Inflammatory/retinol group exhibited the highest multilineage differentiation potential.	Controlled short-term inflammatory/retinol stimuli activate the Wnt/β-catenin pathway, affecting G-MSCs' pluripotency, proliferation, and differentiation. The present findings provide further insights into the inflammatory-regenerative interactions and their modulation potential for G-MSCs-mediated periodontal repair/regeneration
Filaggrin Profilaggrin Keratin 1 Keratins 13 & 19	• RA concentrations altered terminal differentiation of oral keratinocytes by disruption of organized stratification, inhibition of filaggrin/profilaggrin and K1 expression, and stimulation of K13 and K19 expression. • K19 was expressed higher in keratinocytes from non-cornified as compared to those from cornified epithelia. • RA regulation was ultimately dependent on the type of fibroblasts underlying the epithelial cells.	Results indicate that RA regulation of oral epithelial differentiation is mediated by two separate mechanisms: a direct, RA concentration-dependent effect, and an indirect, fibroblast-mediated effect.
KGF	• Gingival and periodontal fibroblasts expressed KGF transcripts in vitro. • Degree of expression was markedly influenced by the presence of RA. • KGF expression is dose-dependent with increasing expression of KGF seen up to about 10^{-8} M RA.	Results point to a role of KGF in maintenance of normal growth and differentiation of gingival epithelia. Lack of KGF expression by periodontal fibroblasts in vivo is expected to hinder apical epithelial migration and thus stabilize the epithelial attachment. Effects of RA on KGF expression in vitro provide an indirect mechanism by which RA may regulate the growth and differentiation of gingival epithelia.
ALP activity *TNSALP* gene expression (bone-type and liver-type)	• After treatment with RA (10^{-6} M) for 4 days, there was a significant increase in ALP activity of hPDL cells. • The increased ALP activity had properties of the *TNSALP* type. • RT-PCR analysis revealed that bone-type mRNA was highly stimulated in hPDL cells by RA treatment, but expression of liver-type mRNA was not detected.	Results indicated that upregulation of ALP activity in hPDL cells by RA was due to the increased transcription of bone-type mRNA of the TNSALP gene.
ALP activity *TNSALP* gene expression (bone-type and liver-type)	• Elevated ALP activity had properties of the TNSALP type. • RT-PCR showed that RA enhanced the expression of bone-type *TNSALP* mRNA in pulp cells. However, the liver-type *TNSALP* mRNA was not detected.	Findings suggest that the high ALP activity of RA-treated dental pulp cells is associated with increased transcription of the bone-type mRNA of the *TNSALP* gene and not with liver-type.

TABLE 3 Effect of vitamin A—animal studies.

Author, year, country, study type	Animal type, sample size	Treatment, form of vitamin application	Study duration
Nishio et al. 2017[97] Canada	Wistar rats *N* = 16	Group 1: Orthodontic tooth movement (OTM) Group 2: Extraction of upper 1st molar + OTM Within each group 1 and 2: Experimental group: Oral gavage isotretinoin (7.5 mg/kg) Control group: Animals with oil solution	37 days (30 days before the surgery + 7 days after the surgery)
Wang et al. 2013[98] China	Mice *N* = 12	Periodontitis model: oral infection with *P. gingivalis* Test group: All-trans retinoic acid (ATRA) every other day (oral gavage) Control group: no ATRA application	

tooth extraction and orthodontic tooth movement, supplementation with a synthesized retinoid (isotretinoin) via daily gavage for 37 days resulted in higher tissue VEGF levels, larger bone medullary spaces with abundant hematopoietic, inflammatory cells and blood vessels as well as enhanced gingival tissue regeneration and re-keratinization.[97] Similarly, in an experimental mouse periodontitis model, induced through oral infection with *P. gingivalis*, oral all-trans retinoic acid (ATRA) supplementation demonstrated the ability to reduce *P. gingivalis*-induced alveolar bone resorption, halt periodontal inflammation, inhibit Th17 responses, enhance Treg cell responses, reduce RANKL expression on CD^+ T cells, decrease IL-17 levels, reduce inflammatory cell infiltration into the periodontal tissues, and increase IL-10 and TGF-β1 levels, thereby impeding the periodontitis-associated alveolar bone destruction. These ATRA-induced immunomodulatory events, favoring wound healing/regeneration, were linked to a reduced level of $CD4^+$ retinoid-related orphan receptor γτ positive cells and increased $CD4^+$ forkhead box P3 positive cells in the mice cervical lymph nodes[98] (Table 3).

Clinical trials results

Clinical studies exploring the effects of vitamin A supplementation on periodontal healing/regeneration are limited. In a randomized, double-blind trial, 42 systemically healthy patients with chronic periodontitis (ages from 25 to 52 years) received either 8 mg per day of oral lycopene, a natural carotenoid present in red-colored fruits especially tomatoes, (test group) or placebo (control group) for 2 months, following full-mouth SRP. Although CAL, PD and serum levels of TNF-α did not differ between both groups, a significant improvement in BOP, MGI, PI, serum IL-1β, and salivary uric acid were notable in favor of the test group.[99] Systemic lycopene supplementation (in the same concentration and application time as described in the previous study) as an adjunct to SRP was investigated on 20 systemically healthy patients (ages from 35 to 50 years) with moderate periodontitis. MGI, PD, CAL, and serum malondialdehyde levels (a marker for oxidative stress) improved after 2 months, yet with a minute relapse in measured parameters noted at 6 months.[100] A similar randomized, controlled, double-blind clinical trial was conducted on the adjunctive effect of lycopene gel (8 mg for 8 weeks) on type 2 diabetes patients with chronic periodontitis in addition SRP as compared to SRP alone. A similar reduction was noted in the test group patients for PD and serum malondialdehyde levels, with an improvement in the glycemic control.[101]

The local usage of a 2% lycopene gel in periodontal defects as an adjunct to SRP was further described. In a placebo-controlled split-mouth clinical trial, 50 smoking and 50 nonsmoking patients (ages from 30 to 55 years) with chronic periodontitis treated with the lycopene gel demonstrated significant improvement in PD and CAL compared to the placebo sites irrespective of their smoking status.[102] Similar results were demonstrated in a further trial on 31 nonsmoking chronic periodontitis patients (ages from 30 to 55 years) with 2% lycopene gel as an adjunct to SRP, improving CAL and PD 6 months post-therapy.[103] Supplementation with a lycopene gel-loaded in solid lipid microparticles and delivered locally with a needle into periodontal defects as an adjunct to SRP in periodontitis patients, significantly decreased the oxidative stress-associated protein carbonyl groups in the gingival crevicular fluid and improved gingival inflammation as well as CAL for up to 6 months post-operatively[104] (Table 4).

Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)	Evaluated parameters	Outcomes: clinical, histological, immunological	Conclusion
Micro-CT Immunohistochemistry	Bone volume *VEGF-C* *COX-2* *IL-1β*	• No significant difference of OTM and bone volume was observed between the control and experimental group. • Alveolar bone of the isotretinoin group revealed more medullary spaces with inflammatory, hematopoietic cells, blood vessels and intense immunolabeling for VEGF-C. • This group also showed faster gingival regeneration.	Isotretinoin did not affect the OTM nor did it cause an alteration in maxillary bone volume. This exogenous acid may contribute to the acceleration of gingival healing.
Histomorphometry Flowcytometry RT-PCR	Alveolar bone resorption (ABR) $CD4^+$ T cells in spleen and cervical lymph nodes (CLN) Th17/Treg cell-related cytokine messenger ribonucleic acid	• ATRA suppressed ABR and inhibited inflammatory cell infiltration into periodontal tissues. • Effects were closely associated with reduced CD4(+) retinoid-related orphan receptor γτ(+) cells and increased CD4(+) forkhead box P3(+) cells in the CLNs. ATRA downregulated IL-17A expression and upregulated IL-10 and TGF-β1 expression in CLNs and *P.gingivalis*-infected gingival tissues.	ATRA modulation of the Th17/Treg imbalance provides protection against periodontitis by enhancing Treg cell activation and inhibiting Th17 cell activation. These results indicate the potential for clinical prevention of periodontitis.

Evidence box	Presence	Effect
Association studies	✓	++
Biological mechanism	✓	++
Animal model "proof of principle"	✓	+/−
Clinical studies with surrogate parameters	✓	++/−
Clinical studies with hard end points	Ø	

3.2 | Vitamin B

3.2.1 | Sources, biological structure and function and roles in periodontal health/prophylaxis and disease

Thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), pantothenic acid (vitamin B5), pyridoxine (vitamin B6), biotin (vitamin B7), folic acid (FA, vitamin B9), and cobalamin (vitamin B12) form the water-soluble vitamin B complex.[34] Each member of the family has a unique structure and function, with no biological function in the body requiring their conjoint presence simultaneously.[35]

Thiamin is essential for the normal muscular and neural functions, especially during the process of energy extraction from glucose.[105] Thus, a deficiency in thiamin greatly reduces the cells capacity to generate energy.[35] Riboflavin is pivotal for growth and development, especially of muscles and hair,[106] with its deficiency being rarely reported due to its presence in adequate amounts in cereals, egg, meat, and milk.[35] Niacin remains to be an important biomolecule for normal enzymatic function and creation of stable collagen structures during wound healing.[107–109] Pantothenic acid contributes to the extraction of energy from fats, carbohydrates, and proteins,[110] the formation of proline (the essential amino acid during collagen formation) from hydroxyproline as well as the promotion of cellular proliferation.[109–112] Pyridoxine remains essential in amino acids metabolism.[113] Biotin is found in a variety of food and is further synthesized by the intestinal microflora. It is involved in carbohydrate, fat and protein metabolism, normal hormonal synthesis, and function, with important roles in erythropoiesis.[35] It is further important for growth, collagen synthesis, digestion, muscle function, and periodontal wound healing.[114–116]

FA is an essential micronutrient involved in the body's methylation reactions, pyrimidine and purine biosynthesis, and the conversion of homocysteine to methionine, pivotal to avert DNA damage as well as to repair damaged DNA. FA has an anti-mutagenesis effect, enhancing apoptosis of dysplastic cells with damaged DNA, through activating the p53 and blocking the Bcl-2 gene.[117,118] Hence, a multitude of ailments have been linked to FA deficiency, including heart diseases, neural tube defects, higher tendency to mutagenesis, and poor wound healing.[117,119,120] Dietary FA could be damaged during food processing and cooking, and it is partially compensated for by FA synthesized by the gut microflora.[117,119,120] In the human body, serum FA is required in a range of 7–39.7 nmol/L, playing a critical role together with cyanocobalamin in erythropoiesis, cardiovascular health, during pregnancy, and in mucosal health. FA deficiency weakens hematopoietic cell functions and increases nuclear staining in the basal cell layer with degeneration and widening of intercellular spaces in the spinous cell layer, negatively affecting the epithelial integrity and barrier functions and predisposing

TABLE 4 Effect of vitamin A—clinical studies.

Author, year, country, study type	Patients, sample size, gender, smoker, diagnosis	Treatment, form of vitamin application	Study duration	Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)
Ambati et al. 2017[100] India Single arm intervention study	N = 20 patients Single arm intervention study Non-smokers CP	Test group: 8 mg of oral lycopene + SRP Control group: SRP	2, 6 months	Serology Clinical measurements
Arora et al. 2013[99] India RCT	N = 42 patients Non-smokers CP	Test group: 8 mg of oral lycopene + SRP Control group: SRP	2 months	Serology Saliva samples Clinical measurements
Chandra et al. 2012[102] India RCT	N = 100 patients (50/group) Smokers and Non-smokers CP	Test group: SRP + local 2% lycopene gel Control group: SRP + placebo	6 months	ELISA Clinical measurements
Chandra et al. 2013[103] India RCT	N = 31 patients Nonsmokers Chronic periodontitis	Test group: SRP + local 2% lycopene gel Control group: SRP + placebo	12 weeks	ELISA Clinical measurements
Reddi et al. 2015[101] India RCT	N = 40 patients Nonsmokers CP Type 2 diabetes mellitus	Test-group: 8 mg of oral lycopene + SRP Control group: SRP	8 weeks	ELISA TBARS assay Clinical measurements
Tawfik et al. 2019[104] Egypt RCT	N = 16 patients 5 males and 11 females Nonsmokers CP	Group I: SRP + local 2% lycopene (LP) loaded solid lipid microparticles (SLMs) delivered with a needle inside the defect Group II: SRP only Group III: No treatment	6 months	Liquid chromatography of GCF Clinical measurements

the oral mucosa to infection, ulceration, gingivitis, periodontitis, and poor wound healing outcomes.[120,121] In an epidemiological study involving 497 nonsmoking subjects, aged 18 years or older, with 20 or more teeth, an inverse relationship was evident between a dietary FA intake and gingival bleeding scores, underlining the importance of FA in promoting gingival health.[122] Still, a double-blinded randomized clinical trial evaluating the impact 2 mg FA oral supplementation twice per day for 30 days on gingivitis, demonstrated no significant differences in gingivitis or plaque scores in favor of the FA as compared to the negative control group. Yet, a significant reduction in gingival crevicular fluid (GCF) exudation was evident in the FA group, pointing at the possibility of a FA-induced subclinical reduction in gingival inflammation.[120] Conversely, a double-blind randomized clinical trial assessed the effectiveness of a topical FA mouth rinse (5 mL of FA mouth rinse; concentration 1 mg/1 mL, twice daily) on established gingivitis, regarding gingival color, bleeding sites and plaque scores. At 4 weeks, the FA group demonstrated a significant improvement in gingival color and bleeding scores as compared to the negative control group, demonstrating a positive impact of the FA mouth rinse on gingival health.[123] As opposed to the previous investigation, these findings suggest that FA could be more effective if applied locally than when administrated systemically in the treatment of gingivitis, even in the absence of nonsurgical periodontal intervention.

Cobalamin is a pivotal cofactor of the methylmalonyl-CoA mutase enzyme, responsible for energy extraction from proteins and fats, and serves as a cofactor for the 5-methyltetrahydrofola

Evaluated parameters	Outcomes: clinical, histological, immunological, microbiological, PROMS	Conclusion
Serum malondialdehyde levels MGI, PD, CAL	• From pretreatment to posttreatment at 2 months, the mean values of all parameters were reduced. • From 2 to 6 months when lycopene was not administered, an increase in the mean values of all the parameters was observed; however, these values were still below baseline values.	There was a reduction in oxidative stress and improvement in clinical parameters following systemic antioxidant therapy along with SRP, which was maintained up to 4 months after discontinuation of lycopene treatment.
Serum IL-1ß, TNF-α Salivary uric acid PI, MGI, PD, CAL, IBD	• Lycopene showed better results after therapy compared to the placebo group with reference to PI ($p=0.004$), MGI ($p=0.002$), BOP ($p=0.021$), salivary IL-1ß ($p=0.05$), and uric acid levels ($p=0.02$). • CAL gain, PD reduction and serum TNF-α value were not statistically significant but showed an improvement compared to the placebo group.	Further longitudinal studies are required to establish the role of lycopene in the management of chronic periodontitis.
GCF 8-hydroxydeoxyguanosine PI, MGI, PD, CAL	• Compared to placebo, lycopene-treated sites in smokers and nonsmokers showed significant reductions in probing depths and gain in CAL. • There was no statistically significant difference in clinical parameters when lycopene-treated sites in smokers and nonsmokers were compared, except for the reduction in the 8-OHdG levels. • The 8-OHdG levels at 1 week and 3 months in sites treated with lycopene in smoker and nonsmoker group were comparable with those in the periodontally healthy control group.	The gel formulation was effective in increasing clinical attachment and reducing gingival inflammation, probing depth, and oxidative injury compared with the placebo in smoking and nonsmoking subjects.
GCF 8-hydroxydeoxyguanosine PI, MGI, PD, CAL	The gel, when delivered to the sites with oxidative stress, was effective in increasing clinical attachment and in reducing gingival inflammation, probing depth, and 8-OHdG levels as compared to the placebo and sham sites.	From this trial conducted over a period of 6 months, it was found that locally delivered lycopene seems to be effective in reducing the measures of oxidative stress and periodontal disease.
Serum malondialdehyde (MDA) CRP GI, PD, CAL	• Inter-group comparison showed group A giving statistically significant results in reducing mean serum MDA levels at 2 months and 6 months, and in reducing mean PD (mm) and mean HbA1c (%) levels at 2 months ($p<0.005$).	Lycopene as an adjunctive treatment was effective in reducing oxidative stress and restoring altered glycemic levels. Further longitudinal studies with a larger sample size are required to establish the role of lycopene in the management of chronic periodontitis.
Protein carbonyl (PC) PI, MGI, PD, CAL, IBD	• SLMs recorded 30 days of drug release with no burst effect. • Patients treated with LP SLMs showed significantly lower levels of PC after SRP compared to those treated with SRP only, in addition to improvement in measured clinical parameters.	Locally delivered LP SLMs along with SRP could have a protective effect over periodontal tissues and it has the ability to decrease oxidative damage of proteins in diseased periodontium.

te-homocysteine methyltransferase enzyme, which catalyzes the formation of 5,10-methylene-methyltetrahydrofolate, important in DNA and thymine synthesis.[124,125] Recurrent aphthous ulceration has been linked to cobalamin deficiency,[126–128] allegedly due to a dysregulation in DNA synthesis, cell-mediated immunity and changes in the oral epithelium. A 6-month study on patients with serum cobalamin deficiency and suffering from recurrent aphthous ulcers, demonstrated a 96% positive response to cyanocobalamin treatment (intramuscular injection 1000 g per day for 7 days then once a week for 1 month and then once a month for 6 months), with a significant increase in serum cobalamin levels and decreased ulceration frequency.[125] Increased CAL loss, with associated higher risk for tooth loss, were found to be linked to low levels of cobalamin[129] (an overview of important biological functions of different members of the vitamin B family is presented in Table 5).

3.2.2 | Roles in periodontal therapy and biological wound healing/regeneration

In vitro studies results

Only limited in vitro studies are available on the impact of various members of the vitamin B family members, isolated or combined, on periodontal cellular attributes. The addition of biotin, in an avidin-biotin combination, to highly porous three-dimensional

hydroxyapatite scaffold, improved the cellular adhesion as well as the strength of the attachment of periodontal ligament fibroblasts to it.[130] The described potential to improve cellular adhesion to tissue engineering scaffolds could be pivotal for any periodontal healing/regeneration approaches (Table 6).

Animal studies results

Limited animal studies were further available on the effect of vitamin B family members, isolated or combined, on periodontal wound healing. Nicotinamide (single intraperitoneal injection, 270 mg/kg body weight) exerted a protective effect in a streptozotocin-induced diabetic rat model, reducing the blood glucose level, body weight loss, tibial bone loss, the amount of the experimental periodontitis-related bone loss and RANKL-positive cells in the periodontal tissues.[131] A further study, compared the effects of riboflavin, nicotinamide, and FA (each at concentrations of 50 mg/kg or 100 mg/kg body weight of each daily for 11 days) on alveolar bone loss, inflammatory cells infiltrate, osteoclast number and serum IL-1β levels in a Wistar rat experimental periodontitis model. Riboflavin, nicotinamide, and FA, supplied to the rats by gastric gavage daily for 11 days at both concentrations reduced the amount of bone loss, the inflammatory cells infiltrate

TABLE 5 Reported functions of Vitamin B family members.

Vitamin B member	Reported functions
Vitamin B1 (thiamin)	• Essential for energy metabolism and the conversion of carbohydrates into glucose • Supports muscular and nerve function • Helps maintain a healthy cardiovascular system • Contributes in the synthesis certain hormones
Vitamin B2 (riboflavin)	• Important in energy production (metabolism of fats, proteins, and carbohydrates) • Involved in antioxidant activity • Converting tryptophan into niacin • Supports healthy muscles, skin, vision, and red blood cell production
Vitamin B3 (niacin)	• Supports energy metabolism and collagen formation • Important in DNA repair and cell signaling • Involved in the production of enzymes necessary for cellular function • Maintaining healthy skin and nerves
Vitamin B5 (pantothenic acid)	• Important in energy production (metabolism of fats, proteins, and carbohydrates) • Important in the production of hormones, cholesterol and red blood cells • Involved in the synthesis of coenzyme A • Important in the conversion of hydroxyproline to proline
Vitamin B6 (pyridoxine)	• Important in metabolism of amino acids, fats and carbohydrates • Important in the synthesis of neurotransmitters and red blood cells • Involved in immune functions and brain development • Involved in homocysteine metabolism
Vitamin B7 (biotin)	• Important in energy production (metabolism of fats, proteins, and carbohydrates) • Essential for the synthesis of fatty acids and erythropoiesis • Important for cell growth and maintenance of healthy hair, skin, and nails • Involved in the regulation of gene expression
Vitamin B9 (folic acid)	• Essential for DNA synthesis, cell division and the production of red and white blood cells • Important during pregnancy for proper fetal development • Involved in homocysteine metabolism
Vitamin B12 (cobalamin)	• Important in metabolism of fats and proteins • Essential for the formation of red blood cells and DNA synthesis • Important for proper nerve and brain functions • Participates in the metabolism of amino acids and fatty acids

TABLE 6 Effect of vitamin B—in vitro studies.

Author, Year, Country, study type	Study material (stem cells, fibroblasts, etc.), sample size	Form of vitamin application	Groups	Methods of evaluation (ELISA, etc.)
Jang et al. 2011[130] Korea	Human periodontal ligament fibroblasts *N* = 8	Avidin-biotin binding system scaffold (ABBS) bound to n-HA	Group 1: ABBS cell seeding Group 2: static cell seeding Group 3: Agitation cell seeding	Cell attachment (WST-1 assay) Scanning electron microscopy

and the number of osteoclasts as compared to the untreated periodontitis controls.[132] Although limited in number, the reported positive effects exerted by members of the vitamin B family in the animal preclinical models, makes them interesting micronutrients with promising potential in the field of periodontal healing/regeneration (Table 7).

Clinical trials results

Limited clinical trials evaluated the impact of the vitamin B family members on periodontal wound healing following surgical or nonsurgical periodontal treatment. A randomized double-masked placebo-controlled clinical trial was conducted on 30 patients taking one capsule a day of either vitamin B complex, consisting of 50 mg of thiamine HCl, riboflavin, niacinamide, d-calcium pantothenate, and pyridoxine HCl, and 50 μg each of d-biotin and cyanocobalamin, and 400 mg of FA, or placebo for 30 days following access flap surgery. Results demonstrated significant CAL gain and PD reduction, in shallow (1–3 mm) and deep pockets (≥6 mm), at 180 days in the vitamin B complex group.[116] In a further randomized controlled clinical trial, 60 periodontitis patients treated with either SRP with systemic FA (400 mg tablet containing FA, calcium, vitamin D, and inactive ingredients) or placebo thrice a day for 4 weeks, while avoiding any dairy products, demonstrated better healing and clinical outcomes with significant improvement in the CAL and gingival recession values in the SRP with FA group as compared to SRP and placebo one[119] (Table 8).

In conclusions, results from in vitro, animal, and clinical studies hint at a positive impact of vitamin B family members on periodontal wound healing/regeneration. Still, the scarcity in studies warrants further conduction of in vitro, animal as well as randomized controlled clinical trials on different members of this family and in various application forms.

Evidence box		
	Presence	Effect
Association studies	✓	++/−
Biological mechanism	✓	+
Animal model "proof of principle"	✓	+
Clinical studies with surrogate parameters	✓	+
Clinical studies with hard end points	Ø	

3.3 | Vitamin C

3.3.1 | Sources, biological structure and function and roles in periodontal health/prophylaxis and disease

Vitamin C (L-ascorbic acid, AA) is a potent water-soluble antioxidant, essential for collagen formation[133] and wound healing, with a multitude of reported positive effects on oral health and periodontal disease prevention.[134,135] AA is believed to support wound healing through pleiotropic mechanisms, including the promotion of neovascularization, VEGF expression, extracellular matrix deposition, cellular renewal, and differentiation, while inhibiting cellular apoptosis.[136] Furthermore, AA boosts the telomerase activity and pluripotency of stem/progenitor cells, osteoblasts and fibroblasts.[137–142] AA stimulates fibroblast migration and keratinocyte proliferation, with the potential for improving the epithelial phenotype.[143] It functions as an enzymatic cofactor for a large family of metalloenzymes involved in the synthesis and stabilization of collagen of the periodontal ligament, gingiva, cement and alveolar bone,[144,145] in the production of peptide hormones and neurotransmitters, and in the regulation of transcription factors, such as hypoxia inducible factor-1.[146,147]

Immunologically, AA favors wound healing through upregulating the intracellular glutathione levels,[148] thereby inhibiting nuclear factor kappa-light-chain enhancer of activated B cells and endothelial cells, which plays important roles in the regulation of gene expression during inflammation.[149] It further possesses anti-histaminic properties, through direct histamine inactivation.[150] AA suppresses ROS production,[30] and promotes anti-inflammatory responses in macrophages,[149,151] governing their responses to CRP, tumor necrosis factor (TNF), interferons (INF), and ILs.[152]

Thus, AA deficiency is believed to be linked to impaired reparative and immune functions.[153,154] Following dietary intake and absorption, AA is transported from the plasma to gingival tissues by a sodium-dependent low-affinity and high-capacity vitamin C transporter 1 (SVCT1).[155,156] As SVCT1 does not function properly at low AA concentrations, it remains pivotal to define the optimal dosage AA required for efficient periodontal wound healing. A daily intake of at least 60 mg AA was recommended by the Food and Nutrition Board of the National Academy of Sciences in the United States for adults.[157] Yet, ideally for the body to function properly, it has been recommended that adults should receive >200 mg of AA from their

Evaluated parameters	Outcomes	Conclusion
Ratio of attached living cells	• Number of periodontal ligament fibroblasts attached was greater for ABBS seeding method than for static or agitating method. • No difference was observed among seeding methods with scanning electron microscopy.	The high-affinity ABBS enhances the ability of periodontal ligament fibroblasts to attach to three-dimensionally constructed n-HA scaffolds.

TABLE 7 Effect of vitamin B- animal studies.

Author, year, country, study type	Animal type, sample size	Treatment, form of vitamin application	Study duration	Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)
Akpinar et al. 2016[132] Turkey	Wistar rats *N* = 64 (male)	Control group Ligated group Intervention groups: RBF50 (RBF, 50 mg/kg daily) NA50 (NA, 50 mg/kg daily) FA50 (FA, 50 mg/kg daily) RBF100 (RBF, 100 mg/kg daily) NA100 (NA, 100 mg/kg daily) FA100 (FA, 100 mg/kg daily). Gastric gavage, daily for 11 days Periodontitis-induced with ligature around left mandibular first molar.	11 days	Clinical Histopathology ELISA
Kim et al. 2014[131] South Korea	Rats *N* = 5–6 animals/group	Group 1: IV application of streptozotocin (STZ) Group2: Intraperitoneal of nicotinamide (NA, 270 mg/kg) 15 min before IV STZ Group 3: untreated controls Periodontitis-induced with ligature around left mandibular first molar, 1 week after injection.	20 days	Accu-check active system Micro-CT Histochemistry Histomorphometry

daily diet.[158] In this context, a dietary intake of AA in concentrations >150 mg per day in combination with fruits, vegetables, β-carotene, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) has been reported to exert beneficial impacts on periodontal healing in nonsmokers following SRP.[69]

AA remains to be the nutrient that received most of the attention of all vitamins, regarding its effect on periodontal healing over the past years, where low circulating or depleted AA levels were reported to be associated with increased risk for gingival inflammation[159,160] as well as periodontal disease onset and progression.[33,45–49,161,162] Case control studies demonstrated lower AA plasma levels in periodontitis patient than in healthy controls, with periodontitis patients who additionally smoke showing lower AA levels than nonsmokers.[163,164] Results of NHANES III demonstrated that a reduced dietary intake of AA was weakly, yet significantly, associated with an increased risk of periodontitis.[47] Additionally, results from NHANES III showed that the occurrence of periodontitis was inversely associated with serum concentration of antioxidants, including AA and various carotenoids, after adjusting for age, gender, ethnicity, smoking status, contraceptive intake, diabetes, income, and education. The study showed a stronger inverse association between AA concentration and sever periodontitis occurrence in a sub-group of never smokers than in the whole sample.[33] Similar results were further demonstrated in data from the fourth Korean National Health and Nutrition Examination Survey.[67]

Unlike the reduction in plasma AA levels following major surgical therapies (e.g., cardiac surgery), as a result of the occurring oxidative stress, a clinical trial demonstrated that nonsurgical periodontal therapy did not significantly alter the plasma AA levels in smokers between the first SRP session and the last one 4 weeks later. These findings were explained by the lower extent of tissue damage and inflammatory reactions with oxidative stress occurring following SRP as compared to these major surgical interventions.[165] In a recent pilot study on 20 patients, AA serum levels were further linked to periodontitis severity and CRP levels. Interestingly, the study demonstrated that AA serum levels were not related to the patient's reported vegetable or fruit intake yet associated with a higher consumption of processed meat. In patient with low AA serum levels a significant positive correlation was evident with the periodontitis stage (severity), demonstrating a further significant inverse correlation with elevated CRP levels.[166] Conversely, a recent cohort investigation examining associations of oral nutrient

Evaluated parameters	Outcomes: clinical, histological, immunological	Conclusion
ABL Serum IL-1β level Presence of inflammatory cells and osteoclasts	• Mean ABL in mandibular first molar was significantly lower in RBF100 than in Control group. • In the Ligated group, ABL was significantly higher than in all other groups. • The ratio of presence of inflammatory cell infiltration in the Ligated group was significantly higher than in the Control group. • The differences in serum IL-1β levels between groups were not statistically significant. • Osteoclasts that were observed in the Ligated group were significantly higher than those of the Control and FA100 groups. The osteoblastic activity in the Ligated group, RBF100, and NA100 groups were shown to be significantly higher than those in the Control group.	Systemic administration of RBF, NA, and FA in different dosages (50–100 mg/kg) reduced ABL in periodontal disease in rats.
Body weight Blood glucose Glucose tolerance Serum insulin Tibia bone loss ABL Number of inflammatory cells, RANKL-positive, area of osteoid	• In STZ-treated rats, hyperglycemia over 300 mg/dL and severe weight loss were observed. • Insulin level was approximately 14% compared to control rats. • STZ-NA-treated rats were impaired in glucose tolerance compared to control rats; body weight and insulin levels were not different. • Tibia bone loss was increased in STZ-treated rats, but significant change was not observed in STZ-NA-treated rats compared to control rats. • In ligatured teeth, ABL was increased in both STZ- and STZ-NA-treated rats compared to control. • ABL, the number of inflammatory cells and RANKL-positive cells in STZ-treated rats were greater than in STZ-NA-treated rats. • Area of osteoid decreased in STZ-treated rats compared to control, but not STZ-NA-treated rats.	Results indicate that STZ- and STZ-NA-treated rats exhibit diabetic characteristics similar to type 1 diabetes mellitus and a pre-diabetic state, respectively, and suggest that the level of bone loss in alveolar bone and tibia depend on diabetic status.

intake and corresponding serum metabolites with clinical severity of human periodontitis showed an opposite relationship, where higher AA dietary intake was associated with the sub-group of periodontitis patients demonstrating higher tooth loss. This conflicting result was explained through the fact that in this cohort, patients suffering from severe forms of periodontitis associated with teeth loss were more aware of their critical situation and subsequently increased their self-administrated dietary amount of AA intake.[167] The inter-individual differences observed in AA absorbance between studies, which additionally influences plasma AA concentrations, may be explained through genetic variations in the AA transporter protein SVCT1.[168] In this context, an investigation suggested that a genetic variance in SLC23A1 encoding SVCT1 could be linked to periodontitis, through reducing the serum availability of AA, irrespective of the individual's dietary intake.[169]

An investigation correlating serum AA levels to *A. actinomycetemcomitans* and *P. gingivalis* IgG antibodies of Finnish and Russian male patients demonstrated a significantly negative correlation between serum AA and *P. gingivalis* IgG levels only. Unlike *A. actinomycetemcomitans* IgG levels, with increasing of AA the number of *P. gingivalis* seropositive subjects decreased.[170] In a male guinea pigs preclinical model, marginal ascorbate deficiency induced a significant reduction in the amount of stimulated saliva as well as increased the plasma and to a lesser degree the salivary cortisol content. The elevated cortisol levels were explained by the previously reported effects that reduced AA levels cause adrenal hypertrophy, with increased cortisol liberation.[171] Yet, apart from the direct impact of a AA deficiency on the body, such elevated cortisol levels would indirectly compromise oral inflammatory responses to dental plaque, reduce collagen formation, decrease bone matrix formation as well as impair periodontal wound healing.[172] Taken together results suggested a strong association between AA and periodontal disease and warranted the need for further in vitro, animal, and clinical intervention studies to further explore the underlying mechanisms.

3.3.2 | Roles in periodontal therapy and biological wound healing/regeneration

In vitro studies results

A number of studies tested the cellular effects of AA as well as the underlying intracellular mechanisms in vitro. AA exerted an

TABLE 8 Effect of vitamin B—clinical studies.

Author, year, country, study type	Patients, sample size, gender, smoker, diagnosis	Treatment, Form of vitamin application	Study duration
Keceli et al. 2020[119] Turkey RCT	*N* = 60 patients (30/group) 37 completed the study (22 females and 15 males) (20 in SRP + FA and 17 in the SRP + P) Nonsmokers Periodontitis	Group 1: SRP + placebo (P) Group 2: SRP + FA	6 months
Neiva et al. 2005[116] USA	*N* = 30 patients (13 males, 17 females) RCT Non-smokers Generalized moderate to severe CP	Group 1: A capsule/day of either Vit-B (50 mg of: thiamine HCl, riboflavin, niacinamide, d-calcium pantothenate, pyridoxine HCl; 50 microg each of d-biotin and cyanocobalamin and 400 mcg of folate) Group 2: Placebo For 30 days following access flap surgery (AFS)	6 months

immunomodulatory protective effect on human PDLSCs and was able to revert the *P. gingivalis*-LPS-induced expression of ROS, NFκB, MyD88, and p300, while restored the expression of miR-210 (implicated in the protection against periodontitis as well as in osteogenesis, angiogenesis, and cell survival).[173,174] Interestingly, a further investigation demonstrated a synergistic effect of IL-11 (10 ng/mL) and AA (50 μg/mL) on the osteoblastic differentiation potential of periodontal ligament cells, with their combined application for 1 week inducing higher collagen I, *RUNX2*, *ALP*, *OCN*, and tissue inhibitor of metalloproteinase-1 production. It appeared that IL-11, combined with AA at the tested concentration and stimulation time, can function as an osteo-promotive cytokine, in line with recent reports on the possible beneficial effects of controlled inflammatory stimuli on periodontal regeneration.[175] The observed effects were linked to an AA-induced inactivation of the intracellular janus kinase/signal transducers and activator of transcription and MAPK signaling pathways.[176] In a further in vitro investigation G-MSCs were stimulated by AA (250 μmol/L) under an uninflamed as well as an experimental inflammatory setup (1 ng/mL IL-1β, 10 ng/mL TNF-α, and 100 ng/mL IFN-γ). AA stimulation improved G-MSCs' stemness, proliferation, and differentiation attributes, associated with a Wnt/β-catenin signaling pathway activation. Apart from firstly enhancing cellular metabolism and *Sox2* and *Oct4A* pluripotency gene expression, inflammation seemed to reduce these AA-induced positive effects.[177]

AA was further extensively applied in the biosynthesis of cell sheets of mesenchymal stem cells from the bone marrow, umbilical cord, stem cells from the apical papilla (SCAP), PDLSCs, and human dental pulp stem cells (DPSCs), with great potential in periodontal regenerative approaches.[1] AA at concentrations of 20 μg/mL predictably produced cell sheets from SCAP, PDLSCs, and DPSCs, with upregulated expressions of the pluripotency markers *OCT4*, *SOX2* and *NANOG* as well as *ALP*, *RUNX2*, *OPN*, osteocalcin (*OCN*), integrin β1, fibronectin, and collagen I.[178,179] Interestingly, DPSCs showed a significantly higher expression of *OCT4* and *TERT* pluripotency markers.[178,179] Ectopically formed tissue, following the AA-induced cell sheets transplantation subcutaneously in nude mice, demonstrated that PDLSCs cell sheets formed directionally arranged tissue fibers, while SACP cell sheets showed the highest mineralization potential.[179] These AA-induced cell sheets further demonstrated elevated telomerase activity.[178] When cultured in a Matrigel

Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)	Evaluated parameters	Outcomes: clinical, histological, immunological, microbiological, PROMS	Conclusion
Clinical parameters ELISA	PI, GI, PD, CAL, GR, GCF (at baseline, (one (PT-1), three (PT-3) and six (PT-6) months for CRP and homocysteine (Hcy) evaluation)	• Compared to SRP + P, CAL was lower in SRP + FA at PT-1 ($p = 0.004$) and PT-3 ($p = 0.035$), whereas GR was lower at only PT-1 ($p = 0.015$). • GCF volume and CRP did not show inter-group differences, whereas Hcy was higher in SRP + FA at PT-3 ($p = 0.044$) and PT-6 ($p = 0.041$). • GCF volume and Hcy showed reduction after treatment in both groups ($p < 0.001$).	Systemic FA intake may be recommended adjunctive to periodontitis treatment for better outcomes. However, its impact mechanisms should be further enlightened.
Clinical parameters BANA test	CAL, BOP, GI, PI Microbial assessment	• Both groups showed comparable levels of PD reduction following AFS (test: −1.57 ± 0.34 mm; control: −1.50 ± 0.21 mm). • Changes in mean CAL were more favorable in Vit-B supplemented subjects (test: +0.41 ± 0.12 mm; control: −0.52 ± 0.23 mm; $p = 0.024$). • Stratified data demonstrated significantly better CAL results for test group in both shallow (test: −0.08 ± 0.03 mm; control: −1.11 ± 0.27 mm; $p = 0.032$) and deep sites (test: +1.69 ± 0.31 mm; control: +0.74 ± 0.23 mm; $p = 0.037$). • No significant differences were observed between groups regarding PI, GI, and BOP. • BANA test values were significantly reduced in both groups after surgical treatment and no significant differences were noted between groups.	Vitamin B complex supplement in combination with AFS resulted in statistically significant superior CAL gains when compared to placebo.

conditioned medium and transplanted subcutaneously in nude mice for 3 months, the cell sheets successfully regenerated periodontal as well as dentin pulp-like structures.[180] Transplantation of AA-stimulated PDLSCs into the back of immunocompromised mice formed significantly higher quantities of ectopic cementum- and bone-like mineralized structures, compared to unstimulated cells.[181] In combination with 10 mM β-glycerophosphate, AA at concentrations of 50 μg/mL is commonly used for osteogenic induction of mesenchymal stem/progenitor cells.[182,183] The AA-induced osteoblastic differentiation in periodontal ligament cells increased the expression of the osteoblastic markers *RUNX2*, *ALP*, *and OCN*, an effect ascribed to an activation of the extracellular signal-regulated kinase (ERK)1/2.[184] Similarly, a further study attributed AA-induced periodontal ligament progenitor cells' differentiation to ERK1/2 pathway activation, relying on an AA-induced upregulation of the PELP1 nuclear receptor co-regulator, with no involvement of JNK or p38 pathways. An inhibition of the ERK1/2 signaling pathway, reduced *RUNX2* expression and mineralized nodule formation.[138] The differentiation inductive potential of AA was further exploited in cementogenesis and cementogenic commitment of human PDLSCs, with an elevation of *cementum attachment protein (CAP)* and *cementum protein 1 (CEMP1)* expression.

In a recent study, sodium ascorbyl phosphate (SAP), a hydrophilic L-ascorbic acid derivative, convertible to free AA by cell phosphatases for sustained release in the medium, was tested at concentrations ranging from 50 to 500 μmol/L on mouse MC3T3-E1 pre-osteoblastic cells. SAP stimulated pre-osteoblastic cellular differentiation, inducing *RUNX2*, *COL1A1*, and *OCN* genes, collagen I secretion and increased extracellular matrix mineralization (especially at concentrations of 200 or 400 μmol/L).[185] In a further study, SHED stimulated with 10 μM AA similarly enhanced *OCT4*, *SOX2*, and *NANOG* expression and their cellular differentiation potential as well as altered their secretom profile, with elevated secretions of VEGF, TGF-α and β, SCF, IGF-1, HGF, b-FGF, EGF, Ang-1&2, DKK-1, BMP-2, PTH, Leptin, ACP5, ALPL, and OPN. It further enhanced the expression of the inflammatory cytokines TNF-α, IL-6, IL-17A, NO, IDO, SDF-1, and PGE2, while also increasing the anti-inflammatory cytokine IL-10.[186] The described alterations in the secretom expression profile could open interesting potentials for therapeutic approaches.[187] Porous decalcified dentin matrices loaded with human

dental follicle cell sheets, subsequently induced by 50 mg/mL AA for 9–11 days and ectopically transplanted in the renal capsule of nude mice, formed periodontal like tissues with bone, fibers organized perpendicular to the dentin and positive staining for OPN and periostin after 8 weeks.[188] Taken together these findings for pronounced positive effects on regenerative attributes of periodontal cells and the elucidation of the underlying cellular mechanisms appeared to provide first evidence for an explanation of the observed association of dietary AA intake with periodontal health. Thus, further animal and clinical studies were warranted (Table 9).

Animal studies results

Limited animal studies exist on the effects of AA on periodontal healing/regeneration. AA demonstrated remarkable regenerative potential in animal experimental models. Human AA-induced DPSCs cell sheets transplanted into a miniature swine's preclinical periodontitis model demonstrated improved periodontal tissue healing as compared to DPSCs cellular injection, with significantly higher amounts of regenerated bone volumetrically.[189] Similarly, AA-induced PDLSCs cell sheets in combination with flap surgery showed remarkable periodontal regeneration in a miniature pig periodontitis model, with regularly arranged periodontal ligament fibers inserted histologically into newly formed cementum and higher amount of bone formation, compared to dissociated PDLSCs on a gelfoam scaffold.[178] Although limited in number, these studies further accentuated the positive regenerative attributes of AA reported earlier on cellular level (Table 10).

Clinical trials results

Although evidences collected from epidemiological, in vitro, and animal studies suggested a positive effect of AA supplementation on periodontal treatment outcomes, the results of the clinical intervention studies were very modest and mostly failed to show an additional benefit of AA systemic supplementation on periodontal therapeutic outcomes. An earlier clinical trial demonstrated no additional beneficial effect of adjunctive 250 mg q.i.d. AA in mega doses 1 week prior to SRP on the healing response to nonsurgical periodontal therapy.[190] Similarly, in a clinical trial, 2000 mg per day of AA given orally to patients undergoing SRP for 4 weeks had no influence on periodontal outcomes (CAL, PD, BOP, PI, and GI) or TAOC plasma levels.[191]

Yet, although systemic AA supplementation failed to show additional effect on periodontal wound healing, local AA application seemed to represent an interesting alternative with promising results. In a recent study, AA in a concentration of 250 μg/mL was amalgamated into platelets rich fibrin (PRF) and incorporated directly into intra-osseous defects of stage-III/grade C periodontitis patients with open flap debridement (OFD). Compared with PRF alone, the AA/PRF group demonstrated significantly better recession reduction and linear radiographic defect fill over a 6-month follow-up period.[192] These results shed light on a new perspective, employing local AA application as an adjunct to periodontal wound healing following periodontal surgical therapeutic interventions. Yet, the absence of beneficial effect of AA systemic supplementation on periodontal therapeutic outcomes underlines the multifactorial intricacy of the body's biological systems and negates the simplistic unifactorial therapeutic approaches. It further hints at the fact that, unlike under idealized in vitro conditions, certain vitamins could need additional cofactors and micronutrients to exert their expected beneficial effects on the periodontium in vivo (Table 11).

Evidence box

	Presence	Effect
Association studies	✓	++/−
Biological mechanism	✓	++
Animal model "proof of principle"	✓	++
Clinical studies with surrogate parameters	✓	−−/+
Clinical studies with hard end points	Ø	

3.4 | Vitamin D

3.4.1 | Sources, biological structure and function and roles in periodontal health/prophylaxis and disease

Vitamin D is a fat-soluble vitamin, which functions through vitamin D receptors (VDR), a member of the steroid nuclear receptor superfamily, universally expressed on nucleated cells. Biologically, 90% of vitamin D is produced through sunlight ultraviolet-B exposure of the subcutaneous cholesterol-like precursor (7-dehydrocholesterol) to pre-vitamin D, which isomerizes to cholecalciferol (vitamin D3). The rest is obtained from dietary sources (e.g., fatty fish, fish oils, egg yolk, and dairy products).[193] Vitamin D2 stems from plant sources.[194] Both vitamin D3 and D2 are biologically inactive. Through a process driven mainly by the parathyroid hormone (PTH) and growth hormone, vitamin D undergoes 25-hydroxylation in the liver to 25(OH)D3 (calcidiol, half-life 2–3 weeks), followed by hydroxylation in the kidneys or nonrenal sites, including alveolar macrophages, osteoblasts, lymph nodes, placenta, colon, breast, and keratinocytes to its most active form, 1,25(OH)D3 (calcitriol, half-life of 4–6 h).[194]

The ability of periodontal cells to self-produce vitamin D was demonstrated in vitro. Following incubation of primary cultures of human gingival fibroblasts and periodontal ligament cells with the 25-hydroxylase substrate vitamin D3, 25(OH)D3 was detectable, followed by the production of 1,25(OH)D3. A knockdown experiment confirmed that the vitamin D activating enzyme 1α-hydroxylase (CYP27A1) mRNA was responsible for the production of 1,25(OH)D3 extra-skeletally in these cells. Interestingly, challenging both cell types by IL-1β and *P. gingivalis*-LPS strongly induced CYP27A1 mRNA expression.[195] The amount of vitamin D in the human organism is determined through identifying the concentration of its main circulating metabolite 25(OH)D3 in the plasma. A concentration below 37.5 nmol/L denotes vitamin D deficiency, while a concentration above 200 nmol/L signifies hypervitaminosis.[196] To exert an effect

on the periodontium, the plasma concentration of 25(OH)D3 should reach 90–100 nmol/L.[197]

Apart from its endocrine actions, facilitating intestinal calcium absorption and osteoclast activity, vitamin D further functions though VDR present on a variety of immunological and nonimmunological cells in an autocrine mechanism, regulating gene signaling/expression, hormone/protein synthesis, immune/inflammatory responses, and cellular turnover.[198,199] In this context, vitamin D deficiency was linked to the occurrence of a multitude of diseases, including autoimmune diseases, cancer, diabetes, myopathies, metabolic syndromes, osteoporosis, infections, cardiovascular disease, and chronic obstructive pulmonary disorder (COPD).[194,200,201] Silencing of VDR was shown to result in hyperresponsiveness of macrophages to LPS.[202]

Vitamin D received significant attentiveness as an anti-inflammatory agent,[203] with a relationship reported between periodontitis and low serum vitamin D levels, especially in adults of the middle and higher age groups.[204–206] Thus, it was suggested that a vitamin D supplementation starting from the middle age could be beneficial in preventing periodontal disease progression, due to its pleiotropic functions and given that aging itself could pose a risk factor for bone health disorders (e.g., osteoporosis).[37] A study on 85 postmenopausal women demonstrated that compared to age and sex matched controls, periodontal disease appeared to be more common in them, combined with a lower vitamin D as well as a higher RANKL and osteoprotegerin (OPG) expression. It appears that the osteoclastic activation associated with low vitamin D levels could represent a common risk factor linking both diseases together.[207] A similar correlation was demonstrated between serum 25(OH)D3 and periodontal health status in type 1 diabetes mellitus patients[208] and postmenopausal women,[209] where low serum 25(OH)D3, denoting vitamin D deficiency and an unbalanced immune response, could pose a common risk factor for the enhanced progression potential of the diseases. A case–control study, examining the relationship between serum 25(OH)D3 and periodontitis in pregnant women between 14 and 26 weeks of gestation, concluded that serum 25(OH)D3 levels below 75 nmol/L were associated with maternal periodontal disease. The authors concluded that vitamin D supplementation could represent an important strategy to improve oral health of pregnant women.[210] A further case–control study explored the association between serum 25(OH)D3 levels, periodontal health and COPD, demonstrating that lower serum 25(OH)D3 levels were significantly associated with poor periodontal health and COPD.[211]

A cross-sectional study examining the association between total vitamin D intake and periodontal health in 562 older veteran men demonstrated that subjects who received vitamin D ≥ 800 IU per day had a lower risk having severe periodontitis (PD ≥5 mm on more than one tooth and CAL ≥6 mm at more than two tooth, and moderate-to-severe alveolar bone loss ≥40% at more than sites), whereas those receiving <400 IU per day of vitamin D suffered from a more advanced level of alveolar bone resorption.[212] In a randomized controlled trial, vitamin D was given in three doses of 2000, 1000, and 500 IU per day and compared to a placebo group regarding changes in gingival scores for up to 3 months, demonstrating a dose-dependent anti-inflammatory effect. Gingivitis scores changed in direct relationship to the dose of vitamin D supplementation, where the group receiving the higher supplement dose demonstrated the fastest resolution in gingivitis scores followed by the lower dose groups in a dose-dependent manner. In contrast to the placebo group, where serum 25(OH)D3 concentration increased by 0.87 nmol/L over the 3 months period, the consumption of 500 IU increased serum 25(OH)D3 by 32.03 nmol/L, the consumption of 1000 IU by 42.12 nmol/L and the consumption of 2000 IU by 74.22 nmol/L.[213] It was further postulated, that vitamin D levels may be crucial during post-surgical periodontal healing and that baseline deficiencies cannot be reversed through a 6 weeks vitamin D supplementation at the time of surgery, as it may take up to 3 months of supplementation for serum 25(OH)D3 levels to normalize.[214] Conversely, a presurgical 4- to 6-week supplementation with a mixture of micronutrients was reported to increase 25(OH)D3 levels from an average plasma level of 24.76 ng/mL to 50.11 ng/mL.[215] Still, lower serum vitamin D levels (<30 ng/mL) were demonstrated to be marginally associated with a slightly nonsignificantly reduced periodontal healing following non-surgical periodontal therapy.[216]

A multitude of clinical studies demonstrated the protective role of vitamin D against periodontal disease as well as the direct association between periodontal health and serum 25(OH)D3 levels. A 5-year prospective clinical study suggested that vitamin D and calcium supplementation reduced the risk of tooth loss in elderly individuals.[217] Findings from the NHANES III implied that serum 25(OH)D3 levels were negatively correlated with CAL loss in men and women 50 years and older.[218] Similarly, a further linear correlation was evident between serum 25(OH)D3 and gingival inflammation independent of race, gender or being a user/nonuser of further vitamins/minerals supplements, where individuals with the highest serum 25(OH)D3 levels demonstrated 20% less BOP than those with the lowest levels.[219] A cohort study correlated serum 25(OH)D3 levels, PD, CAL, BOP, body mass index (BMI), current smoking status, and smoking history (packyears). Although 25(OH)D3 deficiency was significantly associated with periodontal disease, serum 25(OH)D3 levels did not correlate directly with CAL, PPD, or BOP.[220]

An earlier study exploring the relationship between serum adipokines, vitamin D, and clinical and microbiological parameters in 56 periodontitis patients, demonstrated positive correlations between adiponectin and vitamin D as well as between IL-6 and leptin, a negative correlation between IL-6 and vitamin D as well as leptin and vitamin D, but did not show associations between adiponectin, vitamin D, IL-6 or leptin and clinical or microbial parameters. Although periodontal therapy improved clinical and microbiologic parameters, it did not influence the levels of adiponectin, vitamin D, IL-6, or leptin.[221]

It was reported that vitamin D performs its major anti-inflammatory effect, through inhibition of the MAPK signaling, thereby reducing the production of a multitude of inflammatory cytokines, including IL-6, IL-17, and TNF-α.[222] Vitamin D further possess the ability to switch the macrophage's phenotype from the M1

(pro-inflammatory phenotype) to the M2 (anti-inflammatory phenotype).[223] The immunomodulatory effects of vitamin D were further demonstrated in an in vitro experiment, where cultures of human periodontal ligament cells were treated with 1,25(OH)D3, *P. gingivalis* or their combination. While *P. gingivalis* significantly upregulated IL-6 and IL-8 expression, 1,25(OH)D3 inhibited IL-8 expression especially when combined with *P. gingivalis*. Yet 1,25(OH)D3 was not able to affect the IL-6 expression with or without *P. gingivalis* stimulation.[224]

TABLE 9 Effect of vitamin C—in vitro studies.

Author, year, country, study type	Study material (stem cells, fibroblasts, etc.), Sample size	Form of vitamin application	Groups	Methods of evaluation (ELISA, etc.)
Bhandi et al. 2021[186] Saudi Arabia	SHED *N* = 5	In culture medium	Group 1: SHED + AA (10 μg/mL) Group 2: SHED	Cytometric bead array RT-PCR
Fawzy El-Sayed et al. 2020[177] Germany	G-MSCs *N* = 5	In culture medium	Control group: basic medium Inflammatory group: basic medium with IL-1β (1 ng/mL), TNF-α (10 ng/mL), and IFN-γ (100 ng/mL), AA group: basic medium with AA (250 μmol/L) Inflammatory/AA: AA added to the inflammatory group.	ELISA Cell count Histochemistry RT-PCR
Gauthier et al. 2017[181] USA	hPDLSCs *N* = 20	In culture medium	Group 1: hPDLSCs + AA (20 μg/mL) Group 2: hPDLSCs	RT-PCR Subcutaneous transplantation in immunocompromised mice Immunohistochemistry
Hu et al. 2020[179] China	DPSCs PDLSCs SCAP *N* = 5	In culture medium	Group 1: DPSCs + AA (20 μg/mL) Group 2: PDLSCs + AA (20 μg/mL) Group 3: SCAP + AA (20 μg/mL)	RT-PCR Subcutaneous transplantation in nude mice Immunofluorescence Immunohistochemistry

A further study investigating a newly discovered vitamin D analog, eldecalcitol (ED-71), as a potential therapeutic agent in periodontitis, demonstrated the ability of ED-71 to counteract *P.gingivalis*-LPS-induced NLRP3 inflammasome-dependent pyroptosis (a type of caspase-1-dependent inflammatory apoptosis) in human gingival fibroblasts. Results further revealed that the pyroptosis-inhibition effect was mediated through activating the intracellular Nrf2/HO-1 pathway.[225] 25(OH)D3 treatment reduces serum inflammatory

Evaluated parameters	Outcomes	Conclusion
Human Growth Factor Panel (*Ang-2*, *EGF*, *Ang-1*, *FGF-basic*, *HGF*, *IGF-1*, *TGF-β*, *SCF*, *TGF-α*, *and VEGF*) Human Immune Panel (*PGE-2*, *SDF-1*, *TGF-β1*, *IDO*, *NO*, *CCL2*, *IL-17A*, *IL-6*, *IL-10*, *and TNF-α*) Human Bone Metabolism Panel (*OPG*, *OPN*, *ALP*, *ACP5*, *Leptin*, *RANKL*, *PTH*, *BMP-2*, *DKK-1*)	• Treatment with AA enhanced secretion of growth factors, anti-inflammatory cytokines, and factors related to bone metabolism. • AA altered SHED secretom profile, with elevated secretions of the anabolic growth factors *VEGF*, *TGF- α&β*, *SCF*, *IGF-1*, *HGF*, *b-FGF*, *EGF*, *Ang-1&2*, *DKK-1*, *BMP-2*, *PTH*, *Leptin*, *ACP5*, *ALP* and *OPN*. • It further enhanced the expression of inflammatory cytokines as *TNF-a*, *IL-6*, *IL-17A*, *NO*, *IDO*, *SDF-1* and *PGE2*, while also increasing the anti-inflammatory cytokine *IL-10*	SHEDs displayed enhanced multifaceted activity, which may have applications in regenerative therapy.
β-catenin levels Cellular proliferation Colony-forming Pluripotency genes Multilineage differentiation.	• *β*-Catenin significantly decreased intracellularly in all experimental groups ($p=0.002$). • AA group exhibited significantly higher cellular counts on days 3, 6, 7, 13 ($p<0.05$) and the highest CFUs at 14 days [median-CFUs (Q25/Q75); 40 (15/50), $p=0.043$]. • Significantly higher *Nanog* expression was noted in AA group [median gene-copies/PGK1 (Q25/Q75); 0.0006 (0.0002/0.0007), $p<0.01$]. • Significant multilineage differentiation abilities, especially into osteogenic and chondrogenic directions, were evident in the AA group	AA stimulation enhances G-MSCs' stemness, proliferation, and differentiation properties, effects which are associated with a Wnt/*β*-catenin signaling pathway activation. Apart from initially boosting cellular metabolism as well as Sox2 and Oct4A pluripotency marker expression, inflammation appeared to attenuate these AA-induced positive effects.
Expression of *CAP*, *CEMP1*, *BSP*, *OCN*	• RT-PCR analysis showed that cementogenic CAP was expressed only slightly higher in STRO-1^+/$CD146^+$, STRO-1^-/$CD146^+$ and STRO-1^-/$CD146^-$ subpopulations than in the original cell pool, while CEMP1 expression in these subpopulations was not different from the original pool. • Under the stimulation with osteogenic medium, CAP and CEMP1 were downregulated while osteogenic BSP and OCN were upregulated. • CAP and CEMP1 were upregulated by AA treatment. – Transplantation of AA-treated PDLSCs into immunocompromised mice resulted in significantly more ectopic cementum- and bone-like mineral tissues. • Immunohistochemical analysis of the ectopic growth showed that CAP and CEMP1 were mainly expressed in the mineral tissue and in some cells of the fibrous tissues.	Osteogenic stimulation is not inductive but appears to be inhibitory of cementogenic pathways, whereas AA induces cementogenic lineage commitment by PDLSCs and may be a useful stimulus for cementogenesis in periodontal regeneration.
Expression of collagen I, fibronectin, integrin β1, vimentin *NANOG*, *OCT4*, *SOX2* and *TERT* gene expression	• No differences were found in histological structure and extracellular matrix protein expression between DPSCs, PDLSCs and SCAP sheets. • DPSCs sheet showed higher expression of OCT4 and TERT than PDLSCs and SCAP sheets. • All three cell sheets displayed ability of mineral tissue formation and highly expressed periostin. • The tissue derived from DPSCs sheet showed higher CD31 expression and porous fibers compared with that from the others. • The tissue fibers formed from PDLSCs sheet were directionally arranged, while the tissue derived from SCAP sheet showed highest mineral tissue formation.	Although in vitro DPSCs, PDLSCs and SCAP cell sheets have similar characteristics, their regenerative characteristics in vivo are different, with each showing potential application for regeneration of different tissues.

TABLE 9 (Continued)

Author, year, country, study type	Study material (stem cells, fibroblasts, etc.), Sample size	Form of vitamin application	Groups	Methods of evaluation (ELISA, etc.)
Leon et al. 2007[176] Japan	Human periodontal ligament cells $N=3$	In culture medium	Group 1: Human periodontal ligament cells + IL-11 (10 ng/mL) Group 2: Human periodontal ligament cells + IL-11 (10 ng/mL) + AA (50 μg/mL)	Immunohistochemistry RT-PCR ELISA
Marconi et al. 2021[173] Italy	hPDLSCs $N=6$	In culture medium	Group 1: Untreated hPDLSCs Group 2: hPDLSCs + AA (50 μg/mL) Group 3: hPDLSCs+ *P. gingivalis* lipopolysaccharide (LPS-G (5 μg/mL) Group 4: hPDLSCs + LPS-G (5 μg/mL) +AA (50 μg/mL)	MTT assay Immunofluorescence Western blot microRNA quantization
Okajima et al. 2020[185] Brazil	Mouse pre-osteoblastic cells MC3T3-E1 $N=3$	In culture medium	MC3T3-E1 + sodium ascorbyl phosphate (SAP) at concentrations of 0, 50, 100, 200, 300, 400, 500 μgmol/L	RT-PCR ELISA Immunohistochemistry
Wei et al. 2012[178] China	PDLSCs $N=16$ (human) $N=9$ minipigs	In culture medium	PDLSCs + AA at concentrations of 0, 5, 10, 20, 50 μg/mL	MTT assay Transmission electron microscopy ELISA Western blot Histochemistry Subcutaneous transplantation in nude mice Transplantation in miniature swine periodontal defects

cytokine levels (TNF-α, INF-γ, and IL-6) and alveolar bone loss and it was suggested that through its immunomodulatory effects, 25(OH)D3 positively affects periodontal "inflammaging," reducing the senescence-associated secretory phenotype (SASP) through a SOCS3/STAT-dependent mechanism, and inactivating NF-kB pro-inflammatory signaling in diabetics.[226] Furthermore, 25(OH) D3 boosted PTPN2 expression, an intracellular tyrosine-specific phosphatase, that regulates immune and inflammatory responses in diabetic patients, as a protein downstream of the VDR.[227] The 25(OH) D3-enhanced PTPN2 and VDR expression decreased the expression of TLR4 and the JAK1/STAT3 inflammatory signaling proteins in the gingival epithelium, through their dephosphorylation.[228,229]

Conversely, it was also demonstrated that vitamin D could exert pro-inflammatory and antimicrobial actions.[230,231] Following stimulation with 1,25(OH)D3, VDR and the 1-α-hydroxylase gene were activated and cathelicidin secretion and LL-37 (a potent inhibitor of intracellular

Evaluated parameters	Outcomes	Conclusion
ALP activity Gene expression of RUNX2, OCN and BSP Type 1 collagen and tissue inhibitor of metalloproteinase-1 production	• IL-11 enhanced ALP activity and RUNX2, OCN and BSP gene expression in the presence of AA. • IL-11 induced type 1 collagen and tissue inhibitor of metalloproteinase-1 production in periodontal ligament cells. • Type 1 collagen inhibitor completely inhibited the ALP activity enhanced by IL-11 and AA. • Furthermore, janus kinase/signal transducers and activator of transcription and MAPK signaling inhibitors reduced IL-11/AA-induced ALP activity in periodontal ligament cells.	IL-11/AA-induced osteoblastic differentiation of periodontal ligament cells through type 1 collagen production and janus kinase/signal transducers and activator of transcription, and MAPK signaling pathways were involved in this process. Findings suggest that IL-11 may function as an osteo-promotive cytokine, stimulating osteoblastic differentiation of periodontal ligament cells mainly through the synthesis of type 1 collagen and possibly by the induction of tissue inhibitor of metalloproteinase-1.
Cell proliferation ROS analysis DNMT1, NFκB, MyD88, and p300 miR-210	• Cells co-treated with LPS-G and AA showed restoration of cell proliferation. • The expression of NFκB, MyD88, and p300 was upregulated in LPS-G exposed cells, while the expression was attenuated in co-treatment with AA. • DNMT1 expression is attenuated in cells exposed to the inflammatory stimulus. – The level of miR-210 was reduced in stimulated cells, while the expression was evident in the hPDLSCs co-treated with LPS-G and AA.	AA could enhance a protective effect in in vitro periodontitis model, downregulating the inflammatory pathway and ROS generation and modulating the miR-210 level.
Gene expression of RUNX2, COL1A1, Osteocalcin (BGLAP2) Collagen I	• SAP at concentrations from 50 to 500 μmol/L does not influence preosteoblast cell viability, but stimulates their differentiation, observed by the induction of RUNX2, COL1A1, and BGLAP2; by the higher secreted levels of collagen I; and by increase in the mineralization of the extracellular matrix in cells exposed to this agent at 200 or 400 μmol/L, compared with those not exposed.	The incorporation of SAP into local release devices, membranes/scaffolds or biomaterials, could favor bone tissue formation and therefore periodontal healing.
Cell proliferation/survival Telomerase activity RT-PCR	• AA at 20 μg/mL was capable of forming cell sheets and inducing telomerase activity in PDLSCs, with upregulated expression of extracellular matrix type I collagen, fibronectin, and integrin β1, stem cell markers Oct4, Sox2 and Nanog and osteogenic markers RUNX2, ALP, OCN. – Under AA treatment, PDLSCs can form cell sheet structures because of increased cell matrix production.	PDLSCs sheets demonstrated a significant improvement in tissue regeneration compared with untreated control dissociated PDLSCs and offered an effective treatment for periodontal defects in a swine model. The development of AA-mediated mesenchymal stem cell sheets may provide an easy and practical approach for cell-based tissue regeneration.

LPS signaling) production increased up to 13 folds. Furthermore, triggering receptor expressed on myeloid cells-1 (TREM-1), a pivotal amplifier of the innate immune response in macrophages, was upregulated 16 folds, and the activation on the gingival epithelial cells increased their IL-8 mRNA levels, with antimicrobial activity against *A. actinomycetemcomitans*.[230] An imbalance in vitamin D-mediated functions due to its relative deficiency could further put the body at an increased risk of infection.[232,233] Aggressive forms of periodontitis were found to be associated with high serum 25(OH)D3 levels and its binding protein. In this context, polymorphism in the VDR was reported to be associated with aggressive forms of periodontal disease.[234–236] Additionally, an increase in IL-6, leukocytes and neutrophils was observed. This increase was ascribed to a VDR polymorphism as well as to an increased 25-hydroxylase activity of periodontal cells during acute inflammation, with 25(OH)D3 concentration in the periodontal pockets reaching 300 times greater levels than those in the plasma.[237]

TABLE 10 Effect of vitamin C—animal studies.

Author, year, country, study type	Animal type, sample size	Treatment, form of vitamin application	Study duration	Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)
Hu et al. 2016[189] China	Miniature swine *N* = 12	Group 1: AA-induced hDPSCs sheets Group 2: hDPSCs injection	12 weeks	Scanning electron microscopy Transmission electron microscopy Clinical, Radiological Quantitative histology RT-PCR
Wei et al. 2012[178] China	Miniature swine *N* = 9	Flap surgery with either: Group 1: AA-induced autologous PDLSCs sheets Group 2: UpCell dish PDLSCs sheet Group 3: Gelfoam scaffolds + dissociated autologous PDLSCs with gelfoam.	12 weeks	MTT assay Transmission electron microscopy ELISA Western blot Histochemistry Subcutaneous transplantation in nude mice Transplantation in miniature swine periodontal defects

TABLE 11 Effect of vitamin C—clinical studies.

Author, year, country, study type	Patients, sample size, gender, smoker, diagnosis	Treatment, Form of vitamin application	Study duration	Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)
Abou Sulaiman & Shehadeh 2010[191] Syria RCT	*N* = 60 patients (30 diagnosed with chronic periodontitis (ChP) and 30 matched controls) Females: 42 Males: 18 Non-smokers	ChP group randomly allocated ChP1: (15 patients SRP + AA (2000 mg/day for 4 weeks) ChP2: (15 patients SRP alone)	1 month	ABTS assay Clinical measurements
Elbehwashy et al. 2021[192] Egypt RCT	*N* = 20 patients (10 in each group) Females: 17 Males: 3 Stage-III/grade C periodontitis Non-smokers	Test group: OFD + PRF + AA (250 μg/mL) Control group: OFD + PRF	6 months	Clinical measurements Radiographic measurements
Woolfe et al. 1984[190] USA RCT	*N* = 10 patients (5 in experimental and 5 in control group) Gingivitis Non-smokers	Group 1: 250 mg AA q.i.d. Group 2: placebo SRP done after 1 week supplementation of the tables	7 weeks	Blood samples Clinical measurements (Baseline, 2, 6 and 7 weeks after) Gingival biopsy taken at week 6

Evaluated parameters	Outcomes: clinical, histological, immunological	Conclusion
Volume of bone regeneration Human β-globin gene Hight of bone regeneration Percentage of bone regeneration	• After 12 weeks, both hDPSCs sheet treatment and hDPSCs injection significantly improved periodontal tissue healing clinically in comparison with control group. • The volume of regenerative bone in the hDPSCs sheet group ($52.7 \pm 4.1\,mm^3$) was significantly larger than in the hDPSCs injection group ($32.4 \pm 5.1\,mm^3$) ($p < 0.05$). • Percentage of bone in the periodontium in hDPSCs injection group was $12.8 \pm 4.4\%$, while $17.4 \pm 5.3\%$ in the hDPSCs sheet group ($p < 0.05$).	Both hDPSCs injection and cell sheet transplantation significantly regenerated periodontal bone in swine. The hDPSCs sheet had more bone regeneration capacity compared with hDPSCs injection.
Cell proliferation/survival Telomerase activity RT-PCR Histology	• AA at 20 μg/mL was capable of forming cell sheets and inducing telomerase activity in PDLSCs, with upregulated expression of extracellular matrix type I collagen, fibronectin, and integrin β1, stem cell markers Oct4, Sox2, and Nanog as well as osteogenic markers *RUNX2*, *ALP*, *OCN*. • Under AA treatment, PDLSCs can form cell sheet structures because of increased cell matrix production. • PDLSCs sheets demonstrated a significant improvement in tissue regeneration compared with untreated control dissociated PDLSCs and offered an effective treatment for periodontal defects in a swine model.	The development of AA-mediated mesenchymal stem cell sheets may provide an easy and practical approach for cell-based tissue regeneration.

Evaluated parameters	Outcomes: clinical, histological, immunological, microbiological, PROMS	Conclusion
Plasma TAOC levels measured by an at baseline and 1-month post-therapy. PD, CAL, BOP, GI, PI	• Plasma TAOC levels were significantly lower in ChP patients than controls ($p < 0.001$). • The periodontal therapy resulted in increasing plasma TAOC and improvements in clinical measures among both ChP1 and ChP2 groups ($p < 0.001$). • The adjunctive dose of vitamin C did not offer additional effect ($p > 0.05$).	ChP is significantly associated with lower levels of plasma TAOC. Non-surgical periodontal therapy seems to reduce the oxidative stress during the periodontal inflammation. However, the use of adjunctive vitamin C still needs further investigation.
PD, CAL, RD, FMBS, FMPS RLDD, RDBD	• OFD + AA/PRF and OFD + PRF showed no differences regarding FMBS or FMPS ($p > 0.05$). • -OFD + AA/PRF demonstrated significant RD reduction of 0.90 ± 0.50 mm and 0.80 ± 0.71 mm at 3 and 6 months, while OFD + PRF showed RD reduction of 0.10 ± 0.77 mm at 3 months, with an RD increase in 0.20 ± 0.82 mm at 6 months ($p < 0.05$). • OFD + AA/PRF and OFD + PRF demonstrated significant RLDD reduction (2.29 ± 0.61 mm and 1.63 ± 0.46 mm; $p < 0.05$) and RDBD-increase ($14.61 \pm 5.39\%$ and $12.58 \pm 5.03\%$; $p > 0.05$). • Stepwise linear regression analysis showed that baseline RLDD and FMBS at 6 months were significant predictors of CAL reduction ($p < 0.001$).	OFD + PRF with/without AA significantly improved periodontal parameters 6 months post-surgically. Augmenting PRF with AA additionally enhanced gingival tissue gain and radiographic defect fill.
Plasma AA levels PD, CAL, GI, PI	Correlations between the clinical parameters and the ascorbic acid levels at the different time periods revealed no significant differences between the vitamin and the placebo groups	The use of megadoses of vitamin C in normal human subjects does not have a predictable or strong effect on the gingival response to initial therapy.

3.4.2 | Roles in periodontal therapy and biological wound healing/regeneration

In vitro studies results

A number of in vitro studies explored the immunomodulatory and regenerative effects of vitamin D on cellular level. The inductive potential of 1,25(OH)D3 on proliferation, differentiation, and matrix mineralization of MC3T3-E1 osteoblast-like cells was investigated in vitro. At low concentration (10^{-12} M), 1,25(OH)D3 demonstrated favorable anabolic effects with increased cellular proliferation, *ALP*, *Col-I*, *OCN* and VDR expression and no notable effect on the degree of calcification.[238] A further study compared 10 nM of 1,25(OH)D3 to enamel matrix derivatives (EMD) regarding their osteogenic and cementogenic potential on human PDLSCs. In contrast to EMD, 1,25(OH)D3 resulted in higher expressions of most cementogenic genes, including *RUNX2*, *TGF-β1*, *BMP-2*, *BMP-4*, *collagen I*, *ALP*, *bone sialoprotein (BSP)*, *OPN*, *osteocalcin (OCN)*, and Wnt signaling negative modulators (*SFRP1* and *DKK1*) along with higher mineralized nodule formation. In contrast, EMD stimulated stronger CEMP1 and CAP protein formation, with weaker mineralization. The study concluded that cementogenic factors could be inversely correlated to mineralization factors, with EMD and 1,25(OH)D3 acting at different stages during cementogenesis.[239]

Human periodontal ligament cells treated with 1,25(OH)D3 elevated *OPN* and *OCN* mRNA expression at 24 h and ALP activity at 48 h. Interestingly, the *E. coli* LPS-induced *IL-6* and *CXCL1* transcripts were attenuated by 30 ng/mL 1,25(OH)D3 for 24 h, yet with no effect on *E. coli*-LPS-induced *IL-1β* and *MCP-1* mRNA expression.[240] To further explore the osteoinductive potential of 1,25(OH)D3 under inflammatory conditions and elaborate on the underlying intracellular mechanism, human PDLSCs were cultured with *P. gingivalis*-LPS or 1,25(OH)D3 (in isolation or combined) in osteogenic inductive media and the expression levels of osteoblastic markers and Periodontal ligament-associated protein-1 (PLAP-1) examined. PLAP-1, also known as asporin, is a member of leucine-rich repeat proteoglycan family and act as a key regulator in the homeostasis of periodontal tissues, with the ability to negatively affect the mineralization of PDLSCs.[241] *P. gingivalis*-LPS (10 μg/mL) inhibited osteoblastic differentiation and upregulated *PLAP-1* expression, an effect that was reversed by 1,25(OH)D3 and mediated through VDR elements identified in the *PLAP-1* promotor region. 10 nM of 1,25(OH)D3 restored collagen I, *ALP and RUNX2*, while downregulated *PLAP-1* expression.[242] Combined results of these studies shed an interesting insight into the periodontal immunomodulatory and regenerative properties of vitamin D, with its clear ability to exert its anabolic effects even in the presence of strong bacterial virulence factors and pro-inflammatory cytokines (Table 12).

Animal studies results

The osteogenic potential of a single topical application of 40×10^{-9} μL 1,25(OH)D3 soaked on collagen, tested for regeneration of 2 × 2 mm diameter alveolar defects, demonstrated no additional benefits in bone regeneration, even in vitamin D deficient rats after 1 and 3 weeks.[243] It appears that for vitamin D to perform its regenerative and immunomodulatory actions, multiple applications are required. I further questions the hypothesis if a topical application of vitamin D would exert similar effects to systemic administration.

Thus, a study on a type 2 diabetic mice periodontitis model demonstrated that multiple intraperitoneal 5 μg/kg 25(OH)D3 injections (every 2 days till sacrifice) could attenuate periodontal destruction. The mechanism of 25(OH)D3 action was thought to be mediated through elevating the antimicrobial peptide cathelicidin production by gingival epithelial cells, reducing TLR4 expression, and elevating the VDR expression, aside from reducing fasting blood glucose levels.[244] The immunomodulatory and anti-inflammatory properties of vitamin D were evident in a further animal rat model investigating the effect of 25(OH)D3 on periodontitis and COPD. 25(OH)D3 was applied intraperitoneal for 8 weeks in animals with periodontitis or periodontitis with COPD. 25(OH)D3 treatment significantly alleviated inflammation by decreasing the serum levels of nuclear factor κB ligand (RANKL), TNF-α and IL-1 and increasing IL-10, while reducing alveolar bone loss and slightly improving lung function.[245] In a further study in a rat periodontitis model, the immunomodulatory effect of 1,25(OH)D3 on T cells was further evident. Calcitriol through oral gavage, suppressed alveolar bone resorption, decreased alveolar bone loss as well as inflammatory cell infiltration in response to the *E. coli*-LPS-induced periodontitis. Furthermore, IL-17 levels were decreased, while IL-4 and IL-10 levels were increased in the *E. coli*-LPS-injected regions. In the peripheral blood, the percentages of Th2 and Treg cells increased, while the percentages of Th1 and Th17 cells decreased in rats receiving 1,25(OH)D3.[246] It appears that vitamin D, as reported earlier, possess the ability to "shape" the acquired immune response, selectively stimulating specific Th-cell subsets and inhibiting other Th cells.[198] Through attenuation of the development of Th17, strongly implicated in periodontitis progression with the production of variety of pro-inflammatory cytokines as IL-1 and IL-6,[247] vitamin D performs a protective effect on the periodontium, while fostering the periodontal reparative/regenerative events though its cellular inductive properties portrayed above (Table 13).

Clinical trials results

Surprisingly, although vitamin D intake has been linked to maintenance of periodontal health following initial periodontal therapy,[248,249] rarely any clinical intervention tested its effect in isolation in a randomized controlled trial's setting. To explore the combined importance of daily vitamin D and calcium administration with or without PTH in serum vitamin D-sufficient or insufficient patients on the periodontal healing following surgical interventions, a longitudinal clinical trial, correlated serum 25(OH)D3 quantities, self-administrated daily subcutaneous injections of 20 μg of PTH, oral vitamin D (800 IU) and oral calcium (1000 mg), 3 days prior to surgery and 6 weeks afterward, to periodontal outcomes in 40 patients diagnosed with severe periodontitis. Results demonstrated that subjects with untreated vitamin D deficiencies showed less favorable results with lower CAL gain, greater residual PD, and less resolution of intrabony defects for up to 1 year following periodontal surgery. Yet, 25(OH)D3 levels had no significant effect on CAL and PD improvements in PTH patients

at 1-year follow-up. Intrabony defect resolution was higher in PTH-treated vitamin-D-sufficient as opposed to deficient patients, while BOP was lower in PTH-treated vitamin-D sufficient as opposed to deficient individuals.[250] Results of this study give important insights into the interaction between the triad of PTH, vitamin D, and calcium, and it remains to be controversial if the observed effects can be ascribed to only one corner of this triad, or represents a result of their conjoint biological actions. In contrast to the remarkable findings from in vitro and preclinical animal studies, the absence of results from well-planned randomized controlled clinical trials, testing the adjunctive effect of vitamin D on the clinical outcomes of nonsurgical as well as surgical periodontal therapeutic approaches, delineates an important knowledge gap in periodontal regenerative knowledge (Table 14).

Evidence box		
	Presence	Effect
Association studies	✓	++
Biological mechanism	✓	++
Animal model "proof of principle"	✓	++/−
Clinical studies with surrogate parameters	✓	+
Clinical studies with hard end points	Ø	

3.5 | Vitamin E

3.5.1 | Sources, biological structure and function and roles in periodontal health/prophylaxis and disease

Vitamin E, first described in 1922 for its positive effects on reproduction in rats,[251] is a plant-derived, lipid-soluble biomolecule. It comprises eight compounds, namely α-, β-, γ-, δ-tocopherols and their corresponding four α-, β-, γ-, δ-tocotrienols. Tocopherols are soluble in ethanol and aprotic solvents, insoluble in water and viscous at room temperature. The most active and abundant form of vitamin E in humans is α-tocopherol.[252] Only plants and photosynthetic organisms can synthesize vitamin E, and it is found in green leafy vegetables, seeds, fruits, rice, palm oil, annatto oil, poultry, meat, fish, nuts, cereals.[253–255]

Physiologically, vitamin E represents an essential micronutrient, which has been successfully applied in medicine, cosmetics, pharmaceutics, and foods. The recommended daily dose of Vitamin E is 15 mg (22.4 IU). Similar to vitamins A and C, vitamin E is one of the key extracellular antioxidants, especially active against free radical-mediated lipid peroxidation, playing a pivotal role in stabilizing the cell membrane by terminating free radical reactions.[256] It is involved in regulating enzyme activity, cellular signaling, gene expression, and cell proliferation. Additionally, vitamin E inhibits platelet coagulation and is implicated in the prevention of a number of diseases, including cardiovascular diseases, neurological disorders, age-related skin and eye deterioration, and infertility.[257]

Vitamin E has received more attention in the past two decades regarding its potential role in periodontal health and disease. It was suggested that vitamin E could improve periodontal health primarily through reestablishing the redox status balance, minimizing the inflammatory responses and promoting wound healing.[258] Vitamin E diets in various concentration administered for 35 days in rats exposed to stress, showed a significant protective effect on stress-related bone loss.[259] Aside from earlier results, that failed to demonstrate a positive effect of local vitamin E application on gingivitis or periodontitis severity,[260] or a difference in serum α-tocopherol levels between periodontitis and nonperiodontitis patients,[261] a significant association was evident between the severity of periodontitis and insufficient systemic micronutrient (A, B1, C, and E, iron, folate and phosphorus) intake.[262] Additionally, favorable effects of vitamin E in relation to periodontal health and inflammation control were reported, where reduced vitamin E levels were identified in periodontitis patients compared to periodontally healthy ones.[259] In contrast to the analysis of results from the NHANES III survey showing no association between vitamin E levels and periodontitis,[33] data from the US NHANES survey collected between 2011 and 2014 demonstrated that lower vitamin E intake was significantly associated with increased severity of periodontitis.[262] An analysis of the previous NHANES data from 1999 to 2001, further demonstrated that reduced α-tocopherol levels (yet within the normal range) were nonlinear inversely associated with the severity of periodontitis in adults (after adjusting for possible cofounders).[263] In a community-based study on elderly Japanese citizens (≥71 years old) low serum levels of α-tocopherol were significantly associated with a greater number of teeth with CAL loss of ≥3 mm over a period of 8 years (1999–2007), suggesting that low α-tocopherol serum levels could represent a risk factor for periodontitis.[45] A further study conducted over a period of 2 years by the same group, reported an inverse association between high vitamin E intake and the number of teeth with periodontal diseases progression (CAL loss of ≥3 mm).[45] Also, in an Indian population significantly reduced vitamin E plasma concentrations and erythrocyte membrane lipid peroxidation were registered in periodontitis patients compared to periodontally healthy subjects.[163] Vitamin E supplementation for 12 weeks reduced periodontal inflammation in female patients with chronic periodontitis.[264] Overall, the observed associations suggested that vitamin E could be a key factor linked to improved periodontal health status, yet its mechanism of action is still to be clarified.

3.5.2 | Roles in periodontal therapy and biological wound healing/regeneration

In vitro studies results

In light of the association studies linking vitamin E to periodontal health, a number of in vitro studies were conducted to investigate the possibly underlying cellular mechanisms. In this context, the

TABLE 12 Effect of vitamin D—in vitro studies.

Author, year, country, study type	Study material (stem cells, fibroblasts, etc.), Sample size	Form of vitamin application	Groups	Methods of evaluation (ELISA, etc.)
Hong et al. 2021[239] Taiwan	hPDLCs *N* = 6	In culture medium	Control: OM medium: α-MEM + 10% FBS + 10 mM β-glycerophosphate + 10^{-7} M dexamethasone) Group 1: OM + AA (100 μM) Group 2: OM + calcitriol (10 nM) Group 3: OM + calcitriol (100 nM) Group 4: OM + EMD (50 μg/mL) Group 5: OM + EMD (100 μg/mL)	RT-PCR Alkaline phosphatase activity Immunofluorescence assay Alizarin red and Von Kossa staining Dispersive x-ray spectrometry
Kim et al. 2018[238] Korea	MC3T3-E1 osteoblast-like cells *N* = 6	In culture medium	Negative control: α-MEM +10% FBS Positive control: α-MEM +10% FBS + 10 mM β-glycerophosphate + 50 μg/mL ascorbic acid Experimental group: α-MEM + 10% FBS + 10 mM β-glycerophosphate + 50 μg/mL ascorbic acid + serial dilution (10^{-4}, 10^{-6}, 10^{-8}, 10^{-10}, 10^{-12}, 10^{-14} M) of 1,25-dihydroxyvitamin D_3.	MTT assay Alkaline phosphatase activity assay ALP staining Alizarin red staining RT-PCR
Nebel et al. 2015[240] Sweden	hPDLCs *N* = 4	In culture medium	Control: hPDLCs Test group 1: hPDLCs + 30 ng/mL vitamin D3 for 24 h Test group 2: hPDLCs + 30 ng/mL vitamin D3 for 48 h Test group 3: hPDLCs + *E. coli*-LPS (1 μg/mL) for 4 h +vitamin D3 (0.3–300 ng/mL) for 24 h	RT-PCR Alkaline phosphatase activity
Zhang et al. 2020[242] China	hPDLCs *N* = 3	In culture medium	Group 1: osteogenic induction (OI) medium Group 2: OI + LPS (0, 1, 10, 20, 50 μg/mL) Group 3: OI + LPS (0, 1, 10, 20, 50 μg/mL) +1,25$(OH)_2D_3$ (0, 0.1, 1, 10 nM)	Western blot RT-PCR Transplantation in Wistar rat periodontitis model

effects of vitamin C and α-tocopherol, separately or combined, were examined in reverting the cytotoxic effects of nicotine and cotinine (at different concentrations) on MG-63 osteoblast-like cells and human gingival fibroblasts (HGFs) in vitro. Vitamin E proved to be significantly more effective than vitamin C in enhancing cell viability, migration, proliferation, and apoptosis reduction of cells exposed to these toxins.[265] Similarly, accelerated wound healing and proliferation rate of human gingival and periodontal ligament fibroblasts, accompanied by an increased synthesis of collagen type I up to 72 h, in response of α-tocopherol was demonstrated.[266] Coating implant

Evaluated parameters	Outcomes	Conclusion
BMP-2, *BMP-4*, *BSP*, *CAP*, *Cbfa1*, *CEMP1*, *DKK1*, *IL-6*, *OCN*, *OPG*, *OPN*, *RUNX2*, *SFRP1*, *TGF-β1*, *VDR* Mineralization Ca, P, Ag	• 10 nM calcitriol enhanced most cementogenic gene expression, TGF-β1, BMP-2 and BMP-4, RUNX2, Type I collagen, *ALP*, *BSP*, *OPN*, *OCN*, *CEMP1*, and *CAP*, and Wnt signaling negative modulators, *SFRP1* and *DKK1*, along with highest ALP activity and mineralization formation in hPDLCs. • Only moderate CEMP1 protein was observed. In contrast, EMD stimulated stronger *CEMP1* and *CAP* protein, but presented weaker mineralization capacity, hinting at the possibility that strong stimulation of mineralization might dominate cemetogenic-specific factors and vice versa.	Calcitriol demonstrated great osteoinductivity, and potential to induce cementogenic gene expression by initiating hPDLC differentiation and promoting mineralization. Compared with calcitriol, EMD promoted cemento-inductivity in hPDLCs at a later time point via highly expressed CEMP1 and CAP protein, but with less mineralization. Thus, calcitriol and EMD could provide differential enhancement of cemento-induction and mineralization, likely acting at various differentiation stages.
Cellular proliferation *ALP* *Col-I* *OCN* VDR Mineralization	• 1,25-dihydroxyvitamin D_3 did not inhibit cell growth and rate of cell proliferation was higher than in positive control group at all concentrations. • ALP activity was higher than in positive control group at low concentrations of 1,25-dihydroxyvitamin D_3 (10^{-10}, 10^{-12}, and 10^{-14} M). • RT-PCR showed that the gene expression levels of *ALP*, *Col-I*, *OCN*, and VDR were higher at a low concentration of 1,25-dihydroxyvitamin D_3 (10^{-12} M). • Alizarin red staining after treatment with 1,25-dihydroxyvitamin D_3 (10^{-12} M) showed no significant differences in the degree of calcification. In contrast to the positive control group, formation of bone nodules was induced in early stages of cell differentiation.	1,25-dihydroxyvitamin D_3 positively affects cell differentiation and matrix mineralization. Therefore, it may function as a stimulating factor in osteoblastic bone formation and can be used as an additive in bone regeneration treatment.
Cell count *OPN*, *OCN*, *IL-1β*, *IL-6*, *MCP1*,	• Treatment with 30 ng/mL of vitamin D3 for 24 h had no effect on PDL cell number and morphology but increased OPN and OCN mRNA expression by about 70 and 40%, respectively. • Treatment with vitamin D3 for 48 h enhanced hPDLCs • ALP activity by about two times. • Stimulation with LPS (1 μg/mL) for 4 h increased hPDLCs • IL-6 cytokine and chemokine ligand 1 (CXCL1) chemokine mRNA expression several fold. • LPS-induced increase in IL-6 and CXCL1 transcripts was attenuated by vitamin D3 (30 ng/mL). • Treatment with vitamin D3 (3–300 ng/mL) for 24 h reduced the LPS-evoked increase in hPDLCs • IL-6 protein by about 50%. Vitamin D3 (30 ng/mL) had no effect on LPS-induced *IL-1β* and *MCP-1* mRNA expression.	Vitamin D3 promotes osteogenic differentiation but also downregulates inflammation promoter-induced IL-6 cytokine and CXCL1 chemokine expression in hPDLCs, suggesting that vitamin D3 stimulates bone regeneration and antagonizes inflammation in human periodontal tissue.
Col1, *RUNX2*, *ALP*, *PLAP-1*,	• Data showed that LPS inhibited osteoblastic differentiation and induced the expression of PLAP-1 in hPDLSCs. • Increasing addition of 1,25$(OH)_2D_3$ reversed the LPS-induced inhibition of osteoblastic differentiation of hPDLSCs through the suppression of PLAP-1 expression. • A potential VDR elements within the PLAP-1 promoter region was identified and shown to bind with VDR by chromatin immunoprecipitation (ChIP) assays. This negative region was also found to mediate suppressor reporter gene activity.	1,25$(OH)_2D_3$ could enhances the osteogenic differentiation of hPDLSCs under inflammatory condition through inhibiting PLAP-1 expression transcriptionally.

surfaces with vitamin E and UV-irradiated vitamin D precursors (for their activation) reduced the inflammatory response and extracellular matrix breakdown in HGFs cultures, with increased levels of collagen III α1, fibronectin mRNAs, increased TIMP-1 on mRNA and protein levels, and decreased level of IL-8 mRNA.[267] A cellular protective effect, with reduction in the oxidative damage and limited hydroxyl radical development, was evident in human epithelial cells pretreated with the vitamin E prior to H_2O_2 exposure. Still, a vitamin E pretreatment was not able to reverse the deleterious effects of H_2O_2 on the cell cycle of exposed cells.[268]

TABLE 13 Effect of vitamin D—animal studies.

Author, year, country, study type	Animal type, sample size	Treatment, form of vitamin application	Study duration	Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)
Bi et al. 2019[246] China	Sprague Dawley rats $N=30$ (10/group)	Control group: LPS-induced periodontitis group (10 mg/mL *E. coli*-LPS-injected in the gingival sulcus, LPS group) Experimental group: LPS-induced periodontitis treated+ calcitriol group (0.2 μg/kg day oral gavage) (LPS+ Cal group).	4 weeks	Micro-CT Histology Immunohistochemistry Flow cytometric analysis
Fügl et al. 2015[243] Austria	Sprague Dawley rats $N=60$	2 mm diameter single defects in maxilla and mandible Control group: No treatment Vitamin depletion group 1: No treatment Vitamin depletion group 2: Calcitriol-soaked collagen	3 weeks	Histomorphometry Micro-CT Serology
Han et al. 2019[245] China	Sprague Dawley rats $N=50$ (10/group)	Normal group (*N*) Periodontitis group (P) COPD/periodontitis group (CP), Periodontitis/25-OHD3 treatment group (PV), COPD/periodontitis/25-OHD3 treatment group (CPV).	21 weeks COPD induced at 5 weeks of age Periodontitis-induced at 12 weeks of age (*P. gingivalis*)	Whole-body flow plethysmography ELISA Stereomicroscopy
Zhang et al. 2020[242] China	Wistar rats $N=80$	Group A: normal control Group B: 1,25(OH)2D3 Group C: periodontitis only Group D: 1,25(OH)2D3 + periodontitis. Oral gavage	14 days	Immunohistochemistry
Zhou et al. 2018[244] China	BKS.Cg-Dock7m$^{+/+}$Lepr*db*/Nju mice $N=30$	Diabetes group (D) Diabetes/periodontitis group (DP, orally inoculated *P. gingivalis*) Diabetes/periodontitis group treated by 25-OHD_3 (intraperitoneal injection, 5 μg/kg every 2 day)	12 weeks	Serology ELISA Dot blot analysis Stereomicroscopy Immunohistochemistry

Positive cellular protective effects were further reported for α-tocopherol when HGFs were exposed for 24 and 48 h to *P. gingivalis*-LPS. α-tocopherol increased HGFs' proliferation and secretion of human β-defensin-1 and 2, decreased secretion of IL-1β and IL-6, and enhanced the cellular healing rate.[269] The anti-inflammatory activity of all forms of tocopherols (α-, β-, γ-, δ-) was further evident in a

Evaluated parameters	Outcomes: clinical, histological, immunological	Conclusion
ABL Osteoclasts presence TRAP-positive cells RANKL/OPG ratio *IFN-γ, IL-4, IL-17, IL-10* Th1, Th2, Th17, Treg cells in peripheral blood	• Calcitriol decreased ABL in response to LPS injection and inflammatory cell infiltration. • Analysis of osteoclast number and RANKL and OPG expression showed that bone resorption activity was largely suppressed in response to calcitriol administration, along with decreased IL-17 levels but increased IL-4 and IL-10 levels in periodontal tissues (the LPS-injected region). • Similarly, the percentages of Th2 and Treg cells in peripheral blood increased, but the percentages of Th1 and Th17 cells decreased in rats receiving calcitriol.	Calcitriol can be used to inhibit bone loss in experimental periodontitis, likely via the regulation of local and systemic Th cell polarization.
Bone formation Serum levels of 1,25(OH)D3	• Bone formation rate significantly increased within the observation period in all groups. • Bone regeneration was higher in the maxilla than in the mandible. • Bone regeneration was lower in the control group compared to vitamin depletion groups, with no significant effects by local administration of calcitriol (micro-CT mandible $p=0.003$, maxilla $p<0.001$; histomorphometry maxilla $p=0.035$, mandible $p=0.18$).	Vitamin D deficiency does not necessarily impair bone regeneration in the rat jaw and a single local calcitriol application does not enhance healing.
Measurement of lung functions RANKL, *TNF-α*, *IL-1*, *IL-10* ABL	• Results showed that 25-OHD3 treatment significantly alleviated inflammation by decreasing the serum levels of RANKL, TNF-α and IL-1 and increasing that of IL-10, while reducing alveolar bone loss and slightly improving lung function.	Findings suggest that vitamin D supplementation could be a new clinical approach for the treatment of COPD and periodontitis.
Anti-PLAP-1 antibody	• PLAP-1 expression was significantly upregulated in periodontal ligament of rats with periodontitis and the oral administration of 1,25(OH)2D3 attenuated *PLAP-1* expression by periodontal ligament cells to normal levels.	$1,25(OH)_2D_3$ could enhances the osteogenic differentiation under inflammatory condition through inhibiting PLAP-1 expression transcriptionally.
Fasting blood glucose $25\text{-}OHD_3$ *IL-1* Cathelicidin Alveolar bone loss VDR, TLR4, CAP18	• $25\text{-}OHD_3$ intraperitoneal injection attenuated periodontal inflammation by promoting cathelicidin production in gingival epithelia and reducing fasting glucose of diabetic mice. • Dotblotting of serum showed cathelicidin secretion was consistent with $25\text{-}OHD_3$ treatment. • Immunochemistry exhibited enhanced expression of cathelicidin and vitamin D receptors along with reduced expression of TLR4 in diabetic mice. • Stereomicroscopy showed less ABL when injected with $25\text{-}OHD_3$.	The study complemented the mechanism of cathelicidin and extended knowledge of $25\text{-}OHD_3$'s role in diabetic periodontitis.

murine model of macrophage-like cells stimulated with LPS, TNF-α, or *P.gingivalis* fimbriae, with a significant increase in *COX2* mRNA expression. Yet, significantly higher anti-inflammatory effects were demonstrated by β-, γ-, δ- tocopherols compared with the α- form, suggesting their potential useful role in preventing oral and periodontal diseases.[270] Taken together findings form in vitro studies

TABLE 14 Effect of vitamin D- clinical studies.

Author, year, country, study type	Patients, sample size, gender, smoker, diagnosis	Treatment, form of vitamin application	Study duration	Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)
Bashutski et al. 2011[250] USA RCT	N = 40 patients Females: 25 Males: 15 Smokers: 15 Former smokers: 12 Never smokers: 13 Sever periodontitis	Open flap debridement Group 1: Daily calcium (1000 mg) + vitamin D (800 IU) + self-administered teriparatide subcutaneous injections (20 μg PTH) Group 2: Daily calcium + vitamin D + placebo 3 days prior to surgery and continued for 6 weeks after surgery	1 year	Clinical Radiographic Serology

clearly outline remarkable cellular protective, antioxidant, immunomodulatory, and anabolic effects of vitamin E, that warranted further exploration in vivo (Table 15).

Animal studies results

The effect of vitamin E supplementation, specifically α-tocopherol, on gingival wound healing and alveolar bone loss has been investigated in a limited number of animal preclinical studies. Improved wound healing was evident histologically in a rat gingivectomy model receiving 60 IU d-α-tocopherol acetate daily for 14 days as compared to their untreated controls.[271] Similarly, significant suppression of alveolar bone loss and increased collagen fiber formation was observed in rats with ligature-induced periodontitis, where α-tocopherol had been administered systemically for 4 weeks.[272] However, another study underlined the fact that although vitamin E did not prevent alveolar bone loss, it prompted a decreased inflammatory reaction and immunoreactivity to an inducible isoform nitric oxidase synthase (iNOS), with reduced oxidative damage in an experimental periodontitis rat model[273] (Table 16). Combined, results of these studies underlined the vitamin E antioxidative, immunomodulatory and reparative/regenerative propensity.

Clinical trials results

A very limited number of studies explored effects of Vitamin E following nonsurgical periodontal therapy. In an investigation exploring the effect of a 3-month vitamin E supplementation (oral supplementation of 200 mg (300 IU) vitamin E every other day) following SRP, statistically significant higher improvements in PI, GI, BOP, PD and CAL as well as higher serum superoxide dismutase levels, depicting antioxidant activity, were observed in the vitamin E group compared with controls.[274] Contrarily, in a split-mouth study, 2 months following SRP, no significant differences for PD nor the total antioxidant capacity were notable between the vitamin E (200 IU daily for 2 months) and the control group, yet with significantly lower mean CAL loss demonstrated in the vitamin E group[275] (Table 17). This limited availability of clinical investigations warrants the conduction of further high quality randomized controlled trails, testing vitamin E at different concentrations and administration forms and frequencies as an adjunct to surgical as well as nonsurgical periodontal therapies. In light of the encouraging in vitro and preclinical animal findings, it remains plausible to assume that vitamin E holds many unexplored therapeutic options in the field of periodontology.

Evidence box		
	Presence	Effect
Association studies	✓	++
Biological mechanism	✓	++/−
Animal model "proof of principle"	✓	++/−
Clinical studies with surrogate parameters	✓	+
Clinical studies with hard end points	Ø	

3.6 | Vitamin K

3.6.1 | Sources, biological structure and function and roles in periodontal health/prophylaxis and disease

Vitamin K is a fat-soluble vitamin of herbal origin, found in many fruits, vegetables (avocado, broccoli, kiwi, green grapes, and lettuce) and oils (olive oil, soybean, and canola oil). It belongs to a group of biomolecules necessary for the synthesis of several proteins involved in blood coagulation and body hemostasis processes.[276] Biologically, it is implicated in the regulation of calcium metabolism, cell proliferation and growth, inflammatory reactions and oxidative stress.[277] Vitamin K is responsible for the production of vitamin K-dependent proteins (VKDP), including the seven proteins involved in blood coagulation (II, IIV, IX, X, protein S, protein X, protein Z), the four proteins of the transmembrane Gla family, namely OCN (bone Gla protein), matrix Gla protein, growth arrest specific 6 protein (Gas 6) and protein S.[278-280]

Two natural forms of vitamin K exist, namely vitamin K1 (phylloquinone or phytonadione) and vitamin K2 (menaquinone). While Vitamin K1, stored in the liver, is mainly responsible for the synthesis of coagulation proteins, vitamin K2 is distributed in the entire body, and its activity is related to γ-glutymyl carboxylase and anti-NF-kB

Evaluated parameters	Outcomes: clinical, histological, immunological, microbiological, PROMS	Conclusion
IBD resolution PD, CAL, BOP	• Placebo patients with baseline vitamin D deficiency [serum 25(OH)D, 16–19 ng/mL] had significantly less CAL gain (−0.43 mm vs. 0.92 mm, $p < 0.01$) and PD reduction (0.43 mm vs. 1.83 mm, $p < 0.01$) than vitamin-D-sufficient individuals. • Vitamin D levels had no significant impact on CAL and PD improvements in PTH patients at 1 year, but infrabony defect resolution was greater in PTH-treated vitamin-D-sufficient vs. -deficient individuals (2.05 mm vs. 0.87 mm, $p = 0.03$), while BOP was lower in PTH-treated vitamin-D-sufficient vs. -deficient individuals ($p < 0.01$).	Vitamin D deficiency at the time of periodontal surgery negatively affects treatment outcomes for up to 1 year. Analysis of these data suggests that vitamin D status may be critical for post-surgical healing.

activity.[281,282] A synthetic water-soluble analog of vitamin K, namely K3 (menadione) was introduced with the ability to be converted in the liver into vitamin K2.[283,284] The chemical formulation of vitamin K2 relies on the MK-n formula, and comprises of a naphthoquinone ring and a side chain of isoprenoid of variable lengths ("n") in saturated or unsaturated forms.[278] Except for MK-4, found in liver, fish, milk, eggs, vegetables, and primarily synthesized by conversion of vitamin K3 and secondly from dietary phylloquinone,[285,286] all menaquinones are synthesized by bacteria.

Vitamin K promotes bone formation and inhibits bone resorption. It endorses osteoblasts proliferation and differentiation, prevents their apoptosis, especially the Fas-mediated apoptosis[287,288] and improves osteoblastic functions.[281] Vitamin K2 promotes alkaline phosphatase activity, expression of bone anabolic markers (i.e., OCN)[287,289-291] and osteoblast transition to osteocytes.[292] Additionally, vitamin K, especially vitamin K2, through direct and indirect pathways hinders bone resorption,[287,290] by exerting an inhibitory effect on osteoclasts formation and functions[293-295] and through interfering with the expression of various proteins involved in osteoclastic activity, including RANKL or OPG, or cells (tartrate-resistant acid phosphatase positive cells (TRAP+)) or inflammatory cytokines such as PGE2 and IL-1α.[294,295] Evidence from studies of vitamin K on osteoblast and osteoclast activity in the entire body, may possibly imply a similar effect of vitamin K on oral osteoblast and osteoclast activity.[296,297]

The impact of vitamin K on periodontal health or disease has been little investigated so far. The effect of vitamin K on normal or dysbiotic subgingival oral microbiome sampled from periodontally healthy and diseased patients, respectively, was examined.[298] Vitamin K alone or combined with hemin added to a nutrient-rich medium (modification of SHI medium, brain-heart infusion (BHI), and three sucrose concentrations (0%, 0.05%, and 0.1%)) was evaluated compared to a nutrient-limited medium composed of saliva and 5%, 10%, or 20% inactivated human serum, determining the biomass of the microbiome, its variability and 16 s rRNA profile, as well as its richness and diversity. Additionally, the dysbiosis was quantified using the subgingival dysbiosis index. The microbiome in periodontitis patients showed irrespective of the used medium, higher species richness, α-diversity and were clustered with their inoculum separate from the health-derived microbiomes, with vitamin K showing no evident effect on these variables. Nonetheless, vitamin K seemed to have an impact on the biomass, with the medium supplemented with vitamin K and hemin demonstrating the highest biomass.

3.6.2 | Roles in periodontal therapy and biological wound healing/regeneration

In vitro studies results

Only a single investigation tested the effect of vitamin K on periodontal cells. A study investigated the effect of vitamin K2, specifically its MK-4 form, on the osteogenic differentiation potential of PDLSCs, demonstrating an enhanced osteogenic aptitude in presence of MK-4, with a significant upregulation of osteogenic genes and proteins like ALP, RUNX2, OCN, and osterix (Sp7 transcription factor). Blocking the Wnt/β-catenin signaling pathway with XAV-939, reversed the effect of MK-4, suggesting that the MK-4 effect was mediated through the activation of the Wnt/β-catenin signaling pathway[299] (Table 18). Further investigations are recommended in this filed to test the effect of different forms, concentrations and applications forms of vitamin K on the spectrum of periodontal and inflammatory cells in vitro.

Animal studies results

Similar to the rarity in in vitro studies, only one animal preclinical study was identified testing the effect of vitamin K on periodontal therapy. Possible anti-inflammatory effects of vitamin K were evaluated in a murine animal model in experimentally induced periodontitis.[300] No effects were evident regarding the systemic administration of vitamin K2 alone or in conjunction with vitamin D3 concomitant to SRP, regarding changes in alveolar bone level, the levels of the cytokines IL-1β, IL-10, serum bone alkaline phosphatase (B-ALP), tartrate-resistant acid phosphatase 5b (TRAP-5b), and Ca^{2+} levels (Table 19). Similar to the above recommendation regarding in vitro studies, further animal studies are needed to explore effects of different forms, concentrations, and applications forms of vitamin K to determine the ideal formulation for in vivo clinical periodontal therapeutic applications in human.

TABLE 15 Effect of vitamin E—in vitro studies.

Author, year, country, study type	Study material (stem cells, fibroblasts, etc.), sample size	Form of vitamin application	Timepoint of evaluation	Methods of evaluation (ELISA, etc.)
Chapple et al. 2013[341] UK	Neutrophiles isolated from venous blood of periodontally healthy volunteer ($N=9$–12 patients) stimulation of cells with PMA (phorbol myristate acetate, or with Ig-opsonised *F. nucleatum*, with *S. aureus* or PBS (control) in the presence /absence of vitamins	α-tocopherol (1 mmol/L) In culture medium Alone or in combination with AA (10 μL) compared to AA alone	30 min pre-stimulation and 150 min post-stimulation	Dye exclusion (trypan blue) Enhanced chemiluminescence assay: isoluminol or luminol
Derradjia et al. 2015[269] Canada	Primary HGFs $N=5$	In culture medium α-tocopherol (0, 50, 100, 200 μM) ± 1 μg/mL LPS (*P.gingivalis* LPS)	24, 48 h	Optical microscopy MTT assay ELISA In vitro scratch wound assay
Murakami et al. 2013[270] Japan	Murine macrophage-like cell line RAW264.7 $N=$n.r.	In culture medium α-, β-, γ- and δ-tocopherol solution	72 h (48 h culture +24 h incubation)	Cell counting kit (CCK-8) Real-time PCR
Nizam et al. 2014[266] Turkey	Primary cultures of human GFs and PDLFs $N=$n.r.	In culture medium 60 μM α-tocopherol Or combined with 5×10^{-9} M, 10×10^{-9} M, and 50×10^{-9} M Se	24, 48, 72 h	Metabolic XTT assay ELISA New wound healing model (evaluation on photographs)
Royack et al. 2000[268] USA	Human oral epithelial cells exposed to H_2O_2 2 primary cell lines	In culture medium Vitamin E dissolved in ethanol 5 treatments: Control, Ethanol only, Vitamin E only, H_2O_2 only Vitamin E followed by H_2O_2.	Flow cytometry: 72 h Electron paramagnetic resonance analysis: at 5, 15, 25, 35 min Organotypic technique: 4–5 days	Electron paramagnetic resonance analysis Flow cytometry Organotypic technique
Satue et al. 2015[267] Spain	Coated titanium implants and titanium disks HGFs were cultured (passage 7 and 8 after isolation) HGFs density: 2.3×10^4 cells/cm^3 $N=6$	Implants: 10 μL 0.2 nM 7-DHC + vitamin E disks: 0.18 pmol vitamin E/disk Followed by UV radiation and incubation for 48 h at 23°C to allow D3 synthesis from 7-DHC onto the Ti surface	After 3 days of culture: cytotoxicity, cell morphology, gene expression, protein quantification, wound healing, inflammatory response After 14 days: gene expression, protein quantification	Cytotoxicity: assessing lactate dehydrogenase activity Cell morphology: confocal microscopy Gene expression: rt-PCR Inflammatory response: ELISA for TIMP-1, MMP-1 Wound healing: assay on confluent monolayers of grown HGFs, Expression of RNA of *MMP1*, *ACTA2*, *EDN1*, *TGFB1* genes

Evaluated parameters	Outcomes	Conclusion
Cell viability ROS production Isoluminol production	• Total and extracellular unstimulated, baseline ROS generation was unaffected by α-tocopherol; it was inhibited by ascorbate and a combination of both micronutrients. • Fcγ-receptor (Fcγ-R)-stimulated total or extracellular ROS generation was not affected by the presence of individual micronutrients. • Vitamin combination significantly reduced extracellular FcγR-stimulated ROS release. • Neither micronutrient alone inhibited TLR-stimulated total ROS the vitamin combination inhibited TLR-stimulated total ROS. • Ascorbate and the micronutrient combination (without α-tocopherol) inhibited extracellular ROS release by TLR-stimulated cells.	Micronutrient effects in vivo could be beneficial in reducing collateral tissue damage in chronic inflammatory diseases, such as periodontitis, while retaining immune-mediated neutrophil function.
Cell adhesion Cell growth IL-1β, IL-6 Human β defensins 1 and 2 Fibroblast migration	• No adverse effect on cell adhesion and morphology • α-tocopherol increased fibroblast proliferation with/without LPS • No effect on IL-1β, IL-6 secretion • After exposure to *P. gingivalis* LPS, α-tocopherol significantly decreased IL-1β, IL-6, increased human β defensins 1 and 2 • α-tocopherol increased healing rate of fibroblasts from 12 up to 48 h	α-tocopherol may play an active role in countering the damaging effect of LPS by reducing inflammatory cytokines, increasing β-defensins and promoting fibroblast growth, migration, and wound healing.
Cytotoxicity (EC_{50}) of tocopherols toward RAW cells Effect on expression of COX2 mRNA stimulated with *E. coli*-LPS, TNFα or *P. gingivalis* fimbriae	• Each tocopherol showed similar low toxicity • Significant ($p < 0.01$) inhibition of COX2 expression in RAW cells following exposure to with *E. coli*-LPS, TNFα or *P. gingivalis* fimbriae • β-, γ-, δ- tocopherols showed significantly greater inhibitory effects ($p < 0.05$) than α-tocopherol	Tocopherols exhibit anti-inflammatory activity. β-, γ- and δ-tocopherol have particularly more potent anti-inflammatory activity than α-tocopherol. Tocopherols may have potential utility for prevention of periodontal and chronic oral diseases.
Cell viability Cell proliferation bFGF Collagen type I synthesis Wound healing model	• α-tocopherol significantly increased the healing rate of PDFLs at 12 h • α-tocopherol increased bFGF and collagen type I release from GFs and PDLFs at 24, 48, 72 h • α-tocopherol/Se combination significantly enhanced the proliferation rate of both cells at 48 h, decreased the proliferation of PDLFs at 72 h, and increased the healing rate of GFs at 12 h and PDLFs at 12 and 48 h. • bFGF and collagen type I synthesis was also increased in both cell types at 24, 48, and 72 h by α-tocopherol/Se combination.	α-Tocopherol and α-tocopherol/Se combination is able to accelerate the proliferation rate and wound healing process and increase the synthesis of bFGF and collagen type I from both GFs and PDLFs.
Hydroxyl radical Cell cycle distribution Cell morphology	• Hydroxyl radicals' concentration in H_2O_2-treated cells decreased over a period of time. • In vitamin E pretreated cells, initial hydroxyl radical concentration was less than H_2O_2-only group. • Rate of hydroxyl radical degradation was comparable in both situations • Cell cycle analysis: H_2O_2-treated cells differed from normal cells: % of cells in the G1 phase decreased (34.3 vs. 61.2% in control); the S phase increased (35.5 vs. 15.6% in control). • Cells pretreated with vitamin E before exposure to H_2O_2 showed similar alteration in cell cycle as those treated to H_2O_2 alone. • Cells treated only with vitamin E showed similar results to the control group. • Organotypic cultures treated with H_2O_2 alone or pretreated with vitamin E before exposure: nuclear hyperchromatism, loss of maturation and prominent nucleoli; the features were consistent with premalignant epithelial transformation.	H_2O_2 produced hydroxyl radicals and altered the cell cycle. Vitamin E may have the potential to reduce oxidative damage caused by hydroxyl radicals.
Cytotoxicity, cell morphology, gene expression, protein quantification, wound healing, inflammatory response	• Beneficial effect of UV-radiated 7-DHC:VitE -coated Ti implants on HGFs • UV-irradiated 7-DHC and vitamin E coating: Increased collagen III α1 and fibronectin mRNAs Decreased the level of interleukin-8 mRNA. Increased TIMP-1 (mRNA and protein levels) Decreased RANKL mRNA in HGFs	UV-irradiated 7-DHC:VitE-coated Ti implants have a positive effect on HGFs in vitro showing a reduction in the inflammatory response and extracellular matrix breakdown.

TABLE 15 (Continued)

Author, year, country, study type	Study material (stem cells, fibroblasts, etc.), sample size	Form of vitamin application	Timepoint of evaluation	Methods of evaluation (ELISA, etc.)
Soeta et al. 2010[360] Japan	Osteoblasts isolated from the rat calvaria *N* = 6	In culture medium α-tocopherol (100, 200 μM) and δ-tocopherol (2, 20 μM) for 3 days	14 days	Lowry's methods Reverse transcription PCR
Torshabi et al. et al. 2017[265] Iran	HGF1-PI 1 Human osteoblast-like cell-line MG-63 *N* = 3	In culture medium α-tocopherol-albumin conjugate (0.1–100 mM) + Vitamin C (0.1–4 mM) + Nicotine (5 mM) + or + Cotinine (5 mM)	0, 24, 48 h	MTT assay In vitro scratch test/wound healing assay Rt-PCR

Clinical trials results

So far, to the best of our knowledge, no clinical human studies were performed where vitamin K has been implemented in therapeutic protocols of periodontal disease.

Evidence box		
	Presence	Effect
Association studies	Ø	
Biological mechanism	✓	+
Animal model "proof of principle"	✓	–
Clinical studies with surrogate parameters	Ø	
Clinical studies with hard end points	Ø	

3.7 | Coenzyme Q_{10}

3.7.1 | Sources, biological structure and function and roles in periodontal health/prophylaxis and disease

Coenzyme Q_{10} (CoQ_{10}, 2,3 dimethoxy-5-methyl-6-decaprenyl benzoquinone) was firstly isolated from beef heart mitochondria, with properties of reversible oxidation and reduction.[301] CoQ_{10} is a lipid-soluble micronutrient, synthesized in mammals and plants. It is composed of two functional groups: a benzoquinone "head" and an isoprene side chain "tail". The lipid-soluble side chain consists of ten isoprene units with a total of 50 carbon atoms.[302] CoQ_{10} can be internally synthesized by humans or obtained from dietary sources, including fish and meat, with highest concentrations identified in tissues with a high energy turnover (i.e. heart, brain, liver, kidney).[303] It is only sparsely absorbed in the intestine due to its lipophilic nature. However, after its gut uptake it circulates through the lymphatic system and ultimately lands in the blood circulation.[304] CoQ_{10} found in an oxidized ("ubiquinone") and a reduced ("ubiquinol") form, is an important antioxidant and plays an essential role in mitochondrial ATP production, being the main carrier for the electron transfer in the respiratory chain.[305–308] Apart from its intercellular antioxidant activity, CoQ_{10} has also been recognized to have an effect on gene expression, with an impact on the overall tissue metabolism.[309,310]

For over two decades, CoQ_{10} has been widely used as a dietary supplement for maintaining health without safety concerns or limitation on the daily dosage. Several diseases have been associated with CoQ_{10} deficiency and have been shown to benefit from its supplementation. Primary and secondary CoQ_{10} deficiencies are found in mitochondrial diseases, fibromyalgia, cardiovascular disease, neurodegenerative diseases, cancer, diabetes mellitus, and male infertility.[311] Limited evidence exists for a possible association between periodontal disease and CoQ_{10} deficiency.[312] Yet, considering the fact that during periodontitis, periodontal pathogens may induce an overproduction of ROS, antioxidants like CoQ_{10} may counteract such ROS production and reduce the associated periodontal tissues' degradation. In this context, suboptimal CoQ_{10} levels were found in about 80% of the gingival biopsies from periodontitis patients.[313] Earlier clinical studies further showed an improvement of chronic periodontitis and salivary secretion following systemic CoQ_{10} supplementation.[314,315]

3.7.2 | Roles in periodontal therapy and biological wound healing/regeneration

In vitro studies results

The antioxidant effects of CoQ_{10} in relation to nicotine have been studied on human periosteal fibroblasts and osteoblasts obtained

Evaluated parameters	Outcomes	Conclusion
ALP activity BSP mRNA OCN Nanocalcified nodules	• After 3 days, significant decrease in the alkaline phosphatase activity of the cultured osteoblasts induced by both vitamin forms • After 14 days, no significant change in ALP activity and expression of bone sialoprotein mRNA in the osteoblasts treated with the vitamins for 3 days • Expression of osteocalcin mRNA was decreased by treatment of α-tocopherol (100, 200 μM) and δ-tocopherol (2, 20 μM) at Days 4 and 7. • At Day 14, expression of osteocalcin mRNA was decreased only with treatment of 200 μM α-tocopherol. • Noncalcified nodules were decreased by treatment of α-tocopherol (200 μM) and δ- tocopherol (20 μM) at day 7. • Treatment of α-tocopherol and δ-tocopherol showed No significant change of formation of calcified nodules at Day 14.	Vitamin E inhibits differentiation of osteoblasts especially from early stage to osteoid-producing stage.
Cell viability and proliferation Cell migration Gene expression of apoptosis-related genes (BAX, BCL2, CASP3) compared to control gene GAPDH	• Significantly greater dose-dependent negative effects of nicotine on morphology, viability, proliferation and migration of MG-63 and HGF cells than cotinine. • Vitamin E improved statistically significantly more cell viability, proliferation, and migration, reduced more cell apoptosis than vitamin C in cells exposed to nicotine/ cotinine than vitamin C.	Vitamin C and specially vitamin E (systemically/locally) may be helpful in regeneration and repair of oral soft and hard tissues in smokers

from periodontally diseased patients. Incubation of human periosteal fibroblasts and osteoblasts in the presence of $CoQ_{10,}$ pycnogenol or phytoestrogens showed a significant stimulating effect on physiologically active steroid metabolites even in the presence of nicotine as opposed to cellular incubation solely with the toxin. Thus, it seems that CoQ_{10}, pycnogenol or phytoestrogens could exert protective cellular antioxidative effects, reversing the catabolic effects of nicotine[316] (Table 20). In light of the very limited availability of in vitro studies, further investigations are warranted to explore the effects of different CoQ_{10} concentrations and application forms on periodontal cells.

Animal studies results

In a rat aging model, systemic supplementation with CoQ_{10} ameliorated the exacerbated age-related alveolar bone loss associated with life-long n-6 polyunsaturated fatty acid diet (low in CoQ_{10}). Moreover, genetic analysis revealed that CoQ_{10} supplementation restored the biogenesis and age-related increase in some mitochondrial components in gingival cells, probably due to improved oxidative and respiratory balance.[317]

Positive effects on periodontitis have been further observed for the topical application of reduced CoQ_{10} ($rCoQ_{10}$) in a murine aging model, with significantly lower oxidative DNA damage, lower tartrate-resistant acid phosphatase-positive osteoclasts, and reduced expression of NLRP3, caspase-1, 8-OHdG, ASC, IL-1β, and NF-κB as compared with the control group without $rCoQ_{10}$. Additionally, the age-dependent serum increase of 8-OHdG were significantly reduced in the experimental group[318] (Table 21). Taken together, these results suggest positive periodontal immunomodulatory effects of CoQ_{10} in both systemic as well as topical application.

Clinical trials results

Effects of CoQ_{10} on periodontal healing were mostly investigated in the context of nonsurgical treatment of chronic periodontitis. Subgingival application of CoQ_{10} as an adjunct to SRP demonstrated significant additional reductions in bleeding index, PI, PD and CAL gains, 3 and 6 months following treatment compared to SRP alone.[314,319-325] Recently, a new delivery system was established in the form of a nanomicelles formulation (NM_{Q10}) encapsulating CoQ_{10}. The entrapped CoQ_{10} in spherical-shaped NM_{Q10} gel delivery system was tested clinically, following SRP for the treatment of chronic periodontitis,[325] demonstrating significant improvements in GI, PI, PPD, and BOP with enhanced antioxidant activity as compared to SRP alone.[325] Conversely, other studies, although demonstrating a beneficial affect between baseline and follow-up, failed to show a significant beneficial effect of such adjunctive CoQ_{10} application as compared to SRP alone.[326-328] The contradictorily results could be explained on the basis of the differences in study designs (i.e., split-mouth studies vs. parallel group studies, number of patients, power, sample size, etc.), carrier type, treatment duration and tested CoQ_{10} concentrations.

Systemic CoQ_{10} administration, at doses of 30 mg administered twice daily for 3 months[329] or 120 mg per day for three months,[330] demonstrated improvements in periodontal clinical parameters in periodontitis patients. Similarly, two studies evaluating systemic CoQ_{10} effects, either 30 mg twice daily for three months[331] or 100 mg per day for 1 month,[332] in type 2 diabetic patients suffering from moderate to severe chronic periodontitis, demonstrated significant improvement in clinical parameters,[331,332] and lower inflammatory cytokine levels (MMP8)[331] (Table 22). Taken together clinical findings on CoQ_{10} suggest it could hold an interesting potential as an adjunct to periodontal therapeutic approaches.

TABLE 16 Effect of vitamin E—animal studies.

Author, year, country, study type	Animal type, sample size	Treatment, form of vitamin application	Study duration
Asman et al 1994[342] Sweden	Rats Controls (PBS): $N=5$ Vitamin E 3 mg ($N=2$) Vitamin E 15 mg ($N=2$) Vitamin E + Se: 1 mg vitE + 20 μg Se ($N=6$) 1.5 mg vitE + 30 μg Se ($N=3$) 3 mg Vitamin E + 60 μg Se ($N=3$) 6 mg Vitamin E + 120 μg Se ($N=2$) For injection in sponge): Controls: $N=15$ Vitamin E + Se: 2 mg VitE + 40 μg Se ($N=7$), 3 mg vitE + 60 μg Se ($N=2$)	α-tocopherol undiluted or in combination with Se Administered subcutaneous or injected in collagen sponges (containing homologous ^{3}H collagen powder) at the neck every 2 days between the 8th and 18th day after sponge implantation	8, 18 days
Bas et al. 2021[272] Turkey	Rat $N=40$ (10/group)	Experimental periodontitis (ligature-induced, 4 weeks) Group A: Se Group B: α-tocopherol (α-T) Group C: Se + α-T Group D: control (saline) Vitamin E form: α-tocopherol acetate once a day for 4 weeks (intraperitoneal)	4 weeks
Carvalho et al. 2013[273] Brasil	Wistar rats $N=18$/group	Ligature-induced periodontitis 4 treatment groups: 2 control groups with periodontitis OR sham surgery two test groups: Vitamin E + surgery OR Vitamin E + periodontitis Oral administration of vitamin E (500 mg/kg) for 9 days	11 days
Kim and Shklar 1984[271] USA	Albino rats ($N=40$)	Standardized gingivectomy between mandibular incisor teeth Group 1: Gingivectomy Group 2: Gingivectomy +60 I.U. of d-α-tocopheryl acetate daily, orally ($n=20$) Group 3: animals not wounded ($n=10$) Group 4: animals not wounded + 60 I.U. of d-α-tocopheryl daily ($n=10$)	Four animals: groups 1, 2 sacrificed at 1, 2, 4, 7, 14 days following gingivectomy Two animals: groups 3, 4 sacrificed at similar times.

Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)	Evaluated parameters	Outcomes: clinical, histological, immunological	Conclusion
Light microscopy (ingrowth of granulation tissue in sponges) Scintillation counting in urine (breakdown of implanted radioactivity caused by sponge induced granulation tissue)	Ingrowth of granulation tissue in sponges (without collagen) Collagen degradation monitored as total radioactivity DPM/mg Collagen in excreted urine	• Similar reduction in radioactivity (in urine) in the combination vitamin E + Se (for the groups with 1 mg, 1.5 mg, 3 mg vitamin E) • a higher dose induced a higher radioactivity • Vitamin E alone had negligible effect on radioactivity • Vitamin E + Se injected subcutaneously had no detectable effect on the development of granulation tissue as compared to controls • Vitamin E + Se injected into the sponge arrested the ingrowth of granulation tissue and after 10 days of treatment there was no detectable ingrowth of fibroblasts or capillaries in half of the sponge (arrested the maturation of the granulation tissue)	Vitamin E and Se are potential inhibitors of the free oxygen radicals from phagocytic inflammatory cells. Thus, it was suggested that these radicals may play a role in the collagen destruction by granulation tissues, as in periodontitis
Image analysis method in connective tissue under epithelium (*N* of iNOS, CD95 positive cells, and collagen fibers) Immunohistochemistry ELISAof serum IL-1β, IL-6, IL-4.	ABL Inflammatory cell infiltrate Collagen density Nitric oxide synthase *N* of iNOS, *N* of CD95 cells, *N* collagen fibers serum IL-1β, IL-6, IL-4.	• Se + αT significantly suppressed ABL compared with the control group ($p < 0.05$). Other groups showed no statistical significance. • *N* gingival collagen fibers in Se and αT tended to be higher than in the control group • Se: *N* iNOS+ cells were smaller than in control ($p < 0.05$) (iNOS important inflammation marker and tissue destruction). The other groups showed only a tendency toward lower iNOS levels ($p > 0.05$) • Serum IL-6, IL-1β, IL-4 levels were not significantly different among groups • α-tocopherol alone or in combination did not alter the cytokines levels	Se has been concluded to inhibit inflammation of the gum due to reduction in iNOS. Se and αT can have a remarkable important role in preventing ABL, particularly in combination.
Elevated plus-maze (EPM) test Morphometry and immunohistochemistry	Anxiety ABL Lipid perioxidation quantification Activity of enzyme superoxide dismutase TNF-α iNOS	• Experimental periodontitis-induced a marked inflammatory process and intense ABL. • Treatment with vitamin E decreased inflammatory reaction, prevented malondialdehyde formation and reduced the immunoreactivity to iNOS, but did not decrease ABL. • Vitamin E had an anxiogenic effect on rats with or without periodontitis.	Vitamin E may have potential to reduce oxidative damage and inflammatory response in experimental periodontitis but does not prevent ABL. Attention should be given to indiscriminate use of vitamin E due to the risk of causing anxiety in patients.
Gingival healing (grossly) Histologically (hematoxylin and Eosin stain + Mallory CT stain)	Healing time Healing tissue quality Wound size remaining Autopsy of major organs	• Animals receiving vitamin E healed more rapidly, with almost complete restoration of gingiva by 7 days. • Complete healing was seen in both control and experimental groups by 14 days.	Vitamin E was shown to accelerate gingival wound healing in experimental animals.

TABLE 17 Effect of vitamin E—clinical studies.

Author, year, country, study type	Patients, sample size, gender, smoker, diagnosis	Treatment, form of vitamin application	Study duration	Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)
Behfarnia et al. 2021[275] Iran Controlled clinical trial	*N* = 16 patients with periodontitis	Control group: 41 teeth, SRP Test group: 42 teeth, SRP +200 IU Vitamin E daily for 2 months	2 months	TAC Kit
Singh et al. 2014[274] India RCT	*N* = 38 patients CP (8 males, 30 femals) Age: 37.5 years (17–58 years) and *N* = 22 controls (systemically and periodontally healthy) (6 males, 16 females) Age: 22.5 years (22–50 years)	Randomization: Test group 1: SRP Test group 2: SRP + 200 mg (300 IU) vitamin E every 2 days for 3 months	3 months	SOD assay ELISA

TABLE 18 Effect of vitamin K—in vitro studies.

Author, year, country, study type	Study material (stem cells, fibroblasts, etc.), Sample size	Form of vitamin application	Timepoint of evaluation	Methods of evaluation (ELISA, etc.)
Cui et al. 2021[299] China	PDLSCs from premolars *N* = 40	In culture medium Vitamin K2 (MK-4, menaquinone-4)	7, 14 days	Flow cytometry Cell counting kit-8 (CCK8) Colony formation assays Alizarin Red S staining qRT-PCR Western blot

TABLE 19 Effect of vitamin K—animal studies.

Author, year, country, study type	Animal type, sample size	Treatment, form of vitamin application	Study duration	Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)
Aral et al. 2015[300] Turkey Controlled, parallel group	Rats with experimentally induced periodontitis *N* = 72 divided into 6 groups	Vitamin K2 (30 mg/kg) + SRP (group 5) vs. Healthy (group 1) Periodontitis (group 2) SRP (group 3) SRP + vitamin D3 (2 μg/kg; group 4) SRP + Vitamin K2 + Vitamin D3 (group 6) Vitamin K2 (menatetrenone) was administered daily for 10 days in corn oil vehicle.	18 days (10 days vitamin administration)	Histological analysis (Masson trichrome) + light microscopy ELISA

TABLE 20 Effect of coenzyme Q_{10}- in vitro studies.

Author, year, country, study type	Study material (stem cells, fibroblasts, etc.), sample size	Form of vitamin application	Timepoint of evaluation	Methods of evaluation (ELISA, etc.)
Figuero et al. 2006[316] UK	Human periosteal fibroblasts from periodontally diseased patients Osteoblasts Cells were incubated previously with 14c-testosterone *N* = 8	In culture medium CoQ_{10} (20 μg/mL) alone or in combination with nicotine (250 μg/mL) Compared to Pycnogenol (150 μg/mL) and phytoestrogens (10 and 40 microg/ml) alone or in combination with nicotine (250 μg/mL)	24 h	Combined gas chromatography–mass spectrometry

Evaluated parameters	Outcomes: clinical, histological, immunological, microbiological, PROMS	Conclusion
Saliva TAC (total antioxidant capacity) PD, CAL (6 teeth/person)	• 2 months after SRP, no significant differences for the mean changes in TAC ($p = 0.14$) or PD changes ($p = 0.33$) • Mean CAL loss in test was significantly less than the control group ($p = 0.03$).	Vitamin E supplementation with SRP can reduce the inflammatory process of periodontitis and improve periodontal clinical indices and decrease the amount of attachment loss.
Superoxide dismutase (SOD) activity in serum and saliva Periodontal parameters: PI, GI, BOP, PD, CAL	• SOD activity in both serum ($p < 0.05$) and saliva ($p < 0.001$) was lower in patients with CP compared with controls (cross-sectional evaluation compared to healthy controls). • 3 months after SRP, SOD activity improved in both treatment groups: • Serum SOD improvement in TG-2 was higher than in TG-1 ($p < 0.001$) • Higher improvement in periodontal parameters in TG-2 than TG-1 ($p < 0.001$). • Serum SOD levels in TG-2 increased above the level of the control group.	Systemic and local SOD levels are lowered in CP. Adjunctive vitamin E supplementation improves periodontal healing as well as antioxidant defense.

Evaluated parameters	Outcomes	Conclusion
ALP Extracellular matrix mineralization mRNA of ALP Protein expression: ALP, Runx2, OCN, Osterix Correlation to Wnt/β-catenin signaling pathway	• 10^{-5} M MK-4 significantly promoted the osteogenic differentiation of PDLSCs. • Gene and protein expressions of ALP, Runx2, OCN, and Osterix were all upregulated compared with control. • After blocking the Wnt/β-catenin signaling pathway with XAV-939, the effect of MK-4 was apparently reversed.	MK-4 can promote osteogenic differentiation of PDLSCs, which is likely related to the activation of the Wnt/β-catenin signaling pathway.

Evaluated parameters	Outcomes: clinical, histological, immunological	Conclusion
IL-1β, IL-10 Serum bone-ALP (B-ALP) Tartrate-resistant acid phosphatase 5b (TRAP-5b) Ca level ABL	• ABL in the periodontitis group were significantly greater than those in the other five groups. • No significant differences were found in gingival IL-1β and IL-10, serum B-ALP, TRAP-5b, calcium and ABL between the groups receiving SRP with vitamins and the group receiving SRP alone.	Vitamin D3 and K2 alone or in combination did not affect gingival IL-1β and IL-10, serum B-ALP and TRAP-5b levels, or alveolar bone compared with conventional periodontal therapy alone.

Evaluated parameters	Outcomes	Conclusion
Radioactive steroid metabolites (DHT)	• Incubation of osteoblasts and periosteal fibroblasts with CoQ_{10}, Pycnogenol® or phytoestrogens stimulated the synthesis of the physiologically active androgen DHT. • DHT was significantly reduced in response to nicotine compared to control values ($p < 0.001$ for phytoestrogens). • Nicotine + CoQ_{10}, Pycnogenol® or phytoestrogens increased yields of DHT compared with incubation with nicotine alone in both cell types.	Addition of antioxidants such as CoQ_{10} or Pycnogenol® and phytoestrogens could reverse the catabolic effects of nicotine in human periosteal fibroblasts and osteoblasts.

TABLE 21 Effect of coenzyme Q_{10}—animal studies.

Author, year, country, study type	Animal type, sample size	Treatment, form of vitamin application	Study duration
Varela-Lopez et al. 2016[317] Spain	Rat aging model *N* = 72 (*N* = 12/group/sacrifice)	Randomized to 3 groups: based on 3 different dietary fat sources n-6, n-3 polyunsaturated fatty acid or monounsaturated fatty acids (olive oil-VQ-group, sunflower -SQ group or fish oil-FQ group) 50 mg/kg/day CoQ_{10}	24 months (animal age) Sacrifice at 6, 24 months
Yoneda et al. 2013[318] Japan	Rat aging model *N* = 34 (Aged 2 months *N* = 6 and 4 months *N* = 18) All of the 2-month-old rats and six of the 4-month-old rats were sacrificed 12 remaining 4-month-old rats received topically applied ointment with or without 1% rCoQ10 on the gingival surface until they reached 6 months of age.	Test: *N* = 6, 4 months old (with Q_{10}) Control: *N* = 6, 4 months old (without Q_{10}) 1% Topical application of a reduced form of co-enzyme Q_{10} ($rCoQ_{10}$)	2 months

Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)	Evaluated parameters	Outcomes: clinical, histological, immunological	Conclusion
Quantitative rt-PCR Histology (methylene blue) High sensitivity multiplexed beads immunoassays (Milliplex MAP) Lipid peroxidation analysis -ELISA Gas–liquid chromatography	ABL OPG, RANKL IL-1β, IL-6 Lipid peroxidation Fatty acid profile of the three fat sources	• Age-related ABL (differences between 24 and 6 months of age) were 139 ± 28 μm -VQ group, 163 ± 38 μm -SQ group, 97 ± 57 μm – FQ group. • No significant differences between groups. • CoQ_{10} diminished the exacerbated age-related alveolar bone loss associated to n-6 polyunsaturated fatty acid diet • IL-1β: higher levels at 6 and 24 months for the FQ group • Higher cytokine circulating levels for old animals in all 3 dietary groups • At 6 months, SQ showed the lowest levels • RANKL: significant age-related changes with lower values for VQ and FQ groups • OPG: similar plasma levels at 6 months, and increased significantly with age (SQ and FQ-highest levels) • Gene expression analysis suggests that involved mechanisms might be related to a restored capacity of mitochondria to adapt to aging in gingival cells from rats fed on n-6 polyunsaturated fatty acid. Could be due to an age-related increase of the rate of mitochondrial biogenesis and a better oxidative and respiratory balance in these animals.	Supplementation with CoQ_{10} could counteract the negative effects of n-6 polyunsaturated fatty acid on ABL associated to age.
Histology (hematoxylin eosin) Immunohistochemical staining for 8-OHdG Tartrate-resistant acid phosphatase (TRAP) Real-time PCR	8-OHdG tartrate-resistant acid phosphatase (TRAP) IL-1β, NLRP3, caspase-1, apoptosis-associated speck-like protein (ASC), nuclear factor (NF)-κB, β-Actin	• Rats showed an age-dependent increase in circulating oxidative stress. • No significant differences for ABL between the 2 groups at 6 months. • No pathological changes: extension of blood vessels or increased *N* of inflammatory cells. • The periodontium in the experimental group exhibited low expression levels of 8-OHdG and TRAP, as compared with that in the control group • Gene expression of NLRP3, caspase-1, ASC, IL-1β, NF-κB, in periodontal tissues was significantly lower in the experimental group as compared to control ($p < 0.05$) • Serum levels of 8-OHdG tended to increase with age, and these values were significantly lower in the experimental than control group. • rCoQ10 decreased oxidative DNA damage and tartrate-resistant acidphosphatase-positive osteoclasts in the periodontal tissue at 6 months of age as compared to control. • The same conditions lowered gene expression of caspase-1 and interleukin-1β in the periodontal tissue. • Nod-like receptor protein 3 inflammasomes were less activated in periodontal tissues from rCoQ10-treated rats as compared to control. • Serum levels of CoQ_{10} (mean ± SD) at 6 months of age were 0.018 ± 0.002 μg/mL – control group and 0.023 ± 0.002 μg/mL experimental group. • There was a significant difference in the serum total CoQ_{10} levels of the control and experimental group ($p < 0.05$).	rCoQ10 suppresses age-related inflammatory reactions and osteoclast differentiation by inhibiting oxidative stress.

TABLE 22 Effect of coenzyme Q_{10}—clinical studies.

Author, year, country, study type	Patients, sample size, gender, smoker, diagnosis	Treatment, form of vitamin application	Study duration
Barakat & Attia 2019[319] Saudi Arabia Split-mouth	$N = 20$ patients Age: 40 ± 4.33 years (25–60 years) CP	Test: SRP + CoQ_{10} subgingivally + periodontal dressing Control: SRP	1 month
Chatterjee et al. 2012[320] India Split-mouth, randomized	$N = 30$ patients Age: n.r. Gingivitis	SRP in 2 quadrants Topical application of CoQ_{10} in a scaled + unscaled quadrant for 28 days Group A: SRP Group B: SRP + CoQ_{10} Group C: CoQ_{10} Group D: none	28 days
Chug et al 2020[328] India Split-mouth, randomized	$N = 30$ patients ($N = 25$ sites/treatment group) Age: ≥30 years old CP	Test: SRP + subgingival application of CoQ_{10} Control: SRP + methyl cellulose Both groups received periodontal dressing for 15 days Surgical treatment with Sticky Bone in nonresponding sites	12 months evaluation (recall 1, 3, 6, 12 months) 6 months after surgical treatment were Sticky Bone was placed
ElBarbary et al. 2022[329] Egypt RCT	$N = 32$ patients Stage II grade B periodontitis	Test: SRP + systemic 30 mg CoQ_{10} 2 times/day for 3 months Control: SRP	3 months
Ghasemi et al. 2022[332] Iran RCT	Controlled diabetic patients $N = 42$ CP	Test: SRP + systemic CoQ_{10} (100 mg) once/day for 30 days Control: SRP	4 weeks
Hanioka et al. 1994[314] Japan Cohort study	$N = 10$ patients Age: mean: 48.3 years (35–61 years) All male Adult periodontitis	Test: Topical application of CoQ_{10} for 3 weeks ± SRP Topical application CoQ_{10} was applied in 20 pockets once a week for 6 weeks in total In remaining 10 sites (control): application of soybean oil	6 weeks (reevaluation at 3, 6 weeks)
Hans et al. 2012[321] India Split-mouth	$N = 12$ patients Age: 22–55 years Both genders CP	Quadrant wise topical application once: • extra-sulcular • intra-pocket alone • intra-pocket + SRP • SRP only Perio-Q_{10} gel	6 weeks

Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)	Evaluated parameters	Outcomes: clinical, histological, immunological, microbiological, PROMS	Conclusion
Clinical measurements	BI, PI, PD, CAL	• Significant improvement of all parameters compared to baseline in both groups • Significant intergroup differences favoring SRP + CoQ_{10} for all clinical parameters	CoQ_{10} gel intra-pocket applications packed by periodontal dressing provide precious clinical outcomes and considered as a useful adjunctive agent with non-surgical periodontal therapy
Clinical evaluation	GI, BI, PI	• Reduction in gingival, bleeding, and plaque scores at the sites where CoQ_{10} was applied. • Significant reduction in mean ± SD gingival, bleeding, and plaque scores at 28th day for groups A, B, and C when compared with baseline	Promising results were obtained after application of CoQ_{10} alone or as adjunct to SRP for treatment of plaque induced gingivitis.
Clinical evaluation	PI, GI, PD, CAL	• Significant improvement for plaque index, gingival index, and PD compared to baseline • Increase in values of plaque and PD at 6 months and significant increase in values of gingival and plaque index, and PD seen at 12 months, • No significant difference in values was seen at 12 months and baseline.	Coenzyme Q10 does not aid in the treatment of periodontitis
Clinical measurements ELISA	PD, CAL MMP9	• Significant decrease in PD and gain in CAL in both groups • Significant better clinical results for the test group. • Significant decrease in MMP-9 levels in GCF was detected in both groups • Significant higher decrease in the test group at 1 and 3 months	The antioxidant action of CoQ_{10} provided an added benefit to SRP for PD, CAL and MMP9 GCF levels.
Clinical evaluation	PI, GI, BOP, CAL, PD	• PD, CAL, BOP, and PI indices in the CoQ_{10} group were significantly lower than the control group • GI was similar in both groups with a significant decrease after therapy in both groups	Systemic administration of Q_{10} adjunctive to SRP in patients with controlled diabetes and CP might accelerate the treatment process and significantly reduce the pocket depths
Clinical measurements Periotron Periotest Enzymatic assay system	gingival crevicular fluid flow PD, CAL, PI, BOP, MGI Peptidase activity	• In the first 3-week period, significant reductions in gingival crevicular fluid flow, probing depth and attachment loss were found only at experimental sites. • Significant decreases in the plaque index, gingival • crevicular fluid flow, probing depth and attachment loss were found in both groups • Significant improvements in MGI, BOP, and peptidase activity were observed only at test sites.	Topical application of CoQ_{10} improves adult periodontitis not only as a sole treatment but also in combination with traditional nonsurgical periodontal therapy.
Clinical evaluation	PI, GI, GBI, PD, CAL	• Significant reduction ($p < 0.01$) of clinical parameters (plaque index, gingival index, gingival bleeding index, PD, CAL in all four treatment groups • Intra-pocket gel application + SRP showed significant reduction ($p < 0.05$) for PI, GI, GBI, and CAL in comparison to intra-pocket gel alone.	In CP patients, SRP only and with Perio-Q gel showed almost similar clinical results without any stsignificant differences. The study did not provide enough clinical support for the superiority of adjunctive use of Perio-Q gel. It appears that Perio-Q gel in this study may have a potential additive effect.

TABLE 22 (Continued)

Author, year, country, study type	Patients, sample size, gender, smoker, diagnosis	Treatment, form of vitamin application	Study duration
Manthena et al 2015[330] India RCT	$N = 30$ patients ($n = 15$/group) 14 female, 16 male Age: 18–35 years Non-smokers Plaque induced gingivits	Test: SRP + CoQ_{10} Systemic CoQ_{10} (120 mg/day) for 3 months Control: SRP + placebo	3 months
Pranam et al. 2020[326] India Randomized split-mouth study	$N = 16$ patients Age: 30–50 years old Both genders Mild–moderate CP	Sites were randomized to Test: SRP + CoQ_{10} Single application of CoQ_{10} gel in the treatment site at baseline after SRP Control: SRP + placebo	3 months
Raut et al. 2016[322] India Comparative, parallel-arm study	$N = 15$ patients (45 sites) 6 males 9 females Age: 37.4 ± 9.75 years (20–60 years) Moderate–severe CP Smokers with >10 cigarettes/day were excluded	Test I: CoQ_{10} + SRP Single application of CoQ_{10} gel in the treatment site at baseline after SRP Test II: tea tree oil gel + SRP Control: placebo gel + SRP Periodontal dressing was used in all groups	1 month
Raut et al. 2019[323] India RCT	$N = 40$ patients ($n = 20$ /group) Age: 37.4 ± 9.76 years (20–60 years) Moderate–severe CP Only smokers included (≥10 cigarettes/day for min. 5 years)	Test: CoQ_{10} + SRP CoQ_{10} gel for single subgingival application following SRP Control: SRP	3 months
Sale et al. 2014[324] India Comparative, parallel-arm study	$N = 18$ patients Age: 33.8 years (20–55 years) No smokers included Both genders CP	Test I: single supragingival application of CoQ_{10} gel following SRP Test II: single subgingival application of CoQ_{10} gel following SRP Control: SRP	4 weeks
Shaheen et al 2020[325] Egypt Split-mouth, randomized	$N = 15$ patients CP	Q_{10} in nanomicelles (NM_{Q10}) formulation Incorporated in situ gelling systems Treatment: full-mouth SRP Test side: Intrasulcular injection of Q_{10} formulation (F1); injection was repeated every 2nd day for 1 week Control side: No additional treatment	6 weeks

Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)	Evaluated parameters	Outcomes: clinical, histological, immunological, microbiological, PROMS	Conclusion
Clinical evaluation	PI, GI, PD	• Both groups showed marked reduction of clinical periodontal parameters at 1 and 3 months when compared to baseline. • No significant difference in PI and PD between the two groups at any given time period • Test group showed significant difference in gingival inflammation at 1 month and 3 months when compared to control group.	The use of systemic CoQ_{10} adjunctive to SRP showed significant reduction in gingival inflammation when compared to SRP alone
Microcapillary method ELISA Clinical evaluation	Superoxide dismutase (SOD) PD, GI, PI	• Intergroup analysis: no statistically significant difference for clinical parameters at all the time intervals ($p > 0.05$) • Significant increase in the level of SOD in the test group ($p > 0.05$) compared with the control group at 3 months.	Adjunctive use of CoQ_{10} with SRP can boost the antioxidant concentration, but it is not superior to SRP in the treatment of chronic periodontitis
Clinical evaluation	PI, GBI, PD, CAL	PD reduction: Control:0.50 ± 0.2 mm Test I: 2.95 ± 0.20 mm Test II: 2.09 ± 0.15 mm Mean CAL reduction: Control: 0.45 ± 0.22 mm Test I: 2.33 ± 0.04 mm Test II: 2.28 ± 0.09 mm Changes in mean plaque scores: Control 0.67 ± 017 Test I:1.00 ± 0.11 Test II:1.08 ± 0.05 GBI scores: Control 0.92 ± 0.29 Test I:1.08 ± 0.13 Test II: 0.88 ± 0.28	CoQ_{10} and tea tree oil gel proved to be effective in the treatment of chronic periodontitis.
Clinical evaluation	PI, MSBI, PD, CAL	• Significant improvement in all clinical parameters (PD, CAL, MSBI) in the test sites at 3 months compared to the control group • Significant clinical improvements (PD, CAL, PI, MSBI) in both groups at 1 and 3 months.	CoQ_{10} has beneficial effect on smokers with periodontitis when used as an adjunct to SRP
	PI, GBI, PD	• Significant improvement in all clinical parameters in the test sites seen at 4 weeks. • Sites with BOP were reduced more in the test group than in the control group PD: Control: 5 ± 0.84 to 3.66 ± 0.686 mm Test I: from 5.72 ± 0.57 to 3.72 ± 0.826 mm Test II: from 6.33 ± 1.085 to 3.72 ± 0.826 mm	CoQ_{10} can be said to have a beneficial effect on periodontitis when used as an adjunct to SRP.
Biochemical assay Transmission electron microscopy	Gingival tissue (color, size, texture, contour) GI, PI, PD, BOP Total antioxidant capacity (TAOC) MDA-biomarker for lipid peroxidation Lipid peroxide	• Only SRP determined improvement of the periodontal parameters. • Values of T-AOC and lipid peroxide diminished by 21.5 and 23.8%, respectively. • SRP combined with local application of NMQ10 resulted in a significant improvement of periodontal parameters • The assayed biomarkers proved enhanced antioxidant activity over SRP alone	NMQ10 can be suggested as a promising nanosystem as an approach to support the management of chronic periodontitis.

(Continues)

TABLE 22 (Continued)

Author, year, country, study type	Patients, sample size, gender, smoker, diagnosis	Treatment, form of vitamin application	Study duration
Sharma et al. 2016[327] India Split-mouth	N = 24 patients (120 sites) Age: 25–55 years Periodontitis	Test I: intrasulcular application of CoQ_{10} following SRP Test II: intrasulcular 0.8% hyaluronic acid application following SRP Control: SRP alone	6 weeks
Shoukheba et al. 2019[361] RCT	Diabetes patients (type II, moderately controlled) N = 30 (N = 15/group) 18 females 12 males Age: 30–50 years Generalized moderate CP	Test: CoQ_{10} 30 mg twice/day for 3 months after SRP Control: SRP + placebo	6 months

Evidence box	Presence	Effect
Association studies	✓	+
Biological mechanism	✓	+
Animal model "proof of principle"	✓	+
Clinical studies with surrogate parameters	✓	++/−
Clinical studies with hard end points	Ø	

4 | VITAMINS COMBINATIONS

4.1 | Roles in periodontal health/prophylaxis and disease

Micronutrients provide essential factors and cofactors for several enzymes required for their normal biological functions, structural maintenance, and transport. The close dose-dependent association between micronutrient intake, like vitamin A, B1, B2, E, C, copper, iron, folate, selenium, phosphorus, etc., and a reduced risk and severity of periodontitis has been suggested in various investigations.[34,35,262,333,334] Serum levels of vitamin C, bilirubin, and total antioxidants were inversely associated with periodontitis, especially in severe cases of the large NHANES III cohort, strongly underlining the positive effects of antioxidants on the reduction in the relative risk for periodontitis.[33] A strong inverse association between higher intake of multiple dietary antioxidants and the progression of periodontal disease as well as tooth loss was further evidenced in an older population (age ≥ 75 years).[45] Additionally, aging seems to reduce the body's absorption and production of vitamins and minerals, diminishing thus their protective role on certain anti-inflammatory mechanisms also involved in periodontal disease. In a large population sample from a NHANES examination from 1999 to 2004 with 15 000 subjects, moderate and severe periodontitis forms were found to be associated with lower cis-β-carotene, β-cryptoxanthin, folate, and vitamin D levels. It was noted that for most micronutrients a consistent age-dependent decrease was evident. A further analysis of these data pointed to close interactions between age, periodontitis on one hand, and vitamin D levels in females and FA in certain population races on the other hand.[335] In further regional nutritional survey in Japan, data from 487 nonsmokers demonstrated a statistically significant negative correlation between folate level and BOP, yet with an insignificant association with the Community Periodontal Index.[122]

Possible beneficial adjunctive effects of a multi-vitamin nutritional supplement were reported in a 60-day randomized controlled trial on periodontitis patients. Patients taking multi-vitamin preparations, composed of 250 mg AA, 600 mcg FA, 100 mcg methlycobalamin, 100 mg Echinacea Augustifolia root extract, 50 mg Vitus Vinifera, 25 mg $CoQ_{10,}$ and 5 mg Piper Nigrum per serving, showed significant reduction in their gingival inflammation and plaque scores as well as their PD.[336] A further preparation of 400 mg AA, 10 mg vitamin B6, 30 µg vitamin B12, 60 IU vitamin E, 400 mg FA, 0.5 mg copper, 100 µg selenium, 7.5 mg zinc, 30 mg alpha-lipoic acid, 25 of citrus bioflavonoids, 500 mg green tea extract, 125 mg of phylox blend, 25 mg of quercetin, and 6 mg of β-carotenes reduced gingival inflammation modestly over an 8-week period, with no effects observed on CAL and PD.[337] Positive

Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)	Evaluated parameters	Outcomes: clinical, histological, immunological, microbiological, PROMS	Conclusion
Clinical evaluation	PI, PD, CAL Gingival color change index Eastman interdental BI	• Intragroup analysis of all clinical parameters showed clinically significant results between baseline and 6th week. • No significant intergroup differences	Local application of CoQ_{10} and hyaluronic acid gel in conjunction with SRP may have a beneficial effect on periodontal health in patients with chronic periodontitis.
GCF samples ELISA	GI, BI, PD, CAL MMP8	• Significant decrease in clinical parameters at 6 months compared to baseline in the test group • Control group: the decrease in values for the clinical parameters was not statistically significant excepting the BI • Significant differences for all clinical parameters between the groups favoring the test treatment • In test group there was a significant continuous reduction in MMP8 levels up to 3 months • Significant lower levels of MMP-8 in the test compared to control	Dietary supplementation with CoQ_{10} may provide a low-cost intervention to augment periodontal therapy. Hence, CoQ_{10} as an antioxidant could be used safely as an effective adjunct to oral prophylaxis in treatment of CP in diabetic patients.

effects of micronutrients were also reported in diabetic patients. In a placebo-controlled randomized controlled trial with 196 type 2 diabetes patients receiving micronutrient tablets for 6 months, a statistically significant increase in serum levels of total protein, iron, FA, and hemoglobin and decrease in unsaturated iron-binding capacity (UIBC) levels were observed in the experimental micronutrients group compared to the placebo group. Immunologically, blood concentrations of IgE, $CD4^+$, lymphocytic counts, basophils increased, $CD8^+$ count decreased, and $CD4^+/CD8^+$ increased. This was accompanied by a significantly lower incidence of upper respiratory tract infection, vaginitis, urinary tract infection, gingivitis, and dental ulcers in the test as compared to the placebo group.[338] The supplementary administration of a micronutrient combinations in high stress situations was further tested in 40 healthy students under examination situations. The micronutrient supplementation for 3 months exhibited beneficial effects in terms of reducing the level of certain lipids (triglycerides and LDL) and inflammatory processes (slight reduction of the degree of gingival inflammation).[339] A decrease in inflammatory parameters was also observed in a randomized pilot study with a special low-carb diet and rich in omega-3 fatty acids, in vitamins C and D, antioxidants and fiber for 4 weeks. Inflammatory parameters, including GI, BOP, and periodontal inflamed surface area (PISA), were significantly reduced in the experimental compared to the control group.[340]

Nonetheless, single micronutrient supplementation in relation to periodontal diseases and their treatment have been studied more intensively than combinations. This may be related to the fact that their individual effect can be better pursued in a single-supplemented diet. Yet, micronutrients appear naturally in a rather mixed/combined manner, ideally in the form of a well-balanced unsupplemented diet.

4.2 | Roles in periodontal therapy and biological wound healing/regeneration

4.2.1 | In vitro studies results

The effect of antioxidant micronutrients and α-tocopherol has been investigated on neutrophils isolated from periodontally healthy volunteers stimulated with PMA (phorbol mystrate acetate), or with Ig-opsonized *F. nucleatum*, with *S. aureus* or PBS (control). Although α-tocopherol alone did not affect neither the extracellular nor the total ROS level, the combination between α-tocopherol and ascorbate inhibited ROS production. Additionally, the combination induced a significant inhibition/reduction of the Fcg-receptor-stimulated ROS release, as well as reduced the TLR-stimulated total ROS and the extracellular ROS release by the TLR-stimulated cells. These findings suggested that combined supplementation with micronutrients may be beneficial in reducing periodontal tissue damage and retaining immune-mediated neutrophil function.[341]

A further in vitro study on human gingival and periodontal ligament fibroblasts that included in its analysis also a group of combined α-tocopherol and selenium application, reported an accelerated proliferation rate, wound healing, and increased synthesis of bFGF and collagen type I of both cell types.[266] Improved cell migration and wound healing of human gingival fibroblasts and osteoblast in the presence of nicotine or cotinine was further reported by other working groups, suggesting a beneficial effect for regeneration and repair of the soft and hard tissues in smokers.[265] In a further in vitro investigation, G-MSCs were combinedly stimulated by AA and retinol under an uninflamed as well as an experimental inflammatory setup. Results revealed that AA/retinol reversed the inflammation-mediated decrease in G-MSCs'

TABLE 23 Effect of vitamin combinations—in vitro studies.

Author, year, country, study type	Study material (stem cells, fibroblasts, etc.), sample size	Form of vitamin application	Timepoint of evaluation	Methods of evaluation (ELISA, etc.)
Chapple et al. 2013[341] UK	Neutrophiles isolated from venous blood of periodontally healthy volunteers ($N=20$) Stimulation of cells with PMA (phorbol myristate acetate), or with Ig-opsonised *F. nucleatum*, with *S. aureus* or PBS (control) in the presence /absence of vitamins	In culture medium Combination of α-tocopherol (1 mmol/L) + AA (10 µL) compared to α-tocopherol or to AA	30 min pre-stimulation and 150 min post-stimulation	Dye exclusion (trypan blue) Enhanced chemiluminescence assay: Isoluminol or luminol
Fawzy El-Sayed et al. 2021[142] Germany	G-MSCs $N=5$	In culture medium Control group: basic medium Inflammatory group: basic medium with IL-1β (1 ng/mL), TNF-α (10 ng/mL), and IFN-γ (100 ng/mL), AA/Retinol group: basic medium with AA (250 µmol/L) retinol (20 µmol/L) Inflammatory/ AA/Retinol group: AA/retinol added to the inflammatory group.	3, 5, 14 days	ELISA Cell count Histochemistry RT-PCR Next generation sequencing
Nizam et al. 2014[266] Turkey	Primary cultures of human GFs and PDLFs $N=$ n.r.	In culture medium 60 µM α-tocopherol Or combined with 5×10^{-9} M, 10×10^{-9} M, and 50×10^{-9} M Se	24, 48, 72 h	Metabolic XTT assay ELISA New wound healing model (evaluation on photographs)
Torshabi et al. 2017[265] Iran	HGF1-PI 1 Human osteoblast-like cell-line MG-63 $N=3$	In culture medium α-tocopherol-albumin conjugate (0.1–100 mM) + Vitamin C (0.1–4 mM) + Nicotine (5 mM) + or + Cotinine (5 mM)	0, 24, 48 h	MTT assay In vitro scratch test/wound healing assay Rt-PCR

clonogenic ability and CFUs, amplified chondrogenic differentiation potential, with a higher activation of genes associated with development, proliferation, and migration (*FOS*, *EGR1*, *SGK1*, *CXCL5*, *SIPA1L2*, *TFPI2*, *KRATP1-5*), survival (*EGR1*, *SGK1*, *TMEM132A*), differentiation and mineral absorption (*FOS*, *EGR1*, *MT1E*, *KRTAP1-5*, *ASNS*, *PSAT1*).[142] Again, in this study a conjoint application of micronutrient appeared to be beneficial (Table 23).

4.2.2 | Animal studies results

Limited evidence exists on the benefits of combined vitamin/micronutrient supplementation or application in preclinical animal investigations. Similar to the above-mentioned findings from in vitro studies, selenium in combination with various forms of vitamin E applied systemically or topically has been shown to positively

Evaluated parameters	Outcomes	Conclusion
Cell viability ROS production Isoluminol production	• Total and extracellular unstimulated, baseline ROS generation was unaffected by α-tocopherol; it was inhibited by ascorbate and a combination of both micronutrients. • Fcγ-receptor (Fcγ-R)-stimulated total or extracellular ROS generation was not affected by the presence of individual micronutrients. • Vitamin combination significantly reduced extracellular Fcγ-R-stimulated ROS release. • Neither micronutrient alone inhibited TLR-stimulated total ROS • Vitamin combination inhibited TLR-stimulated total ROS. • Ascorbate and the micronutrient combination (without α-tocopherol) inhibited extracellular ROS release by TLR-stimulated cells.	Micronutrient effects in vivo could be beneficial in reducing collateral tissue damage in chronic inflammatory diseases, such as periodontitis, while retaining immune-mediated neutrophil function.
β-catenin levels Cellular proliferation Colony-forming Stemness genes Multilineage differentiation. Up−/downregulated genes and altered intracellular pathways	• G-MSCs demonstrated all mesenchymal stem/progenitor cells characteristics. • Controlled inflammation with AA/retinol significantly elevated *NANOG* ($p < 0.05$). • AA/retinol-mediated reduction in intracellular phosphorylated β-Catenin was restored through the effect of controlled inflammation ($p < 0.05$). • Cellular proliferation was highest in the AA/retinol group ($p < 0.05$). • AA/retinol counteracted the inflammation-mediated reduction in G-MSCs' clonogenic ability and CFUs. • Amplified chondrogenic differentiation was observed in the inflammatory/AA/retinol group. • At 1 and 3 days, the differentially expressed genes were associated with development, proliferation, and migration (*FOS*, *EGR1*, *SGK1*, *CXCL5*, *SIPA1L2*, *TFPI2*, *KRATP1-5*), survival (*EGR1*, *SGK1*, *TMEM132A*), differentiation and mineral absorption (*FOS*, *EGR1*, *MT1E*, *KRTAP1-5*, *ASNS*, *PSAT1*), inflammation and MHC-II antigen processing (*PER1*, *CTSS*, *CD74*) and intracellular pathway activation (*FKBP5*, *ZNF404*). Less as well as more genes were activated the longer the G-MSCs remained in the inflammatory medium or AA/retinol, respectively.	Results point at possibly interesting interactions between controlled inflammation or AA/retinol affecting stemness, proliferation, and differentiation attributes of G-MSCs.
Cell viability Cell proliferation bFGF Collagen type I synthesis Wound healing model	• α-tocopherol significantly increased the healing rate of PDFLs at 12 h • α-tocopherol increased bFGF and collagen type I release from GFs and PDLFs at 24, 48, 72 h • α-tocopherol/Se combination significantly enhanced the proliferation rate of both cells at 48 h, decreased the proliferation of PDLFs at 72 h, and increased the healing rate of GFs at 12 h and PDLFs at 12 and 48 h. • bFGF and collagen type I synthesis was also increased in both cell types at 24, 48, and 72 h by α-tocopherol/Se combination.	α-Tocopherol and α-tocopherol/Se combination is able to accelerate the proliferation rate and wound healing process and increase the synthesis of bFGF and collagen type I from both GFs and PDLFs.
Cell viability and proliferation Cell migration Gene expression of apoptosis-related genes (BAX, BCL2, CASP3) compared to control gene GAPDH	• Significantly greater dose-dependent negative effects of nicotine on morphology, viability, proliferation and migration of MG-63 and HGF cells than cotinine. • Vitamin E improved statistically significantly more cell viability, proliferation and migration, reduced more cell apoptosis than vitamin C in cells exposed to nicotine/ cotinine than vitamin C.	Vitamin C and vitamin E (systemically/locally) may be helpful in regeneration and repair of oral soft and hard tissues in smokers

affect periodontal healing in animal experimental models. This was evident by an inhibition of ROS release from phagocytic cells in rats, helping thus to diminish the associated collagen destruction.[342] Furthermore, in another study, the same combination resulted in significantly less alveolar bone loss and suppressed gum inflammation by iNOS compared with the control group[272] (Table 24). Results of in vitro and preclinical animal studies suggest that selenium should be regularly administered in the presence of vitamin E to exploit its full potential in periodontal healing/regeneration.

4.2.3 | Clinical studies results

Various anti-inflammatory effects of micronutrient combined with vitamins supplementation were observed in several clinical studies. In a study on postmenopausal women, a multi-nutrient intake consisting of beta carotene, zinc, selenium, and copper for 3 months following SRP, resulted in significant improvement of the enzymatic antioxidant status as well as an significant adjunctive reduction in GI and BOP compared to the control group.[343]

TABLE 24 Effect of vitamin combinations—animal studies.

Author, year, country, study type	Animal type, sample size	Treatment, form of vitamin application	Study duration
Aral et al. 2015[300] Turkey Controlled, parallel group	Rats with experimentally induced periodontitis $N=72$ divided into 6 groups	Vitamin K2 (30 mg/kg) + SRP (group 5) versus Healthy (group 1) Periodontitis (group 2) SRP (group 3) SRP + vitamin D3 (2 μg/kg; group 4) SRP + vitamin K2 + vitamin D3 (group 6) Vitamin K2 (menatetrenone) was administered daily for 10 days in corn oil vehicle.	18 days (10 days vitamin administration)
Asman et al 1994[342] Sweden	Rats Controls (PBS): $N=5$ Vitamin E 3 mg ($N=2$) Vitamin E 15 mg ($N=2$) Vitamin E + Se: 1 mg vitamin E + 20 μg Se ($N=6$) 1.5 mg vitamin E + 30 μg Se ($N=3$) 3 mg vitamin E + 60 μg Se ($N=3$) 6 mg vitamin E + 120 μg Se ($N=2$) For injection in sponge): Controls: $N=15$ Vitamin E + Se: 2 mg vitamin E + 40 μg Se ($N=7$), 3 mg vitamin E + 60 μg Se ($N=2$)	α-tocopherol undiluted or in combination with Se Administered subcutaneously or injected in collagen sponges (containing homologous ^{3}H collagen powder) at the neck every 2 days between the 8th and 18th day after sponge implantation	8, 18 days
Bas et al. 2021[272] Turkey	Rat $N=40$ (10/group)	Experimental periodontitis (ligature-induced, 4 weeks) Group A: Se Group B: α-tocopherol (α-T) Group C: Se + α-T Group D: control (saline) Vitamin E form: α-tocopherol acetate once a day for 4 weeks (intraperitoneal)	4 weeks

In a cross- sectional case–control study, where dietary intakes following SRP were followed up in 86 patients for 16 weeks, it was observed that intakes of fruits, vegetables, β-carotene, vitamin C, α-tocopherol, EPA, and DHA resulted in significant reductions of PD in smokers. However, smokers with a chronic generalized form of periodontitis seemed to have no benefit of this healthy diet.[69]

Combinations of vitamin C, vitamin E, lysozyme, and carbazochrome (CELC) administered for 4 weeks following SRP resulted only in limited adjunctive improvements compared to placebo, yet with a significant reduction of the gingival inflammation.[344] Micronutrients (vitamins A, D, E, K, B1, B2, B6, C, niacin, folate, pantothenic acid, biotin, minerals sodium, potassium, calcium,

Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)	Evaluated parameters	Outcomes: clinical, histological, immunological	Conclusion
Histological analysis (Masson trichrome) + light microscopy ELISA	IL-1β, IL-10 Serum bone-ALP (B-ALP) Tartrate-resistant acid phosphatase 5b (TRAP-5b) Ca level ABL	• ABL in the periodontitis group were significantly greater than those in the other five groups. • No significant differences were found in gingival IL-1β and IL-10, serum B-ALP, TRAP-5b, calcium and ABL between the groups receiving SRP with vitamins and the group receiving SRP alone.	Vitamin D3 and K2 alone or in combination did not affect gingival IL-1β and IL-10, serum B-ALP and TRAP-5b levels, or alveolar bone compared with conventional periodontal therapy alone.
Light microscopy (ingrowth of granulation tissue in sponges) Scintillation counting in urine (breakdown of implanted radioactivity caused by sponge induced granulation tissue)	Ingrowth of granulation tissue in sponges (without collagen) Collagen degradation monitored as total radioactivity DPM/mg collagen in excreted urine	• Similar reduction of radioactivity (in urine) in the combination vitamin E + Se (for the groups with 1 mg, 1.5 mg, 3 mg vitamin E) • A higher dose induced a higher radioactivity • Vitamin E + Se injected subcutaneously had no detectable effect on the development of granulation tissue as compared to controls • Vitamin E + Se injected into the sponge arrested the ingrowth of granulation tissue and after 10 days of treatment there was no detectable ingrowth of fibroblasts or capillaries in half of the sponge (arrested the maturation of the granulation tissue)	Vitamin E and Se are potential inhibitors of the free oxygen radicals from phagocytic inflammatory cells. Thus, it was suggested that these radicals may play a role in the collagen destruction by granulation tissues, as in periodontitis
Image analysis method in connective tissue under connective epithelium Immunohistochemistry ELISA	ABL Inflammatory cell infiltrate Collagen density N gingival collagen fibers Nitric oxide synthase N of iNOS, N of CD95 cells, N collagen fibers Serum IL-1β, IL-6, IL-4.	• Se + αT significantly suppressed ABL compared with the control group ($p < 0.05$). Other groups showed no statistical significance. • *N* gingival collagen fibers in Se and αT tended to be higher than in the control group • Se: N iNOS+ cells was smaller than in control ($p < 0.05$) (iNOS important inflammation marker and tissue destruction). The other groups showed only a tendency toward lower iNOS levels ($p > 0.05$) • Serum IL-6, IL-1β, IL-4 levels were not significantly different among groups • α-tocopherol alone or in combination did not alter the cytokines levels	Se has been concluded to inhibit inflammation of the gum due to iNOS. Se and αT can have a remarkable important role in preventing ABL, particularly in combination.

phosphorous, iron, zinc) administered for 8 weeks following periodontal surgery showed positive results regarding tooth mobility and early periodontal healing, yet with no notable effect on gingival inflammation[345] (Table 25). Combined it appears plausible to conclude that certain vitamins and micronutrient administration in combination at precise concentrations could provide a synergistic periodontal reparative/regenerative effect, exceeding the arithmetic sums of their isolated effects. Yet, in light of the above-mentioned studies (Table 26), further investigations are warranted to test this promising hypothesis.

TABLE 25 Effect of vitamin combinations—clinical studies.

Author, year, country, study type	Patients, sample size, gender, smoker, diagnosis	Treatment, form of vitamin application	Study duration
Daiya et al. 2014[343] India Cohort study	Postmenopausal patients (*N* = 43) Group 1 (*N* = 22, control) Group 2 (*N* = 21, test) Only female Age: 45–55 years CP	Group 1: SRP Group 2: SRP+ systemic micronutrient antioxidants (10 mg beta carotene, 27.5 mg Zn sulphate monohydrate, 70 mcg Se dioxide, 2 mg manganese, 1 mg copper) 1x/day for 3 months	3 months
Dodington et al. 2015[69] Canada cross-sectional, case–control study	*N* = 86 patients 63 nonsmokers 23 smokers Generalized CP	SRP	16 weeks
Hong et al. 2019[344] South Korea RCT	*N* = 100 patients CP	Randomized: Test: SRP+ combinations of vitamin C, vitamin E, lysozyme and carbazochrome (CELC) for 8 weeks Control: SRP+ placebo for the first 4 weeks; next 4 weeks CELC	8 weeks
Lee et al. 2014[345] Korea Cohort study	Natients Test (*N* = 17) Control (*N* = 11) 18 males 10 females Age: 30–65 years	Allocation after periodontal flap surgery to one of the following groups: Test: nutritional supplement drinks for 8 weeks (Vitamins A, D, E, K, B1, B2, B6, C, niacin, folate, pantothenic acid, biotin, minerals sodium, potassium, calcium, phosphorous, iron, zinc). Control: no supplements	8 weeks

Evidence box	Presence	Effect
Association studies	✓	++
Biological mechanism	✓	+
Animal model "proof of principle"	✓	+
Clinical studies with surrogate parameters	✓	++
Clinical studies with hard end points	Ø	

5 | CONCLUDING REMARKS

The above-mentioned findings draw an interesting picture for vitamins, as possible host-modulatory adjunctive biomolecules, with promising potentials in the field of periodontal wound healing/regeneration. Although strong associations were reported between certain vitamins and the prevalence of periodontal disease, in addition to in vitro and animal preclinical studies providing plausible explanation approaches for mechanisms that could

Methods of evaluation (ELISA, immunohistochemistry, histological, etc.)	Evaluated parameters	Outcomes: clinical, histological, immunological, microbiological, PROMS	Conclusion
Clinical evaluation SOD assay kit ELISA	Serum and saliva superoxide dismutase (SOD) activity Periodontal parameters: PD, CAL, BOP, GI, PI	• Salivary and serum SOD values significantly ($p < 0.05$) improved with periodontal treatment. • Improvement in systemic enzymatic antioxidant status • Significant improvement of al periodontal parameters in both groups • Significant reduction in GI and BOP (%) sites was significantly greater in group 2 as compared to group 1.	Adjunctive micronutrient supplements reduce periodontal inflammation and improve the status of systemic enzymatic antioxidants in postmenopausal women.
Block 2005 food frequency questionnaire Supplement questionnaire Clinical evaluation	PD Intakes of fruits, vegetables, β-carotene, vitamin C, α-tocopherol, α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) Serum 25-hydroxyvitamin D concentrations	• In nonsmokers, PD was associated with fruit and vegetable, β-carotene, vitamin C, α-tocopherol, EPA, and DHA intakes ($p < 0.05$). • PD was not significantly associated with ALA intake or serum 25-hydroxyvitamin D concentration. • Significant associations that included supplements (β-carotene, vitamin C, α-tocopherol) were attenuated or lost, depending on the statistical model used. • There were no significant associations within the group of smokers.	Dietary intakes of fruits and vegetables, β-carotene, vitamin C, α -Tocopherol, EPA, and DHA are associated with reduced PD after SRP in nonsmokers, but not in smokers, with chronic generalized periodontitis. These findings may lead to the development of dietary strategies to optimize healing after periodontal procedures.
Clinical evaluation	GI, PD, CAL, PI Gingival recession VAS for discomfort, bleeding and swelling	• 93 patients completed the study. • GI in the test group was significantly decreased after 4 weeks ($p < 0.001$) and 8 weeks ($p < 0.001$). • GI change was significantly decreased in the test group compared to baseline and to control group after 4 weeks ($p = 0.015$). • In the model adjusting for age, gender and visits test group showed 2.5 times GI improvement compared to the control group ($p = 0.022$).	Within the study, CELC showed a significant reduction in gingival inflammation compared with a placebo. Other parameters, however, were similar between groups.
	GI Tooth mobility OHIP-14 Anthropometric parameters: body wight, body fat, fat-free mass, BMI, waist circumference, blood pressure Questionnaire: loss of appetite, dietary intake	• At 1 week, GI values were reduced in the intervention group ($p < 0.05$), and tooth mobility had increased, but to a lesser extent in the intervention group ($p < 0.05$). • At 8 weeks, the intakes of protein, vitamins A and B1, and niacin were increased in the intervention group.	These results demonstrate that nutritional supplementation improves early periodontal healing after surgery

establish a "cause-effect" relationship, interventional trials were not always able to demonstrate such expected positive therapeutic effects. Even with the currently limited number of studies available, some of the trials reported conflicting results, suffered from structural flaws, including sample size, mode of vitamin administration, randomization, and blinding, and have, therefore, a high risk of bias. Many of the trials were even conducted on subpopulations, with factors (as aging, smoking, or menopause), which themselves represent risk factors for bone loss and would benefit most from such vitamin supplementation, but do not represent all aspects of periodontitis populations. The picture depicts how little the current evidence is, on the effect of vitamins on periodontal wound healing, especially in the context of high quality randomized clinical trials, to the extent that for vitamin K no such trials even exist to the best of our knowledge in the published periodontal literature till now. It thereby sheds light on the knowledge gap and highlights potential future research areas in this field. Moreover, it clearly underlines the complexity of the

TABLE 26 Level of evidence for vitamin combinations.

Vitamin combinations	Type of studies	Level of evidence (ranked according to the GRADE system)	Strength	Weakness
Beta carotene + Se + Manganese + Copper	Prospective cohort study	Grade B	First evidence of a positive effect on clinical level Examining associations Adequate study design Hypothesis generation	Lack of large scale RCTs
Fruits, vegetables, β-carotene, vitamin C, α-tocopherol, α-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid	Cross-sectional, case–control study	Grade C	Suggestion of a positive effect on clinical level Examining associations Hypothesis generation	Lack of large scale RCTs
Nutritional supplements drink (Vitamins A, D, E, K, B1, B2, B6, C, niacin, folate, pantothenic acid, biotin, minerals sodium, potassium, calcium, phosphorous, iron, zinc)	Prospective cohort study	Grade B	First evidence of a positive effect on clinical level Examining associations Adequate study design Hypothesis generation	Lack of large scale RCTs
Retinol + vitamin C	In vitro study	Preliminary evidence (not graded)	Controlled laboratory settings Supporting evidence of positive effects on cellular level Proof of principle	Lack of animal and clinical studies
Vitamin B complex (B1, B2, B3, B5, B6, B7, B12) + Calcium	Randomized controlled trial	Grade A	High level of evidence Low risk of bias and proper study design First proof of hypothesis	Lack of multiple large scale RCTs
Vitamin D + Calcium	Randomized controlled trial	Grade A	High level of evidence Low risk of bias and proper study design First proof of hypothesis	Lack of multiple large scale RCTs
Vitamin D + Vitamin K	In vitro study Animal studies	Preclinical evidence (not graded)	Supporting evidence of a positive effect on preclinical level Adequate study design Proof of principle	Lack of clinical studies Animal studies do not directly translate to human clinical practice
Vitamin E + Se	In vitro study Animal studies	Preclinical evidence (not graded)	Controlled laboratory settings Supporting evidence of a positive effect on preclinical level Adequate study design Proof of principle	Lack of clinical studies Animal studies do not directly translate to human clinical practice
Vitamin E + Vitamin C	In vitro studies Randomized controlled trial	Grade A	High level of evidence Low risk of bias and Adequate study design First proof of hypothesis	Lack of multiple large scale RCTs

Note: Green color indicates high level of evidence, yellow color indicates moderate level of evidence, Red color indicates low level of evidence.

Abbreviations: AA, ascorbic acid; ABL, alveolar bone loss; ACP5, acid phosphatase 5; ALP, alkaline phosphatase; biomineralization associated, Ang-1, angiopoietin-1; Ang-2, angiopoietin-2; BANA, N-benzoyl-dL-arginine-2-napthylamide; BI, bleeding index; BMP-2, bone morphogenetic protein-2; BMP-4, Bone morphogenetic protein-4; BOP, bleeding on probing; BSP, bone sialoprotein; CAL, clinical attachment level; CAP, cementum attachment protein; cbfa1, core-binding factor subunit alpha-1; CCL2, C-C Motif Chemokine Ligand 2; CEMP1, cementum protein 1; Col-I, collagen I; COL1A1, collagen type I alpha 1 chain; COX-2, cyclooxygenase 2; CP, chronic periodontitis; DKK-1, Dickkopf WNT Signaling Pathway Inhibitor 1; DNMT1, DNA (cytosine-5)-methyltransferase 1; DPSCs, dental pulp stem cells; EGF, epidermal growth factor; ELISA, Enzyme-linked Immunosorbent Assay; FA, folic acid; FGF, fibroblast growth factor; FMBS, full-mouth bleeding score; FMPS, full-mouth plaque scores; FSP, fibroblast specific protein; GBI, gingival bleeding index; GCF, gingival crevicular fluid; GI, gingival index; G-MSCs, gingival mesenchymal stem/progenitor cells; GR, gingival recession; HCl, hydrochloride; HGF, hepatocyte growth factor; hPDL, Human periodontal ligament; hPDLSCs, Human periodontal ligament stem cells; IBD, intrabony defect; IDO, indoleamine 2,3-dioxygenase; IFN-γ, interferon-γ; IGF-1, insulin like growth factor-1; IL, interleukin; iNOS, inducible isoform of nitric oxidase synthase; IV, intravenous; KGF, Keratinocyte growth factor; MCP1, monocyte chemoattractant protein 1; MGI, modified gingival index; MyD88, myeloid differentiation primary response 88; NA, nicotinamide; MSBI, modified sulcular bleeding index; n-HA, nanohydroxyapatite; NO, nitrous oxide; OCN, osteocalcin; OCT4, octamer-binding transcription factor 4; OPG, osteoprotegerin; OPN, osteopontin; PD, probing depth; PDLFs, periodontal ligament fibroblasts; PDLSCs, periodontal ligament stem cells; PGE-2, prostaglandin E-2; PI, plaque index; PLAPL-1, periodontal-ligament-associated protein-1; PPARγ2, peroxisome proliferator-activated receptor gamma isoform 2; PTH, parathyroid hormone; RA, retinoic acid; RANKL, receptor activator of NF-κB ligand; RBF, riboflavin; RCT, randomized controlled trial; RDBD, radiographic defect bone density; RLDD, radiographic linear defect depth; ROS, reactive oxygen species; ROS, reactive oxygen species; RT-PCR, real-time polymerase chain reaction; RUNX2, Runt-related transcription factor 2; SCAP, stem cells from the apical papilla; SCF, stem cell factor; SDF-1, *stromal cell-derived factor 1*; Se, selenium; SFRP1, *Secreted Frizzled Related Protein 1*; SHED, stem cells from human exfoliated deciduous teeth; SOX2, sex determining region Y-box 2; SP7; Osterix, Sp7 transcription factor; SRP, scaling and root planing; TAOC, total antioxidant capacity; TERT, Telomerase Reverse Transcriptase; TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α; TNSALP, Tissue nonspecific alkaline phosphatase; TRAP, Tartrate-resistant acid phosphatase; VDR, vitamin D receptor; VEGF, vascular endothelia growth factor.

multifactorial periodontal wound healing events under microbial dysbiostic condition, as opposed to controlled in vitro experiments under ideal settings.

Current findings should thus be critically appraised, and an oversimplified explanation and generalization of the described effects strictly avoided. Despite possible positive effects linked to a balanced nutritional improvement or micronutrient supplementation, in case of specific deficiencies (as in people not frequently exposed to the sun, pregnancy, smoking, or alcohol consumption), on daily basis or as adjunct to nonsurgical or surgical periodontal treatment, the current evidence is still unsatisfactory and full of limitations. Most of the trials tested single vitamin preparations at arbitrary specified concentrations, dosage, frequency, and routes of administrations, which does not represent a normal dietary intake of vitamins, ideally in a well-balanced diet in combination with further vitamins, micronutrient, and fibers. The reported treatment effects are further sometimes limited or insufficient in their magnitude in order to determine the best dose, route, frequency, and duration to achieve the desired effects in periodontal wound healing. A definition of such desired effect remains to be further complex in light of the time at which the vitamin is required in the body's physiological systems and the biological effect it can exert in relation to periodontal wound healing events (inflammation, proliferation, granulation tissue formation, maturation). Furthermore, as surgical as well as nonsurgical periodontal therapies are very effective, well-established, and evidence-based treatment modalities themselves, additional benefits of adjunctive therapies may have been partly masked. Regarding vitamins as "nutraceuticals," with the ability to revert periodontal diseases by themselves, is thus not justified.

In this context, it is further critical to determine whether a measured antioxidants or vitamin depletion increases the patient's susceptibility to periodontal disease or if such a depletion arose as a consequence of an active periodontal disease. In this context, it is further not plausible to conclude, especially in the era of "patient tailored" therapeutic approaches, that all patient could be prescribed a specified dose (according to age, weight or BMI) for a certain vitamin in isolation or in combination. Not only does the absence of data on the serum vitamin levels and differences in individual gene polymorphism and vitamin metabolism (nutrigenetics) negate such a simplistic approach, but further the difference in metabolism and biopotency between the individual vitamin isoforms. It is further not possible to link the measured serum effects solely to the periodontal condition, being a part of the complex biological human body systems. Serious side effects of hypervitaminosis, which take a considerable time to appear, are further concerns, limiting a regular recommendation for a consistent daily intake of certain vitamins as an adjunct to periodontal therapy or as a life-long preventive modality for periodontal disease progression. On the other hand, it is plausible to conclude that vitamins when administered in combination or in presence of micronutrients (as copper, iron, or zinc) could exert a synergistic or an antagonistic effect depending on the administrated agents, ingredients of the combination, carrier, and release kinetics, and their synchronized chronological co-presence, which are all research fields currently unexplored, warranting further extensive research. Currently, we are still making the first step in a field that requires great attention for the possibilities it hold in the context of periodontal healing/regeneration.

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CONFLICT OF INTEREST STATEMENT

All authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Mendoza AH, Balzarini D, Alves T, Rovai ES, Holzhausen M. Potential of mesenchymal stem cell sheets on periodontal regeneration: a systematic review of pre-clinical studies. *Curr Stem Cell Res Ther*. 2023;18:958-978.
2. Cho MI, Garant PR. Development and general structure of the periodontium. *Periodontol 2000*. 2000;24:9-27.
3. Seo BM, Miura M, Gronthos S, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet*. 2004;364:149-155.
4. Fawzy El-Sayed KM, Dörfer CE. Gingival mesenchymal stem/progenitor cells: a unique tissue engineering gem. *Stem Cells Int*. 2016;2016:7154327.
5. El-Sayed KMF, Paris S, Becker S, et al. Isolation and characterization of multipotent postnatal stem/progenitor cells from human alveolar bone proper. *J Cranio Maxill Surg*. 2012;40:735-742.
6. Morsczeck C, Gotz W, Schierholz J, et al. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biol*. 2005;24:155-165.
7. El-Sayed KM, Paris S, Graetz C, et al. Isolation and characterisation of human gingival margin-derived STRO-1/MACS(+) and MACS(–) cell populations. *Int J Oral Sci*. 2015;7:80-88.
8. Fawzy El-Sayed KM, Dorfer C, Fandrich F, Gieseler F, Moustafa MH, Ungefroren H. Erratum to: adult mesenchymal stem cells explored in the dental field. *Adv Biochem Eng Biotechnol*. 2013;130:301-302.
9. Fawzy El-Sayed KM, Dorfer C, Fandrich F, Gieseler F, Moustafa MH, Ungefroren H. Adult mesenchymal stem cells explored in the dental field. *Adv Biochem Eng Biotechnol*. 2013;130:89-103.
10. Hughes FJ, Ghuman M, Talal A. Periodontal regeneration: a challenge for the tissue engineer? *Proc Inst Mech Eng H P I MECH ENG H*. 2010;224:1345-1358.
11. Corbella S, Taschieri S, Del Fabbro M, Francetti L, Weinstein R, Ferrazzi E. Adverse pregnancy outcomes and periodontitis: a systematic review and meta-analysis exploring potential association. *Quintessence Int*. 2016;47:193-204.
12. Berlin-Broner Y, Febbraio M, Levin L. Association between apical periodontitis and cardiovascular diseases: a systematic review of the literature. *Int Endod J*. 2016;50:847-859.

13. Pradhan S, Goel K. Interrelationship between diabetes and periodontitis: a review. *JNMA J Nepal Med Assoc*. 2011;51:144-153.
14. Suzuki Y, Nakamura N, Miyabe M, et al. Anti-inflammatory role of glucose-dependent insulinotropic polypeptide in periodontitis. *J Diabetes Invest*. 2016;7:497-505.
15. Abariga SA, Whitcomb BW. Periodontitis and gestational diabetes mellitus: a systematic review and meta-analysis of observational studies. *BMC Pregnancy Childbirth*. 2016;16:344.
16. Tang Q, Fu H, Qin B, et al. A possible link between rheumatoid arthritis and periodontitis: a systematic review and meta-analysis. *Int J Periodontics Restorative Dent*. 2017;37:79-86.
17. Sculean A, Nikolidakis D, Nikou G, Ivanovic A, Chapple IL, Stavropoulos A. Biomaterials for promoting periodontal regeneration in human intrabony defects: a systematic review. *Periodontol 2000*. 2015;68:182-216.
18. Alshoiby MM, Fawzy El-Sayed KM, Elbattawy W, Hosny MM. Injectable platelet-rich fibrin with demineralized freeze-dried bone allograft compared to demineralized freeze-dried bone allograft in intrabony defects of patients with stage-III periodontitis: a randomized controlled clinical trial. *Clin Oral Investig*. 2023;27:3457-3467.
19. Abdulrahman YA, Hosny MM, Elfana A, Fawzy El-Sayed KM. Clinical and radiographic evaluation of low-speed platelet-rich fibrin (PRF) for the treatment of intra-osseous defects of stage-III periodontitis patients: a randomized controlled clinical trial. *Clin Oral Investig*. 2022;26:6671-6680.
20. Simonelli A, Severi M, Trombelli L, Farina R. Minimal invasiveness in the surgical treatment of intraosseous defects: a systematic review. *Periodontol 2000*. 2023;91:20-44.
21. Ribeiro FV, Mehta JJ, Monteiro MF, Moore J, Casati MZ, Nibali L. Minimal invasiveness in nonsurgical periodontal therapy. *Periodontol 2000*. 2023;91:7-19.
22. Sanz M, Jepsen K, Eickholz P, Jepsen S. Clinical concepts for regenerative therapy in furcations. *Periodontol 2000*. 2015;68:308-332.
23. Cekici A, Kantarci A, Hasturk H, Van Dyke TE. Inflammatory and immune pathways in the pathogenesis of periodontal disease. *Periodontol 2000*. 2014;64:57-80.
24. Belibasakis GN, Belstrom D, Eick S, Gursoy UK, Johansson A, Kononen E. Periodontal microbiology and microbial etiology of periodontal diseases: historical concepts and contemporary perspectives. *Periodontol 2000*. 2023. doi:10.1111/prd.12473. Online ahead of print.
25. Chapple IL. Reactive oxygen species and antioxidants in inflammatory diseases. *J Clin Periodontol*. 1997;24:287-296.
26. Waddington RJ, Moseley R, Embery G. Reactive oxygen species: a potential role in the pathogenesis of periodontal diseases. *Oral Dis*. 2000;6:138-151.
27. Gustafsson A, Asman B. Increased release of free oxygen radicals from peripheral neutrophils in adult periodontitis after fc delta-receptor stimulation. *J Clin Periodontol*. 1996;23:38-44.
28. Darden AG, Ries WL, Wolf WC, Rodriguiz RM, Key LL Jr. Osteoclastic superoxide production and bone resorption: stimulation and inhibition by modulators of NADPH oxidase. *J Bone Miner Res*. 1996;11:671-675.
29. Key LL Jr, Wolf WC, Gundberg CM, Ries WL. Superoxide and bone resorption. *Bone*. 1994;15:431-436.
30. Chapple IL, Matthews JB. The role of reactive oxygen and antioxidant species in periodontal tissue destruction. *Periodontol 2000*. 2007;43:160-232.
31. McCauley LK, Nohutcu RM. Mediators of periodontal osseous destruction and remodeling: principles and implications for diagnosis and therapy. *J Periodontol*. 2002;73:1377-1391.
32. Halliwell B. On 'Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts' by Barry Halliwell and John M.C.Gutteridge. *Arch Biochem Biophys*. 2022;726:109320.
33. Chapple ILC, Milward MR, Dietrich T. The prevalence of inflammatory periodontitis is negatively associated with serum antioxidant concentrations. *J Nutr*. 2007;137:657-664.
34. Najeeb S, Zafar MS, Khurshid Z, Zohaib S, Almas K. The role of nutrition in periodontal health: an update. *Nutrients*. 2016;8:530.
35. Neiva RF, Steigenga J, Al-Shammari KF, Wang HL. Effects of specific nutrients on periodontal disease onset, progression and treatment. *J Clin Periodontol*. 2003;30:579-589.
36. Dommisch H, Kuzmanova D, Jonsson D, Grant M, Chapple I. Effect of micronutrient malnutrition on periodontal disease and periodontal therapy. *Periodontol 2000*. 2018;78:129-153.
37. Varela-Lopez A, Navarro-Hortal MD, Giampieri F, Bullon P, Battino M, Quiles JL. Nutraceuticals in periodontal health: a systematic review on the role of vitamins in periodontal health maintenance. *Molecules*. 2018;23:1226.
38. Tanner ACR, Kent R, Van Dyke T, Sonis ST, Murray LA. Clinical and other risk indicators for early periodontitis in adults. *J Periodontol*. 2005;76:573-581.
39. Vogel RI, Lamster IB, Wechsler SA, Macedo B, Hartley LJ, Macedo JA. The effects of Megadoses of ascorbic-acid on Pmn chemotaxis and experimental gingivitis. *J Periodontol*. 1986;57:472-479.
40. Saunders J, Smith T. Malnutrition: causes and consequences. *Clin Med (Lond)*. 2010;10:624-627.
41. Bagley SM. Nutritional needs of the acutely ill with acute wounds. *Crit Care Nurs Clin North Am*. 1996;8:159-167.
42. Boynton PR, Jaworski D, Paustian C. Meeting the challenges of healing chronic wounds in older adults. *Nurs Clin North Am*. 1999;34:921-932, vii.
43. Enwonwu CO. Interface of malnutrition and periodontal diseases. *Am J Clin Nutr*. 1995;61:430S-436S.
44. Wasti J, Wasti A, Singh R. Efficacy of antioxidants therapy on progression of periodontal disease – a randomized control trial. *Indian J Dent Res*. 2021;32:187-191.
45. Iwasaki M, Manz MC, Taylor GW, Yoshihara A, Miyazaki H. Relations of serum ascorbic acid and alpha-tocopherol to periodontal disease. *J Dent Res*. 2012;91:167-172.
46. Kuzmanova D, Jansen IDC, Schoenmaker T, et al. Vitamin C in plasma and leucocytes in relation to periodontitis. *J Clin Periodontol*. 2012;39:905-912.
47. Nishida M, Grossi SG, Dunford RG, Ho AW, Trevisan M, Genco RJ. Dietary vitamin C and the risk for periodontal disease. *J Periodontol*. 2000;71:1215-1223.
48. Iwasaki M, Moynihan P, Manz MC, et al. Dietary antioxidants and periodontal disease in community-based older Japanese: a 2-year follow-up study. *Public Health Nutr*. 2013;16:330-338.
49. Amarasena N, Ogawa H, Yoshihara A, Hanada N, Miyazaki H. Serum vitamin C-periodontal relationship in community-dwelling elderly Japanese. *J Clin Periodontol*. 2005;32:93-97.
50. Iwasaki M, Yoshihara A, Moynihan P, Watanabe R, Taylor GW, Miyazaki H. Longitudinal relationship between dietary omega-3 fatty acids and periodontal disease. *Nutrition*. 2010;26:1105-1109.
51. Iwasaki M, Taylor GW, Moynihan P, et al. Dietary ratio of n-6 to n-3 polyunsaturated fatty acids and periodontal disease in community-based older Japanese: a 3-year follow-up study. *Prostaglandins Leukot Essent Fatty Acids*. 2011;85:107-112.
52. Iwasaki M, Manz MC, Moynihan P, et al. Relationship between saturated fatty acids and periodontal disease. *J Dent Res*. 2011;90:861-867.
53. Figueredo CM, Martinez GL, Koury JC, Fischer RG, Gustafsson A. Serum levels of long-chain polyunsaturated fatty acids in patients with periodontal disease. *J Periodontol*. 2013;84:675-682.
54. Santonocito S, Polizzi A, Palazzo G, Indelicato F, Isola G. Dietary factors affecting the prevalence and impact of periodontal disease. *Clin Cosmet Inv Dent*. 2021;13:283-292.

55. Preston AM. Cigarette smoking-nutritional implications. *Prog Food Nutr Sci*. 1991;15:183-217.
56. Bruno RS, Rainakrishnan R, Montine TJ, Bray TM, Traber MG. Alpha-tocopherol disappearance is faster in cigarette smokers and is inversely related to their ascorbic acid status. *Am J Clin Nutr*. 2005;81:95-103.
57. Stryker WS, Kaplan LA, Stein EA, Stampfer MJ, Sober A, Willett WC. The relation of diet, cigarette-smoking, and alcohol-consumption to plasma Beta-carotene and alpha-tocopherol levels. *Am J Epidemiol*. 1988;127:283-296.
58. Schleicher RL, Carroll MD, Ford ES, Lacher DA. Serum vitamin C and the prevalence of vitamin C deficiency in the United States: 2003-2004 National Health and nutrition examination survey (NHANES). *Am J Clin Nutr*. 2009;90:1252-1263.
59. Linden GJ, McClean KM, Woodside JV, et al. Antioxidants and periodontitis in 60-70-year-old men. *J Clin Periodontol*. 2009;36:843-849.
60. Erlinger TP, Guallar E, Miller ER, Stolzenberg-Solomon R, Appel LJ. Relationship between systemic markers of inflammation and serum beta-carotene levels. *Arch Intern Med*. 2001;161:1903-1908.
61. Bi Y, Gong M, Zhang X, et al. Pre-activation of retinoid signaling facilitates neuronal differentiation of mesenchymal stem cells. *Dev Growth Differ*. 2010;52:419-431.
62. Hore TA. Modulating epigenetic memory through vitamins and TET: implications for regenerative medicine and cancer treatment. *Epigenomics-UK*. 2017;9:863-871.
63. Larange A, Cheroutre H. Retinoic acid and retinoic acid receptors as pleiotropic modulators of the immune system. *Annu Rev Immunol*. 2016;34:369-394.
64. Vogel RI, Fink RA, Schneider LC, Frank O, Baker H. The effect of folic acid on gingival health. *J Periodontol*. 1976;47:667-668.
65. Pack AR, Thomson ME. Effects of topical and systemic folic acid supplementation on gingivitis in pregnancy. *J Clin Periodontol*. 1980;7:402-414.
66. Freeland JH, Cousins RJ, Schwartz R. Relationship of mineral status and intake to periodontal disease. *Am J Clin Nutr*. 1976;29:745-749.
67. Park JA, Lee JH, Lee HJ, Jin BH, Bae KH. Association of some Vitamins and Minerals with periodontitis in a nationally representative sample of Korean young adults. *Biol Trace Elem Res*. 2017;178:171-179.
68. Russell AL. International nutrition surveys: a summary of preliminary dental findings. *J Dent Res*. 1963;42(1)Pt 2:233-244.
69. Dodington DW, Fritz PC, Sullivan PJ, Ward WE. Higher intakes of fruits and vegetables, beta-carotene, Vitamin C, alpha-tocopherol, EPA, and DHA are positively associated with periodontal healing after nonsurgical periodontal therapy in nonsmokers but not in smokers. *J Nutr*. 2015;145:2512-2519.
70. Waerhaug J. Prevalence of periodontal disease in Ceylon. Association with age, sex, oral hygiene, socio-economic factors, vitamin deficiencies, malnutrition, betel and tobacco consumption and ethnic group. Final report. *Acta Odontol Scand*. 1967;25:205-231.
71. Cerna H, Vesely J, Nastoupilova E, Lechner J, Fingerova H, Pohanka J. Periodontium and vitamin E and a in pregnancy. *Acta Univ Palacki Olomuc Fac Med*. 1990;125:173-179.
72. Walston J, Xue Q, Semba RD, et al. Serum antioxidants, inflammation, and total mortality in older women. *Am J Epidemiol*. 2006;163:18-26.
73. Watzl B, Kulling SE, Moseneder J, Barth SW, Bub A. A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. *Am J Clin Nutr*. 2005;82:1052-1058.
74. Erkelens MN, Mebius RE. Retinoic acid and immune homeostasis: a balancing act. *Trends Immunol*. 2017;38:168-180.
75. Abdelhamid L, Hussein H, Ghanem M, Eissa N. Retinoic acid-mediated anti-inflammatory responses in equine immune cells stimulated by LPS and allogeneic mesenchymal stem cells. *Res Vet Sci*. 2017;114:225-232.
76. Yamaguchi M, Igarashi A, Morita S, Sumida T, Sugawara K. Relationship between serum beta-cryptoxanthin and circulating bone metabolic markers in healthy individuals with the intake of juice (Citrus unshiu) containing beta-cryptoxanthin. *J Health Sci*. 2005;51:738-743.
77. Yamaguchi M, Uchiyama S. Beta-Cryptoxanthin stimulates bone formation and inhibits bone resorption in tissue culture in vitro. *Mol Cell Biochem*. 2004;258:137-144.
78. Yamaguchi M. Beta-Cryptoxanthin and bone metabolism: the preventive role in osteoporosis. *J Health Sci*. 2008;54:356-369.
79. Uchiyama S, Yamaguchi M. Inhibitory effect of beta-cryptoxanthin on osteoclast-like cell formation in mouse marrow cultures. *Biochem Pharmacol*. 2004;67:1297-1305.
80. Uchiyama S, Yamaguchi M. Beta-cryptoxanthin stimulates apoptotic cell death and suppresses cell function in osteoclastic cells: change in their related gene expression. *J Cell Biochem*. 2006;98:1185-1195.
81. Pourjafar M, Saidijam M, Etemadi K, Najafi R. All-trans retinoic acid enhances in vitro mesenchymal stem cells migration by targeting matrix metalloproteinases 2 and 9. *Biotechnol Lett*. 2017;39:1263-1268.
82. Zhang S, Chen X, Hu Y, et al. All-trans retinoic acid modulates Wnt3A-induced osteogenic differentiation of mesenchymal stem cells via activating the PI3K/AKT/GSK3beta signalling pathway. *Mol Cell Endocrinol*. 2016;422:243-253.
83. Fawzy El-Sayed KM, Hein D, Dorfer CE. Retinol/inflammation affect stemness and differentiation potential of gingival stem/progenitor cells via Wnt/beta-catenin. *J Periodontal Res*. 2019;54:413-423.
84. Hore TA, von Meyenn F, Ravichandran M, et al. Retinol and ascorbate drive erasure of epigenetic memory and enhance reprogramming to naive pluripotency by complementary mechanisms. *Proc Natl Acad Sci U S A*. 2016;113:12202-12207.
85. Rademaker M. Isotretinoin: dose, duration and relapse. What does 30 years of usage tell us? *Australas J Dermatol*. 2013;54:157-162.
86. Lotan R, Clifford JL. Nuclear receptors for retinoids: mediators of retinoid effects on normal and malignant cells. *Biomed Pharmacother*. 1991;45:145-156.
87. Kautsky MB, Fleckman P, Dale BA. Retinoic acid regulates oral epithelial differentiation by two mechanisms. *J Invest Dermatol*. 1995;104:224-230.
88. Thompson KL, Rosner MR. Regulation of epidermal growth factor receptor gene expression by retinoic acid and epidermal growth factor. *J Biol Chem*. 1989;264:3230-3234.
89. Mackenzie IC, Gao Z. Keratinocyte growth factor expression in human gingival fibroblasts and stimulation of in vitro gene expression by retinoic acid. *J Periodontol*. 2001;72:445-453.
90. San Miguel SM, Goseki-Sone M, Sugiyama E, Watanabe H, Yanagishita M, Ishikawa I. Tissue-non-specific alkaline phosphatase mRNA expression and alkaline phosphatase activity following application of retinoic acid in cultured human dental pulp cells. *Arch Oral Biol*. 1999;44:861-869.
91. San Miguel SM, Goseki-Sone M, Sugiyama E, Watanabe H, Yanagishita M, Ishikawa I. The effects of retinoic acid on alkaline phosphatase activity and tissue-non-specific alkaline phosphatase gene expression in human periodontal ligament cells and gingival fibroblasts. *J Periodontal Res*. 1998;33:428-433.
92. Malladi P, Xu Y, Yang GP, Longaker MT. Functions of vitamin D, retinoic acid, and dexamethasone in mouse adipose-derived mesenchymal cells. *Tissue Eng*. 2006;12:2031-2040.
93. Chadipiralla K, Yochim JM, Bahuleyan B, et al. Osteogenic differentiation of stem cells derived from human periodontal ligaments and pulp of human exfoliated deciduous teeth. *Cell Tissue Res*. 2010;340:323-333.

94. Fawzy El-Sayed KM, Dorfer CE. (*) animal models for periodontal tissue engineering: a knowledge-generating process. *Tissue Eng Part C Methods*. 2017;23:900-925.
95. Fawzy El-Sayed KM, Mekhemar MK, Beck-Broichsitter BE, et al. Periodontal regeneration employing gingival margin-derived stem/progenitor cells in conjunction with IL-1ra-hydrogel synthetic extracellular matrix. *J Clin Periodontol*. 2015;42:448-457.
96. Fawzy El-Sayed KM, Paris S, Becker ST, et al. Periodontal regeneration employing gingival margin-derived stem/progenitor cells: an animal study. *J Clin Periodontol*. 2012;39:861-870.
97. Nishio C, Rompre P, Moldovan F. Effect of exogenous retinoic acid on tooth movement and periodontium healing following tooth extraction in a rat model. *Orthod Craniofac Res*. 2017;20:77-82.
98. Wang LY, Wang JY, Jin Y, Gao H, Lin XP. Oral Administration of all-Trans Retinoic Acid Suppresses Experimental Periodontitis by modulating the Th17/Treg imbalance. *J Periodontol*. 2014;85:740-750.
99. Arora N, Avula H, Avula JK. The adjunctive use of systemic antioxidant therapy (lycopene) in nonsurgical treatment of chronic periodontitis: a short-term evaluation. *Quintessence Int*. 2013;44:399-409.
100. Ambati M, Rani KR, Reddy PV, Suryaprasanna J, Dasari R, Gireddy H. Evaluation of oxidative stress in chronic periodontitis patients following systemic antioxidant supplementation: a clinical and biochemical study. *J Nat Sci Biol Med*. 2017;8:99-103.
101. Reddy PV, Ambati M, Koduganti R. Systemic lycopene as an adjunct to scaling and root planing in chronic periodontitis patients with type 2 diabetes mellitus. *J Int Soc Prev Commun Dent*. 2015;5:S25-S31.
102. Chandra RV, Sandhya YP, Nagarajan S, Reddy BH, Naveen A, Murthy RV. Efficacy of lycopene as a locally delivered gel in the treatment of chronic periodontitis: smokers vs nonsmokers. *Quintessence Int*. 2012;43:401-411.
103. Chandra RV, Srinivas G, Reddy AA, et al. Locally delivered antioxidant gel as an adjunct to nonsurgical therapy improves measures of oxidative stress and periodontal disease. *J Periodontal Implan*. 2013;43:121-129.
104. Tawfik MS, Abdel-Ghaffar KA, Gamal AY, El-Demerdash FH, Gad HA. Lycopene solid lipid microparticles with enhanced effect on gingival crevicular fluid protein carbonyl as a biomarker of oxidative stress in patients with chronic periodontitis. *J Liposome Res*. 2019;29:375-382.
105. Muroyama K, Murosaki S, Yamamoto Y, Ishijima A, Toh Y. Effects of intake of a mixture of thiamin, arginine, caffeine, and citric acid on adiposity in healthy subjects with high percent body fat. *Biosci Biotechnol Biochem*. 2003;67:2325-2333.
106. Kozik A, Korytowski W, Sarna T, Bloom AS. Interactions of flavins with melanin. Studies on equilibrium binding of riboflavin to dopamelanin and some spectroscopic characteristics of flavin-melanin complex. *Biophys Chem*. 1990;38:39-48.
107. Goldberg AC. A meta-analysis of randomized controlled studies on the effects of extended-release niacin in women. *Am J Cardiol*. 2004;94:121-124.
108. Vaxman F, Olender S, Lambert A, Nisand G, Grenier JF. Can the wound healing process be improved by vitamin supplementation? Experimental study on humans. *Eur Surg Res*. 1996;28:306-314.
109. Vaxman F, Olender S, Lambert A, et al. Effect of pantothenic acid and ascorbic acid supplementation on human skin wound healing process. A double-blind, prospective and randomized trial. *Eur Surg Res*. 1995;27:158-166.
110. Sonmez A, Lurie D, Chuong CJ. Effects of pantothenic acid on postoperative adhesion formation in a rat uterine horn model. *Arch Gynecol Obstet*. 2000;263:164-167.
111. Lacroix B, Didier E, Grenier JF. Role of pantothenic and ascorbic acid in wound healing processes: in vitro study on fibroblasts. *Int J Vitam Nutr Res*. 1988;58:407-413.
112. Aprahamian M, Dentinger A, Stock-Damge C, Kouassi JC, Grenier JF. Effects of supplemental pantothenic acid on wound healing: experimental study in rabbit. *Am J Clin Nutr*. 1985;41:578-589.
113. Morrow LE, Grimsley EW. Long-term diuretic therapy in hypertensive patients: effects on serum homocysteine, vitamin B6, vitamin B12, and red blood cell folate concentrations. *South Med J*. 1999;92:866-870.
114. Baez-Saldana A, Zendejas-Ruiz I, Revilla-Monsalve C, et al. Effects of biotin on pyruvate carboxylase, acetyl-CoA carboxylase, propionyl-CoA carboxylase, and markers for glucose and lipid homeostasis in type 2 diabetic patients and nondiabetic subjects. *Am J Clin Nutr*. 2004;79:238-243.
115. Branson JP, Attwood PV. Effects of Mg(2+) on the pre-steady-state kinetics of the biotin carboxylation reaction of pyruvate carboxylase. *Biochemistry*. 2000;39:7480-7491.
116. Neiva RF, Al-Shammari K, Nociti FH Jr, Soehren S, Wang HL. Effects of vitamin-B complex supplementation on periodontal wound healing. *J Periodontol*. 2005;76:1084-1091.
117. Khan S, Rahman SZ. A novel formulation of folic acid gel in the treatment of Desquamative gingivitis. *Bangladesh J Med Sci*. 2020;19:187-188.
118. Cao DZ, Sun WH, Ou XL, et al. Effects of folic acid on epithelial apoptosis and expression of Bcl-2 and p53 in premalignant gastric lesions. *World J Gastroenterol*. 2005;11:1571-1576.
119. Keceli HG, Ercan N, Hendek MK, Kisa U, Mesut B, Olgun E. The effect of the systemic folic acid intake as an adjunct to scaling and root planing on clinical parameters and homocysteine and C-reactive protein levels in gingival crevicular fluid of periodontitis patients: a randomized placebo-controlled clinical trial. *J Clin Periodontol*. 2020;47:602-613.
120. Vogel RI, Fink RA, Frank O, Baker H. The effect of topical application of folic acid on gingival health. *J Oral Med*. 1978;33:22.
121. Dreizen S, Levy BM, Bernick S. Studies on the biology of the periodontium of marmosets: 8. The effect of folic acid deficiency on the marmoset oral mucosa. *J Dent Res*. 1970;49:616-620.
122. Esaki M, Morita M, Akhter R, Akino K, Honda O. Relationship between folic acid intake and gingival health in non-smoking adults in Japan. *Oral Dis*. 2010;16:96-101.
123. Pack ARC. Folate mouthwash – effects on established gingivitis in periodontal patients. *J Clin Periodontol*. 1984;11:619-628.
124. Wickramasinghe SN. Morphology, biology and biochemistry of cobalamin-deficient and folate-deficient bone-marrow cells. *Baillieres Clin Haematol*. 1995;8:441-459.
125. Gulcan E, Toker S, Hatipoglu H, Gulcan A, Toker A. Cyanocobalamin may be beneficial in the treatment of recurrent Aphthous ulcers even when Vitamin B12 levels are Normal. *Am J Med Sci*. 2008;336:379-382.
126. Koybasi S, Parlak AH, Serin E, Yilmaz F, Serin D. Recurrent aphthous stomatitis: investigation of possible etiologic factors. *Am J Otolaryngol*. 2006;27:229-232.
127. Piskin S, Sayan C, Durukan N, Senol M. Serum iron, ferritin, folic acid, and vitamin B12 levels in recurrent aphthous stomatitis. *J Eur Acad Dermatol Venereol*. 2002;16:66-67.
128. Burgan SZ, Sawair FA, Amarin ZO. Hematologic status in patients with recurrent aphthous stomatitis in Jordan. *Saudi Med J*. 2006;27:381-384.
129. Zong G, Holtfreter B, Scott AE, et al. Serum vitamin B12 is inversely associated with periodontal progression and risk of tooth loss: a prospective cohort study. *J Clin Periodontol*. 2016;43:2-9.
130. Jang YJ, Jung IH, Park JC, et al. Effect of seeding using an avidin-biotin binding system on the attachment of periodontal ligament fibroblasts to nanohydroxyapatite scaffolds: three-dimensional culture. *J Periodontal Implan*. 2011;41:73-78.
131. Kim JH, Lee DE, Choi SH, Cha JH, Bak EJ, Yoo YJ. Diabetic characteristics and alveolar bone loss in streptozotocin- and

streptozotocin-nicotinamidetreated rats with periodontitis. *J Periodontal Res*. 2014;49:792-800.
132. Akpinar A, Karakan NC, Alpan AL, Dogan SSA, Goze F, Poyraz O. Comparative effects of riboflavin, nicotinamide and folic acid on alveolar bone loss: a morphometric and histopathologic study in rats. *Srp Ark Celok Lek*. 2016;144:273-279.
133. Russell SB, Russell JD, Trupin KM. Collagen-synthesis in human-fibroblasts – effects of ascorbic-acid and regulation by hydrocortisone. *J Cell Physiol*. 1981;109:121-131.
134. Cagetti MG, Wolf TG, Tennert C, Camoni N, Lingström P, Campus G. The role of vitamins in Oral health. A systematic review and meta-analysis. *Int J Environ Res Public Health*. 2020;17:938.
135. Tada A, Miura H. The relationship between Vitamin C and periodontal diseases: a systematic review. *Int J Environ Res Public Health*. 2019;16:2472.
136. Yang Y, Wang T, Zhang S, et al. Vitamin C alleviates the senescence of periodontal ligament stem cells through inhibition of Notch3 during long-term culture. *J Cell Physiol*. 2021;236:1237-1251.
137. Sato H, Takahashi M, Ise H, et al. Collagen synthesis is required for ascorbic acid-enhanced differentiation of mouse embryonic stem cells into cardiomyocytes. *Biochem Biophys Res Commun*. 2006;342:107-112.
138. Yan Y, Zeng W, Song S, et al. Vitamin C induces periodontal ligament progenitor cell differentiation via activation of ERK pathway mediated by PELP1. *Protein Cell*. 2013;4:620-627.
139. Huang Y, Tang X, Xie W, et al. Vitamin C enhances in vitro and in vivo development of porcine somatic cell nuclear transfer embryos. *Biochem Biophys Res Commun*. 2011;411:397-401.
140. Van Pham P, Tran NY, Phan NL-C, Vu NB, Phan NK. Vitamin C stimulates human gingival stem cell proliferation and expression of pluripotent markers. *In Vitro Cell Dev Biol Anim*. 2016;52:218-227.
141. Fawzy El-Sayed KM, Nguyen N, Dorfer CE. Ascorbic acid, inflammatory cytokines (IL-1β/TNF-α/IFN-γ), or their Combination's effect on Stemness, proliferation, and differentiation of gingival mesenchymal stem/progenitor cells. *Stem Cells Int*. 2020;2020:1-14.
142. Fawzy El-Sayed KM, Bittner A, Schlicht K, et al. Ascorbic acid/retinol and/or inflammatory Stimuli's effect on proliferation/differentiation properties and Transcriptomics of gingival stem/progenitor cells. *Cells*. 2021;10:3310.
143. Pullar JM, Carr AC, Vissers MCM. The roles of Vitamin C in skin health. *Nutrients*. 2017;9:866.
144. D'Aniello C, Cermola F, Patriarca EJ, Minchiotti G. Vitamin C in stem cell biology: impact on extracellular matrix homeostasis and epigenetics. *Stem Cells Int*. 2017;2017:8936156.
145. Van der Velden U. Vitamin C and its role in periodontal diseases – the past and the present: a narrative review. *Oral Health Prev Dent*. 2020;18:115-124.
146. Englard S, Seifter S. The biochemical functions of ascorbic acid. *Annu Rev Nutr*. 1986;6:365-406.
147. Du J, Cullen JJ, Buettner GR. Ascorbic acid: chemistry, biology and the treatment of cancer. *Biochim Biophys Acta*. 2012;1826:443-457.
148. Rahman I, Biswas SK, Jimenez LA, Torres M, Forman HJ. Glutathione, stress responses, and redox signaling in lung inflammation. *Antioxid Redox Signal*. 2005;7:42-59.
149. Mohammed BM, Fisher BJ, Kraskauskas D, et al. Vitamin C promotes wound healing through novel pleiotropic mechanisms. *Int Wound J*. 2016;13:572-584.
150. Subramanian N, Nandi BK, Majumder AK, Chatterjee IB. Role of L-ascorbic acid on detoxification of histamine. *Biochem Pharmacol*. 1973;22:1671-1673.
151. Mohammed BM, Fisher BJ, Huynh QK, et al. Resolution of sterile inflammation: role for vitamin C. *Mediators Inflamm*. 2014;2014:173403.
152. Mikirova N, Casciari J, Rogers A, Taylor P. Effect of high-dose intravenous vitamin C on inflammation in cancer patients. *J Transl Med*. 2012;10:189.
153. Carr AC, Maggini S. Vitamin C and immune function. *Nutrients*. 2017;9:1211.
154. De la Fuente M, Sanchez C, Vallejo C, Diaz-Del Cerro E, Arnalich F, Hernanz A. Vitamin C and vitamin C plus E improve the immune function in the elderly. *Exp Gerontol*. 2020;142:111118.
155. Mandl J, Szarka A, Banhegyi G. Vitamin C: update on physiology and pharmacology. *Br J Pharmacol*. 2009;157:1097-1110.
156. Savini I, Rossi A, Pierro C, Avigliano L, Catani MV. SVCT1 and SVCT2: key proteins for vitamin C uptake. *Amino Acids*. 2008;34:347-355.
157. Ordman AB. Recommendations for vitamin C intake. *JAMA*. 1999;282:2118-2119.
158. Frei B, Birlouez-Aragon I, Lykkesfeldt J. Authors' perspective: what is the optimum intake of vitamin C in humans? *Crit Rev Food Sci Nutr*. 2012;52:815-829.
159. Leggott PJ, Robertson PB, Rothman DL, Murray PA, Jacob RA. The effect of controlled ascorbic-acid depletion and supplementation on periodontal health. *J Periodontol*. 1986;57:480-485.
160. Nakamoto T, McCroskey M, Mallek HM. The role of ascorbic acid deficiency in human gingivitis--a new hypothesis. *J Theor Biol*. 1984;108:163-171.
161. Amaliya A, Laine ML, Delanghe JR, Loos BG, Van Wijk AJ, Van der Velden U. Java project on periodontal diseases: periodontal bone loss in relation to environmental and systemic conditions. *J Clin Periodontol*. 2015;42:325-332.
162. Amaliya, Timmerman MF, Abbas F, et al. Java project on periodontal diseases: the relationship between vitamin C and the severity of periodontitis. *J Clin Periodontol*. 2007;34:299-304.
163. Panjamurthy K, Manoharan S, Ramachandran CR. Lipid peroxidation and antioxidant status in patients with periodontitis. *Cell Mol Biol Lett*. 2005;10:255-264.
164. Staudte H, Kranz S, Volpel A, Schutze J, Sigusch BW. Comparison of nutrient intake between patients with periodontitis and healthy subjects. *Quintessence Int*. 2012;43:907-916.
165. Mathias TM, Silva JF, Sapata VM, Marson FC, Zanoni JN, Silva CO. Evaluation of the effects of periodontal treatment on levels of ascorbic acid in smokers. *J Int Acad Periodontol*. 2014;16:109-114.
166. Munday MR, Rodricks R, Fitzpatrick M, Flood VM, Gunton JE. A pilot study examining Vitamin C levels in periodontal patients. *Nutrients*. 2020;12:2255.
167. Mewes L, Knappe C, Graetz C, et al. Vitamin C and Omega-3 fatty acid intake is associated with human periodontitis-a nested case-control study. *Nutrients*. 2022;14:1939.
168. Cahill LE, El-Sohemy A. Vitamin C transporter gene polymorphisms, dietary Vitamin C and serum ascorbic acid. *J Nutrigenet Nutrigenomics*. 2009;2:292-301.
169. de Jong TMH, Jochens A, Jockel-Schneider Y, et al. SLC23A1 polymorphism rs6596473 in the vitamin C transporter SVCT1 is associated with aggressive periodontitis. *J Clin Periodontol*. 2014;41:531-540.
170. Pussinen PJ, Laatikainen T, Alfthan G, Asikainen S, Jousilahti P. Periodontitis is associated with a low concentration of vitamin C in plasma. *Clin Diagn Lab Immunol*. 2003;10:897-902.
171. Patak P, Willenberg HS, Bornstein SR. Vitamin C is an important cofactor for both adrenal cortex and adrenal medulla. *Endocr Res*. 2004;30:871-875.
172. Enwonwu CO, Sawiris P, Chanaud N. Effect of marginal ascorbic acid deficiency on saliva level of cortisol in the Guinea pig. *Arch Oral Biol*. 1995;40:737-742.
173. Marconi GD, Fonticoli L, Guarnieri S, et al. Ascorbic acid: a new player of epigenetic regulation in LPS-gingivalis treated human periodontal ligament stem cells. *Oxid Med Cell Longev*. 2021;2021:6679708.
174. Pizzicannella J, Cavalcanti M, Trubiani O, Diomede F. MicroRNA 210 mediates VEGF upregulation in human periodontal ligament

stem cells cultured on 3DHydroxyapatite ceramic scaffold. *Int J Mol Sci*. 2018;19:3916.
175. Fawzy El-Sayed KM, Elahmady M, Adawi Z, et al. The periodontal stem/progenitor cell inflammatory-regenerative cross talk: a new perspective. *J Periodontal Res*. 2019;54:81-94.
176. Leon ER, Iwasaki K, Komaki M, Kojima T, Ishikawa I. Osteogenic effect of interleukin-11 and synergism with ascorbic acid in human periodontal ligament cells. *J Periodontal Res*. 2007;42:527-535.
177. Fawzy El-Sayed KM, Nguyen N, Dorfer CE. Ascorbic acid, inflammatory cytokines (IL-1beta/TNF-alpha/IFN-gamma), or their Combination's effect on Stemness, proliferation, and differentiation of gingival mesenchymal stem/progenitor cells. *Stem Cells Int*. 2020;2020:8897138.
178. Wei FL, Qu CY, Song TL, et al. Vitamin C treatment promotes mesenchymal stem cell sheet formation and tissue regeneration by elevating telomerase activity. *J Cell Physiol*. 2012;227:3216-3224.
179. Hu L, Zhao B, Gao Z, et al. Regeneration characteristics of different dental derived stem cell sheets. *J Oral Rehabil*. 2020;47(Suppl 1):66-72.
180. Meng HF, Hu L, Zhou Y, et al. A Sandwich structure of human dental pulp stem cell sheet, treated dentin matrix, and Matrigel for tooth root regeneration. *Stem Cells Dev*. 2020;29:521-532.
181. Gauthier P, Yu ZD, Tran QT, Bhatti FUR, Zhu XF, Huang GTJ. Cementogenic genes in human periodontal ligament stem cells are downregulated in response to osteogenic stimulation while upregulated by vitamin C treatment. *Cell Tissue Res*. 2017;368:79-92.
182. Fawzy El-Sayed KM, Dorfer C, Ungefroren H, Kassem N, Wiltfang J, Paris S. Effect of Emdogain enamel matrix derivative and BMP-2 on the gene expression and mineralized nodule formation of alveolar bone proper-derived stem/progenitor cells. *J Craniomaxillofac Surg*. 2014;42:568-576.
183. Fawzy El-Sayed KM, Paris S, Becker S, et al. Isolation and characterization of multipotent postnatal stem/progenitor cells from human alveolar bone proper. *J Craniomaxillofac Surg*. 2012;40:735-742.
184. Mimori K, Komaki M, Iwasaki K, Ishikawa I. Extracellular signal-regulated kinase 1/2 is involved in ascorbic acid-induced osteoblastic differentiation in periodontal ligament cells. *J Periodontol*. 2007;78:328-334.
185. Okajima LS, Martinez EF, Pinheiro IF, Fonseca Silva AS, Demasi APD. Effect of sodium ascorbyl phosphate on osteoblast viability and differentiation. *J Periodontal Res*. 2020;55:660-666.
186. Bhandi S, Alkahtani A, Mashyakhy M, et al. Effect of ascorbic acid on differentiation, Secretome and Stemness of stem cells from human exfoliated deciduous tooth (SHEDs). *J Pers Med*. 2021;11:589.
187. El Moshy S, Radwan IA, Rady D, et al. Dental stem cell-derived Secretome/conditioned medium: the future for regenerative therapeutic applications. *Stem Cells Int*. 2020;2020:7593402.
188. Feng G, Wu Y, Yu Y, et al. Periodontal ligament-like tissue regeneration with drilled porous decalcified dentin matrix sheet composite. *Oral Dis*. 2018;24:429-441.
189. Hu JC, Cao Y, Xie YL, et al. Periodontal regeneration in swine after cell injection and cell sheet transplantation of human dental pulp stem cells following good manufacturing practice. *Stem Cell Res Ther*. 2016;7:130.
190. Woolfe SN, Kenney EB, Hume WR, Carranza FA Jr. Relationship of ascorbic acid levels of blood and gingival tissue with response to periodontal therapy. *J Clin Periodontol*. 1984;11:159-165.
191. Abou Sulaiman AE, Shehadeh RMH. Assessment of Total antioxidant capacity and the use of Vitamin C in the treatment of non-smokers with chronic periodontitis. *J Periodontol*. 2010;81:1547-1554.
192. Elbehwashy MT, Hosny MM, Elfana A, Nawar A, El-Sayed KF. Clinical and radiographic effects of ascorbic acid-augmented platelet-rich fibrin versus platelet-rich fibrin alone in intra-osseous defects of stage-III periodontitis patients: a randomized controlled clinical trial. *Clin Oral Investig*. 2021;25:6309-6319.
193. van Etten E, Mathieu C. Immunoregulation by 1,25-dihydroxyvitamin D3: basic concepts. *J Steroid Biochem Mol Biol*. 2005;97:93-101.
194. Chang SW, Lee HC. Vitamin D and health – the missing vitamin in humans. *Pediatr Neonatol*. 2019;60:237-244.
195. Liu K, Meng H, Hou J. Activity of 25-hydroxylase in human gingival fibroblasts and periodontal ligament cells. *PloS One*. 2012;7:e52053.
196. Lee WT, Jiang J. The resurgence of the importance of vitamin D in bone health. *Asia Pac J Clin Nutr*. 2008;17(Suppl 1):138-142.
197. Hildebolt CF. Effect of vitamin D and calcium on periodontitis. *J Periodontol*. 2005;76:1576-1587.
198. Stein SH, Livada R, Tipton DA. Re-evaluating the role of vitamin D in the periodontium. *J Periodontal Res*. 2014;49:545-553.
199. Holick MF. The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action. *Mol Aspects Med*. 2008;29:361-368.
200. Janssens W, Lehouck A, Carremans C, Bouillon R, Mathieu C, Decramer M. Vitamin D beyond bones in chronic obstructive pulmonary disease: time to act. *Am J Respir Crit Care Med*. 2009;179:630-636.
201. Solidoro P, Bellocchia M, Facchini F. The immunobiological and clinical role of vitamin D in obstructive lung diseases. *Minerva Med*. 2016;107:12-19.
202. Aziz AS, Kalekar MG, Suryakar AN, et al. Assessment of some biochemical oxidative stress markers in male smokers with chronic periodontitis. *Indian J Clin Biochem*. 2013;28:374-380.
203. Mailhot G, White JH. Vitamin D and immunity in infants and children. *Nutrients*. 2020;12:1233.
204. Jagelaviciene E, Vaitkeviciene I, Silingaite D, Sinkunaite E, Daugelaite G. The relationship between Vitamin D and periodontal pathology. *Medicina-Lithuania*. 2018;54:45.
205. Adegboye ARA, Boucher BJ, Kongstad J, Fiehn NE, Christensen LB, Heitmann BL. Calcium, vitamin D, casein and whey protein intakes and periodontitis among Danish adults. *Public Health Nutr*. 2016;19:503-510.
206. Jimenez M, Giovannucci E, Kaye EK, Joshipura KJ, Dietrich T. Predicted vitamin D status and incidence of tooth loss and periodontitis. *Public Health Nutr*. 2014;17:844-852.
207. Jabbar S, Drury J, Fordham J, Datta HK, Francis RM, Tuck SP. Plasma vitamin D and cytokines in periodontal disease and postmenopausal osteoporosis. *J Periodontal Res*. 2011;46:97-104.
208. Antonoglou G, Knuuttila M, Niemela O, et al. Serum 1,25(OH)D level increases after elimination of periodontal inflammation in T1DM subjects. *J Clin Endocrinol Metab*. 2013;98:3999-4005.
209. Millen AE, Hovey KM, LaMonte MJ, et al. Plasma 25-hydroxyvitamin D concentrations and periodontal disease in postmenopausal women. *J Periodontol*. 2013;84:1243-1256.
210. Boggess KA, Espinola JA, Moss K, Beck J, Offenbacher S, Camargo CA Jr. Vitamin D status and periodontal disease among pregnant women. *J Periodontol*. 2011;82:195-200.
211. Zhou X, Han J, Song Y, Zhang J, Wang Z. Serum levels of 25-hydroxyvitamin D, oral health and chronic obstructive pulmonary disease. *J Clin Periodontol*. 2012;39:350-356.
212. Alshouibi EN, Kaye EK, Cabral HJ, Leone CW, Garcia RI. Vitamin D and periodontal health in older men. *J Dent Res*. 2013;92:689-693.
213. Hiremath VP, Rao CB, Naik V, Prasad KVV. Anti-inflammatory effect of Vitamin D on gingivitis: a dose-response randomised control trial. *Oral Health Prev Dent*. 2013;11:61-69.
214. Vieth R, Chan PC, MacFarlane GD. Efficacy and safety of vitamin D3 intake exceeding the lowest observed adverse effect level. *Am J Clin Nutr*. 2001;73:288-294.
215. Paz A, Stanley M, Mangano FG, Miron RJ. Vitamin D deficiency and early implant failure: outcomes from a pre-surgical

supplementation program on Vitamin D levels and antioxidant scores. *Oral Health Prev Dent*. 2021;19:495-502.
216. Ribeiro L, Araujo NS, Zilli Vieira CL, Dos Santos JN, Cury PR. Impact of serum vitamin D levels on periodontal healing outcomes: a preliminary cohort study. *Int J Dent Hyg*. 2022;21:291-297.
217. Krall EA, Wehler C, Garcia RI, Harris SS, Dawson-Hughes B. Calcium and vitamin D supplements reduce tooth loss in the elderly. *Am J Med*. 2001;111:452-456.
218. Dietrich T, Joshipura KJ, Dawson-Hughes B, Bischoff-Ferrari HA. Association between serum concentrations of 25-hydroxyvitamin D3 and periodontal disease in the US population. *Am J Clin Nutr*. 2004;80:108-113.
219. Dietrich T, Nunn M, Dawson-Hughes B, Bischoff-Ferrari HA. Association between serum concentrations of 25-hydroxyvitamin D and gingival inflammation. *Am J Clin Nutr*. 2005;82:575-580.
220. Laky M, Bertl K, Haririan H, et al. Serum levels of 25-hydroxyvitamin D are associated with periodontal disease. *Clin Oral Investig*. 2017;21:1553-1558.
221. Teles FR, Teles RP, Martin L, Socransky SS, Haffajee AD. Relationships among interleukin-6, tumor necrosis factor-alpha, adipokines, vitamin D, and chronic periodontitis. *J Periodontol*. 2012;83:1183-1191.
222. Zhang Y, Leung DYM, Richers BN, et al. Vitamin D inhibits monocyte/macrophage Proinflammatory cytokine production by targeting MAPK Phosphatase-1. *J Immunol*. 2012;188:2127-2135.
223. Zhang X, Zhou M, Guo Y, Song Z, Liu B. 1,25-Dihydroxyvitamin D(3) promotes high glucose-induced M1 macrophage switching to M2 via the VDR-PPARgamma signaling pathway. *Biomed Res Int*. 2015;2015:157834.
224. Tang X, Pan Y, Zhao Y. Vitamin D inhibits the expression of interleukin-8 in human periodontal ligament cells stimulated with Porphyromonas gingivalis. *Arch Oral Biol*. 2013;58:397-407.
225. Huang C, Zhang C, Yang P, et al. Eldecalcitol inhibits LPS-induced NLRP3 Inflammasome-dependent Pyroptosis in human gingival fibroblasts by activating the Nrf2/HO-1 signaling pathway. *Drug Des Devel Ther*. 2020;14:4901-4913.
226. Wang Q, Zhou X, Zhang P, et al. 25-Hydroxyvitamin D3 positively regulates periodontal inflammaging via SOCS3/STAT signaling in diabetic mice. *Steroids*. 2020;156:108570.
227. Frederiksen B, Liu E, Romanos J, et al. Investigation of the vitamin D receptor gene (VDR) and its interaction with protein tyrosine phosphatase, non-receptor type 2 gene (PTPN2) on risk of islet autoimmunity and type 1 diabetes: the diabetes autoimmunity study in the young (DAISY). *J Steroid Biochem Mol Biol*. 2013;133:51-57.
228. Zhang P, Zhang W, Zhang D, et al. 25-Hydroxyvitamin D3 -enhanced PTPN2 positively regulates periodontal inflammation through the JAK/STAT pathway in human oral keratinocytes and a mouse model of type 2 diabetes mellitus. *J Periodontal Res*. 2018;53:467-477.
229. Wang Q, Li H, Xie H, et al. 25-Hydroxyvitamin D3 attenuates experimental periodontitis through downregulation of TLR4 and JAK1/STAT3 signaling in diabetic mice. *J Steroid Biochem Mol Biol*. 2013;135:43-50.
230. McMahon L, Schwartz K, Yilmaz O, Brown E, Ryan LK, Diamond G. Vitamin D-mediated induction of innate immunity in gingival epithelial cells. *Infect Immun*. 2011;79:2250-2256.
231. Liu PT, Stenger S, Li HY, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science*. 2006;311:1770-1773.
232. Almoudi MMM, Hussein AS, Abu Hassan MI, et al. The antibacterial effects of vitamin D-3 against mutans streptococci: an in vitro study. *Eur Oral Res*. 2021;55:8-15.
233. Hewison M. Antibacterial effects of vitamin D. *Nat Rev Endocrinol*. 2011;7:336-344.
234. Hennig BJW, Parkhill JM, Chapple LLC, Heasman PA, Taylor JJ. Association of a vitamin D receptor gene polymorphism with localized early-onset periodontal diseases. *J Periodontol*. 1999;70:1032-1038.
235. Inagaki K, Krall EA, Fleet JC, Garcia RI. Vitamin D receptor alleles, periodontal disease progression, and tooth loss in the VA dental longitudinal study. *J Periodontol*. 2003;74:161-167.
236. Yoshie H, Kobayashi T, Tai H, Galicia JC. The role of genetic polymorphisms in periodontitis. *Periodontol 2000*. 2007;43:102-132.
237. Zhang X, Meng H, Sun X, et al. Elevation of vitamin D-binding protein levels in the plasma of patients with generalized aggressive periodontitis. *J Periodontal Res*. 2013;48:74-79.
238. Kim HS, Zheng M, Kim DK, Lee WP, Yu SJ, Kim BO. Effects of 1,25-dihydroxyvitamin D3 on the differentiation of MC3T3-E1 osteoblast-like cells. *J Periodontal Implant Sci*. 2018;48:34-46.
239. Hong HH, Chou TA, Hong A, et al. Calcitriol and enamel matrix derivative differentially regulated cemento-induction and mineralization in human periodontal ligament-derived cells. *J Periodontol*. 2021;93:1553-1565.
240. Nebel D, Svensson D, Arosenius K, Larsson E, Jonsson D, Nilsson BO. 1 alpha,25-dihydroxyvitamin D3 promotes osteogenic activity and downregulates proinflammatory cytokine expression in human periodontal ligament cells. *J Periodontal Res*. 2015;50:666-673.
241. Yamada S, Tomoeda M, Ozawa Y, et al. PLAP-1/asporin, a novel negative regulator of periodontal ligament mineralization. *J Biol Chem*. 2007;282:23070-23080.
242. Zhang P, Zhang Y, Liu Q, Zhang Y, Ji Y, Xu X. 1,25(OH)2D3 supports the osteogenic differentiation of hPDLSCs under inflammatory conditions through inhibiting PLAP-1 expression transcriptionally. *Int Immunopharmacol*. 2020;78:105998.
243. Fugl A, Gruber R, Agis H, et al. Alveolar bone regeneration in response to local application of calcitriol in vitamin D deficient rats. *J Clin Periodontol*. 2015;42:96-103.
244. Zhou X, Zhang P, Wang Q, et al. 25-Hydroxyvitamin D3 alleviates experimental periodontitis via promoting expression of cathelicidin in mice with type 2 diabetic mellitus. *J Nutr Sci Vitaminol (Tokyo)*. 2018;64:307-315.
245. Han J, Cheng C, Zhu Z, et al. Vitamin D reduces the serum levels of inflammatory cytokines in rat models of periodontitis and chronic obstructive pulmonary disease. *J Oral Sci*. 2019;61:53-60.
246. Bi CS, Wang J, Qu HL, et al. Calcitriol suppresses lipopolysaccharide-induced alveolar bone damage in rats by regulating T helper cell subset polarization. *J Periodontal Res*. 2019;54:612-623.
247. Adibrad M, Deyhimi P, Ganjalikhani Hakemi M, Behfarnia P, Shahabuei M, Rafiee L. Signs of the presence of Th17 cells in chronic periodontal disease. *J Periodontal Res*. 2012;47:525-531.
248. Miley DD, Garcia MN, Hildebolt CF, et al. Cross-sectional study of vitamin D and calcium supplementation effects on chronic periodontitis. *J Periodontol*. 2009;80:1433-1439.
249. Garcia MN, Hildebolt CF, Miley DD, et al. One-year effects of vitamin D and calcium supplementation on chronic periodontitis. *J Periodontol*. 2011;82:25-32.
250. Bashutski JD, Eber RM, Kinney JS, et al. The impact of Vitamin D status on periodontal surgery outcomes. *J Dent Res*. 2011;90:1007-1012.
251. Evans HM, Bishop KS. On the existence of a hitherto unrecognized dietary factor essential for reproduction. *Science*. 1922;56:650-651.
252. Evan HMEO, Emerson GA. THE CHEMISTRY OF VITAMIN E: TOCOPHEROLS FROM VARIOUS SOURCES. *J Biol Chem*. 1936;113:113-319.
253. Garcia-Closas R, Berenguer A, Jose Tormo M, et al. Dietary sources of vitamin C, vitamin E and specific carotenoids in Spain. *Br J Nutr*. 2004;91:1005-1011.
254. Mene-Saffrane L, Vitamin E. Biosynthesis and its regulation in plants. *Antioxidants (Basel)*. 2017;7:2.

255. Munne-Bosch S. Alpha-tocopherol: a multifaceted molecule in plants. *Vitam Horm*. 2007;76:375-392.
256. Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. *Free Radic Biol Med*. 2007;43:4-15.
257. Sen CK, Khanna S, Roy S. Tocotrienols in health and disease: the other half of the natural vitamin E family. *Mol Aspects Med*. 2007;28:692-728.
258. Shadisvaaran S, Chin KY, Shahida MS, Ima-Nirwana S, Leong XF. Effect of vitamin E on periodontitis: evidence and proposed mechanisms of action. *J Oral Biosci*. 2021;63:97-103.
259. Cohen ME, Meyer DM. Effect of dietary vitamin E supplementation and rotational stress on alveolar bone loss in rice rats. *Arch Oral Biol*. 1993;38:601-606.
260. Cohen RE, Ciancio SG, Mather ML, Curro FA. Effect of vitamin E gel, placebo gel and chlorhexidine on periodontal disease. *Clin Prev Dent*. 1991;13:20-24.
261. Slade EW Jr, Bartuska D, Rose LF, Cohen DW. Vitamin E and periodontal disease. *J Periodontol*. 1976;47:352-354.
262. Luo PP, Xu HS, Chen YW, Wu SP. Periodontal disease severity is associated with micronutrient intake. *Aust Dent J*. 2018;63:193-201.
263. Zong G, Scott AE, Griffiths HR, Zock PL, Dietrich T, Newson RS. Serum alpha-tocopherol has a nonlinear inverse association with periodontitis among US adults. *J Nutr*. 2015;145:893-899.
264. Cerna H, Fiala B, Fingerova H, Pohanka J, Szwarcova E. Contribution to indication of total therapy with vitamin E in chronic periodontal disease (pilot study). *Acta Univ Palacki Olomuc Fac Med*. 1984;107:167-170.
265. Torshabi M, Rezaei Esfahrood Z, Jamshidi M, Mansuri Torshizi A, Sotoudeh S. Efficacy of vitamins E and C for reversing the cytotoxic effects of nicotine and cotinine. *Eur J Oral Sci*. 2017;125:426-437.
266. Nizam N, Discioglu F, Saygun I, et al. The effect of alpha-tocopherol and selenium on human gingival fibroblasts and periodontal ligament fibroblasts in vitro. *J Periodontol*. 2014;85:636-644.
267. Satue M, Gomez-Florit M, Monjo M, Ramis JM. Improved human gingival fibroblast response to titanium implants coated with ultraviolet-irradiated vitamin D precursor and vitamin E. *J Periodontal Res*. 2016;51:342-349.
268. Royack GA, Nguyen MP, Tong DC, Poot M, Oda D. Response of human oral epithelial cells to oxidative damage and the effect of vitamin E. *Oral Oncol*. 2000;36:37-41.
269. Derradjia A, Alanazi H, Park HJ, Djeribi R, Semlali A, Rouabhia M. Alpha-tocopherol decreases interleukin-1beta and −6 and increases human beta-defensin-1 and -2 secretion in human gingival fibroblasts stimulated with Porphyromonas gingivalis lipopolysaccharide. *J Periodontal Res*. 2016;51:295-303.
270. Murakami Y, Kawata A, Koh T, et al. Inhibitory effects of tocopherols on expression of the cyclooxygenase-2 gene in RAW264.7 cells stimulated by lipopolysaccharide, tumor necrosis factor-alpha or Porphyromonas gingivalis fimbriae. *In Vivo*. 2013;27:451-458.
271. Kim JE, Shklar G. The effect of vitamin E on the healing of gingival wounds in rats. *J Periodontol*. 1983;54:305-308.
272. Bas N, Kayar NA, Baba ZF, Avunduk MC, Haliloglu S, Alptekin NO. Systemic treatment with alpha-tocopherol and/or sodium selenite decreases the progression of experimental periodontitis. *Clin Oral Investig*. 2021;25:2677-2688.
273. Carvalho Rde S, de Souza CM, Neves JC, et al. Vitamin E does not prevent bone loss and induced anxiety in rats with ligature-induced periodontitis. *Arch Oral Biol*. 2013;58:50-58.
274. Singh N, Chander Narula S, Kumar Sharma R, Tewari S, Kumar SP. Vitamin E supplementation, superoxide dismutase status, and outcome of scaling and root planing in patients with chronic periodontitis: a randomized clinical trial. *J Periodontol*. 2014;85:242-249.
275. Behfarnia P, Dadmehr M, Hosseini SN, Mirghaderi SA. The effect of Vitamin E supplementation on treatment of chronic periodontitis. *Dent Res J (Isfahan)*. 2021;18:62.
276. Shearer MJ, Newman P. Metabolism and cell biology of vitamin K. *Thromb Haemost*. 2008;100:530-547.
277. Akbari S, Rasouli-Ghahroudi AA. Vitamin K and bone metabolism: a review of the latest evidence in preclinical studies. *Biomed Res Int*. 2018;2018:4629383.
278. Beulens JW, Booth SL, van den Heuvel EG, Stoecklin E, Baka A, Vermeer C. The role of menaquinones (vitamin K(2)) in human health. *Br J Nutr*. 2013;110:1357-1368.
279. Ferland G. The vitamin K-dependent proteins: an update. *Nutr Rev*. 1998;56:223-230.
280. Shearer MJ, Fu X, Booth SL. Vitamin K nutrition, metabolism, and requirements: current concepts and future research. *Adv Nutr*. 2012;3:182-195.
281. Ichikawa T, Horie-Inoue K, Ikeda K, Blumberg B, Inoue S. Steroid and xenobiotic receptor SXR mediates vitamin K2-activated transcription of extracellular matrix-related genes and collagen accumulation in osteoblastic cells. *J Biol Chem*. 2006;281:16927-16934.
282. Krueger T, Westenfeld R, Schurgers L, Brandenburg V. Coagulation meets calcification: the vitamin K system. *Int J Artif Organs*. 2009;32:67-74.
283. Booth SL, Suttie JW. Dietary intake and adequacy of vitamin K. *J Nutr*. 1998;128:785-788.
284. Hamidi MS, Gajic-Veljanoski O, Cheung AM. Vitamin K and bone health. *J Clin Densitom*. 2013;16:409-413.
285. Hirota Y, Tsugawa N, Nakagawa K, et al. Menadione (vitamin K3) is a catabolic product of oral phylloquinone (vitamin K1) in the intestine and a circulating precursor of tissue menaquinone-4 (vitamin K2) in rats. *J Biol Chem*. 2013;288:33071-33080.
286. Myneni VD, Mezey E. Regulation of bone remodeling by vitamin K2. *Oral Dis*. 2017;23:1021-1028.
287. Koshihara Y, Hoshi K, Okawara R, Ishibashi H, Yamamoto S. Vitamin K stimulates osteoblastogenesis and inhibits osteoclastogenesis in human bone marrow cell culture. *J Endocrinol*. 2003;176:339-348.
288. Urayama S, Kawakami A, Nakashima T, et al. Effect of vitamin K2 on osteoblast apoptosis: vitamin K2 inhibits apoptotic cell death of human osteoblasts induced by Fas, proteasome inhibitor, etoposide, and staurosporine. *J Lab Clin Med*. 2000;136:181-193.
289. Akedo Y, Hosoi T, Inoue S, et al. Vitamin K2 modulates proliferation and function of osteoblastic cells in vitro. *Biochem Biophys Res Commun*. 1992;187:814-820.
290. Kim M, Na W, Sohn C. Vitamin K1 (phylloquinone) and K2 (menaquinone-4) supplementation improves bone formation in a high-fat diet-induced obese mice. *J Clin Biochem Nutr*. 2013;53:108-113.
291. Poon CC, Li RW, Seto SW, et al. In vitro vitamin K(2) and 1alpha,25-dihydroxyvitamin D(3) combination enhances osteoblasts anabolism of diabetic mice. *Eur J Pharmacol*. 2015;767:30-40.
292. Atkins GJ, Welldon KJ, Wijenayaka AR, Bonewald LF, Findlay DM. Vitamin K promotes mineralization, osteoblast-to-osteocyte transition, and an anticatabolic phenotype by gamma-carboxylation-dependent and -independent mechanisms. *Am J Physiol Cell Physiol*. 2009;297:C1358-C1367.
293. Akiyama Y, Hara K, Kobayashi M, Tomiuga T, Nakamura T. Inhibitory effect of vitamin K2 (menatetrenone) on bone resorption in ovariectomized rats: a histomorphometric and dual energy X-ray absorptiometric study. *Jpn J Pharmacol*. 1999;80:67-74.
294. Hara K, Akiyama Y, Ohkawa I, Tajima T. Effects of menatetrenone on prednisolone-induced bone loss in rats. *Bone*. 1993;14:813-818.
295. Kameda T, Miyazawa K, Mori Y, et al. Vitamin K2 inhibits osteoclastic bone resorption by inducing osteoclast apoptosis. *Biochem Biophys Res Commun*. 1996;220:515-519.
296. Iwamoto J, Matsumoto H, Takeda T, Sato Y, Liu X, Yeh JK. Effects of vitamin K(2) and risedronate on bone formation and resorption, osteocyte lacunar system, and porosity in the cortical bone of glucocorticoid-treated rats. *Calcif Tissue Int*. 2008;83:121-128.

297. Wu WJ, Kim MS, Ahn BY. The inhibitory effect of vitamin K on RANKL-induced osteoclast differentiation and bone resorption. *Food Funct*. 2015;6:3351-3358.
298. Baraniya D, Naginyte M, Chen T, et al. Modeling Normal and Dysbiotic subgingival microbiomes: effect of nutrients. *J Dent Res*. 2020;99:695-702.
299. Cui Q, Li N, Nie F, Yang F, Li H, Zhang J. Vitamin K2 promotes the osteogenic differentiation of periodontal ligament stem cells via the Wnt/beta-catenin signaling pathway. *Arch Oral Biol*. 2021;124:105057.
300. Aral K, Alkan BA, Saraymen R, Yay A, Sen A, Onder GO. Therapeutic effects of systemic vitamin k2 and vitamin d3 on gingival inflammation and alveolar bone in rats with experimentally induced periodontitis. *J Periodontol*. 2015;86:666-673.
301. Crane FL, Hatefi Y, Lester RL, Widmer C. Isolation of a quinone from beef heart mitochondria. *Biochim Biophys Acta*. 1957;25:220-221.
302. Acosta MJ, Vazquez Fonseca L, Desbats MA, et al. Coenzyme Q biosynthesis in health and disease. *Biochim Biophys Acta*. 2016;1857:1079-1085.
303. Tran MT, Mitchell TM, Kennedy DT, Giles JT. Role of coenzyme Q10 in chronic heart failure, angina, and hypertension. *Pharmacotherapy*. 2001;21:797-806.
304. Palamakula A, Soliman M, Khan MM. Regional permeability of coenzyme Q10 in isolated rat gastrointestinal tracts. *Pharmazie*. 2005;60:212-214.
305. Aberg F, Appelkvist EL, Dallner G, Ernster L. Distribution and redox state of ubiquinones in rat and human tissues. *Arch Biochem Biophys*. 1992;295:230-234.
306. Crane FL. Biochemical functions of coenzyme Q10. *J Am Coll Nutr*. 2001;20:591-598.
307. Ernster L, Dallner G. Biochemical, physiological and medical aspects of ubiquinone function. *Biochim Biophys Acta*. 1995;1271:195-204.
308. Okamoto T, Matsuya T, Fukunaga Y, Kishi T, Yamagami T. Human serum ubiquinol-10 levels and relationship to serum lipids. *Int J Vitam Nutr Res*. 1989;59:288-292.
309. Groneberg DA, Kindermann B, Althammer M, et al. Coenzyme Q10 affects expression of genes involved in cell signalling, metabolism and transport in human CaCo-2 cells. *Int J Biochem Cell Biol*. 2005;37:1208-1218.
310. Schmelzer C, Lindner I, Rimbach G, Niklowitz P, Menke T, Doring F. Functions of coenzyme Q10 in inflammation and gene expression. *Biofactors*. 2008;32:179-183.
311. Garrido-Maraver J, Cordero MD, Oropesa-Avila M, et al. Clinical applications of coenzyme Q10. *Front Biosci (Landmark Ed)*. 2014;19:619-633.
312. Prakash S, Sunitha J, Hans M. Role of coenzyme Q(10) as an antioxidant and bioenergizer in periodontal diseases. *Indian J Pharm*. 2010;42:334-337.
313. Soni SAP, Sharma N, Chander S. Coenzyme Q10 and periodontal health-a review. *Int J Oral Maxillofac Pathol*. 2012;3:21-26.
314. Hanioka T, Tanaka M, Ojima M, Shizukuishi S, Folkers K. Effect of topical application of coenzyme Q10 on adult periodontitis. *Mol Aspects Med*. 1994;15(Suppl):s241-s248.
315. Ryo K, Ito A, Takatori R, et al. Effects of coenzyme Q10 on salivary secretion. *Clin Biochem*. 2011;44:669-674.
316. Figuero E, Soory M, Cerero R, Bascones A. Oxidant/antioxidant interactions of nicotine, coenzyme Q10, Pycnogenol and phytoestrogens in oral periosteal fibroblasts and MG63 osteoblasts. *Steroids*. 2006;71:1062-1072.
317. Varela-Lopez A, Bullon P, Battino M, et al. Coenzyme Q protects against age-related alveolar bone loss associated to n-6 polyunsaturated fatty acid rich-diets by modulating mitochondrial mechanisms. *J Gerontol A Biol Sci Med Sci*. 2016;71:593-600.
318. Yoneda T, Tomofuji T, Ekuni D, et al. Anti-aging effects of coenzyme Q10 on periodontal tissues. *J Dent Res*. 2013;92:735-739.
319. Barakat AK, Attia AM. Clinical evaluation of Co-enzyme Q10 in Management of Chronic Periodontitis Patients: mouth Split study. *Int J Health Sci Res*. 2019;9:69-75.
320. Chatterjee A, Kandwal A, Singh N, Singh A. Evaluation of Co-Q10 anti-gingivitis effect on plaque induced gingivitis: a randomized controlled clinical trial. *J Indian Soc Periodontol*. 2012;16:539-542.
321. Hans M, Prakash S, Gupta S. Clinical evaluation of topical application of perio-Q gel (coenzyme Q(10)) in chronic periodontitis patients. *J Indian Soc Periodontol*. 2012;16:193-199.
322. Raut CP, Sethi KS. Comparative evaluation of co-enzyme Q10 and Melaleuca alternifolia as antioxidant gels in treatment of chronic periodontitis: a clinical study. *Contemp Clin Dent*. 2016;7:377-381.
323. Raut CP, Sethi KS, Kohale B, Mamajiwala A, Warang A. Subgingivally delivered coenzyme Q10 in the treatment of chronic periodontitis among smokers: a randomized, controlled clinical study. *J Oral Biol Craniofac Res*. 2019;9:204-208.
324. Sale ST, Parvez H, Yeltiwar RK, Vivekanandan G, Pundir AJ, Jain P. A comparative evaluation of topical and intrasulcular application of coenzyme Q10 (Perio Q) gel in chronic periodontitis patients: a clinical study. *J Indian Soc Periodontol*. 2014;18:461-465.
325. Shaheen MA, Elmeadawy SH, Bazeed FB, Anees MM, Saleh NM. Innovative coenzyme Q(10)-loaded nanoformulation as an adjunct approach for the management of moderate periodontitis: preparation, evaluation, and clinical study. *Drug Deliv Transl Res*. 2020;10:548-564.
326. Pranam S, Palwankar P, Pandey R, Goyal A. Evaluation of efficacy of coenzyme Q10 as an adjunct to nonsurgical periodontal therapy and its effect on Crevicular superoxide dismutase in patients with chronic periodontitis. *Eur J Dent*. 2020;14:551-557.
327. Sharma V, Gupta R, Dahiya P, Kumar M. Comparative evaluation of coenzyme Q(10)-based gel and 0.8% hyaluronic acid gel in treatment of chronic periodontitis. *J Indian Soc Periodontol*. 2016;20:374-380.
328. Chug A, Shukla S. Placement of sticky bone in patients with generalized periodontitis previously treated with coenzyme Q10. *J Contemp Dent Pract*. 2020;21:156-160.
329. ElBarbary A, Nadim MK, Hussein H. The effect of using coenzyme Q10 with non-surgical periodontal treatment on MMP9 gingival Crevicular fluid level in patients with periodontitis: randomized controlled trial. *Adv Dent J*. 2022;4:84-94.
330. Manthena S, Rao MV, Penubolu LP, Putcha M, Harsha AV. Effectiveness of CoQ10 Oral supplements as an adjunct to scaling and root Planing in improving periodontal health. *J Clin Diagn Res*. 2015;9:ZC26-ZC28.
331. Shoukheba MYM, El-Kholy SE-SM. Coenzyme Q10 food supplement on the treatment of chronic periodontitis in patients with type II diabetes mellitus: a randomized control study. *Egyptian Dent J*. 2019;65:253-261.
332. Ghasemi S, Torab Z, Shirmohammadi A, et al. Evaluation of the effect of coenzyme Q10 supplementation along with scaling and root planing (SRP) on periodontal and gingival indices in controlled diabetic patients. *J Adv Periodontol Implant Dent*. 2022;14:32-37.
333. Li W, Shang Q, Yang D, et al. Abnormal micronutrient intake is associated with the risk of periodontitis: a dose-response association study based on NHANES 2009-2014. *Nutrients*. 2022;14:2466.
334. Chapple IL, Milward MR, Ling-Mountford N, et al. Adjunctive daily supplementation with encapsulated fruit, vegetable and berry juice powder concentrates and clinical periodontal outcomes: a double-blind RCT. *J Clin Periodontol*. 2012;39:62-72.
335. Ebersole JL, Lambert J, Bush H, Huja PE, Basu A. Serum nutrient levels and aging effects on periodontitis. *Nutrients*. 2018;10:1986.
336. Munoz CA, Kiger RD, Stephens JA, Kim J, Wilson AC. Effects of a nutritional supplement on periodontal status. *Compend Contin Educ Dent*. 2001;22:425-428, 430, 432 passim; quiz 440.
337. Harpenau LA, Cheema AT, Zingale JA, Chambers DW, Lundergan WP. Effects of nutritional supplementation on periodontal

parameters, carotenoid antioxidant levels, and serum C-reactive protein. *J Calif Dent Assoc*. 2011;39:309-312, 314–318.
338. Liu Y, Jing H, Wang J, et al. Micronutrients decrease incidence of common infections in type 2 diabetic outpatients. *Asia Pac J Clin Nutr*. 2011;20:375-382.
339. Willershausen B, Ross A, Forsch M, Willershausen I, Mohaupt P, Callaway A. The influence of micronutrients on oral and general health. *Eur J Med Res*. 2011;16:514-518.
340. Woelber JP, Bremer K, Vach K, et al. An oral health optimized diet can reduce gingival and periodontal inflammation in humans – a randomized controlled pilot study. *BMC Oral Health*. 2016;17:28.
341. Chapple IL, Matthews JB, Wright HJ, Scott AE, Griffiths HR, Grant MM. Ascorbate and alpha-tocopherol differentially modulate reactive oxygen species generation by neutrophils in response to FcgammaR and TLR agonists. *Innate Immun*. 2013;19:152-159.
342. Asman B, Wijkander P, Hjerpe A. Reduction of collagen degradation in experimental granulation tissue by vitamin E and selenium. *J Clin Periodontol*. 1994;21:45-47.
343. Daiya S, Sharma RK, Tewari S, Narula SC, Kumar Sehgal P. Micronutrients and superoxide dismutase in postmenopausal women with chronic periodontitis: a pilot interventional study. *J Periodontal Implant Sci*. 2014;44:207-213.
344. Hong JY, Lee JS, Choi SH, et al. A randomized, double-blind, placebo-controlled multicenter study for evaluating the effects of fixed-dose combinations of vitamin C, vitamin E, lysozyme, and carbazochrome on gingival inflammation in chronic periodontitis patients. *BMC Oral Health*. 2019;19:40.
345. Lee J, Park JC, Jung UW, et al. Improvement in periodontal healing after periodontal surgery supported by nutritional supplement drinks. *J Periodontal Implant Sci*. 2014;44:109-117.
346. Mactier H, Weaver LT. Vitamin A and preterm infants: what we know, what we don't know, and what we need to know. *Arch Dis Child Fetal Neonatal Ed*. 2005;90:F103-F108.
347. Hayek A, Djabou M, Mewton N, Bonnefoy-Cudraz E, Bochaton T. Thiamine deficiency as a cause for acute circulatory failure: an overlooked Association in Western Countries. *CJC Open*. 2020;2:716-718.
348. Hustad S, McKinley MC, McNulty H, et al. Riboflavin, flavin mononucleotide, and flavin adenine dinucleotide in human plasma and erythrocytes at baseline and after low-dose riboflavin supplementation. *Clin Chem*. 2002;48:1571-1577.
349. Paulionis LJ. Vitamin status and cognitive function in a long-term care population. *FASEB J*. 2001;15:A276.
350. Wittwer CT, Schweitzer C, Pearson J, et al. Enzymes for liberation of pantothenic acid in blood: use of plasma pantetheinase. *Am J Clin Nutr*. 1989;50:1072-1078.
351. Vollbracht C, Gundling PW, Kraft K, Friesecke I. Blood concentrations of vitamins B1, B6, B12, C and D and folate in palliative care patients: results of a cross-sectional study. *J Int Med Res*. 2019;47:6192-6205.
352. Harthe C, Claustrat B. A sensitive and practical competitive radioassay for plasma biotin. *Ann Clin Biochem*. 2003;40:259-263.
353. Galukande M, Jombwe J, Fualal J, Baingana R, Gakwaya A. Reference values for serum levels of folic acid and vitamin B12 in a young adult Ugandan population. *Afr Health Sci*. 2011;11:240-243.
354. Wahlin A, Backman L, Hultdin J, Adolfsson R, Nilsson LG. Reference values for serum levels of vitamin B12 and folic acid in a population-based sample of adults between 35 and 80 years of age. *Public Health Nutr*. 2002;5:505-511.
355. Plevin D, Galletly C. The neuropsychiatric effects of vitamin C deficiency: a systematic review. *BMC Psychiatry*. 2020;20:315.
356. Bischoff-Ferrari HA. Optimal serum 25-hydroxyvitamin D levels for multiple health outcomes. *Adv Exp Med Biol*. 2008;624:55-71.
357. Traber MG, Vitamin E. Inadequacy in humans: causes and consequences. *Adv Nutr*. 2014;5:503-514.
358. Shearer MJ. Vitamin K deficiency bleeding (VKDB) in early infancy. *Blood Rev*. 2009;23:49-59.
359. Mantle D, Dybring A. Bioavailability of coenzyme Q(10): an overview of the absorption process and subsequent metabolism. *Antioxidants (Basel)*. 2020;9:386.
360. Soeta S, Higuchi M, Yoshimura I, Itoh R, Kimura N, Aamsaki H. Effects of vitamin E on the osteoblast differentiation. *J Vet Med Sci*. 2010;72:951-957.
361. Shoukheba MYME-KS. Coenzyme Q10 food supplement on the treatment of chronic periodontitis in patients with type II diabetes mellitus: a randomized control study. *EDJ*. 2019;65:253-261.

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