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REVIEW ARTICLE

Bone regeneration in implant dentistry: Which are the factors affecting the clinical outcome?

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1 | INTRODUCTION

Bone regeneration procedures have been performed together with implant placement since the early days of implant dentistry with the aim to re-establish an adequate alveolar ridge dimension before implant placement (staged technique), or with the aim to regenerate peri-implant bone simultaneous to implant placement (simultaneous, one-stage regeneration) and allow a prosthetically driven implant-supported rehabilitation.[1,2]

A plethora of hard tissue augmentation techniques has been documented for alveolar ridge regeneration/augmentation, including guided bone regeneration (GBR), onlay grafting, combinations of onlay and interpositional grafting, distraction osteogenesis, ridge splitting, and free vascularized autografts.[2,3]

While nowadays implant survival is considered highly predictable, continuous efforts are directed to fine-tune the long-term success of implant rehabilitations and to lower the risk of complications during healing.[4] This is of relevance in cases where implant rehabilitations require a certain level of bone regeneration, as the predictability and stability of the regenerated bone may play a crucial role in the success and survival of implant rehabilitations. Despite the fact that regenerative procedures have been applied for several decades, there are still knowledge gaps and uncertainties in relation to how the type of implant surface/design and biomaterials employed, as well as different surgery-related (e.g., submerged vs. unsubmerged healing), prosthesis-related (e.g., abutment characteristics, platform switching, etc.) and patient-related (e.g., systemic health, oral hygiene, compliance, etc.) factors can impact on the expected outcomes (Figure 1).

This manuscript aims to provide a clinically oriented review that could guide clinicians in the decision-making process related to bone regeneration in implant dentistry. In particular, it will critically appraise and explore the main factors that may have an impact on peri-implant bone regeneration, trying to focus, whenever possible, specifically on bone regeneration procedures performed simultaneously to implant placement to treat dehiscences, fenestrations or for bone contouring.

2 | BIOLOGY OF BONE REGENERATION

From a biological point of view, a deep knowledge of the key steps and molecular events taking place during bone regeneration is of utmost importance to understand how different local and systemic factors may influence the regenerative process.

Our current understanding on the cascade of events taking place during bone regeneration and the key signaling pathways regulating this complex biological process has significantly improved over time thanks to a more refined selection of the experimental models and advancements in the methods of analyses employed.[5] Almost 30 years ago Schenk et al.[6] described from a histological point of view that the use of a barrier membrane can guide the formation and maturation of bone tissue in dogs' mandibular defects by recapitulating the same steps occurring during intramembranous osseous

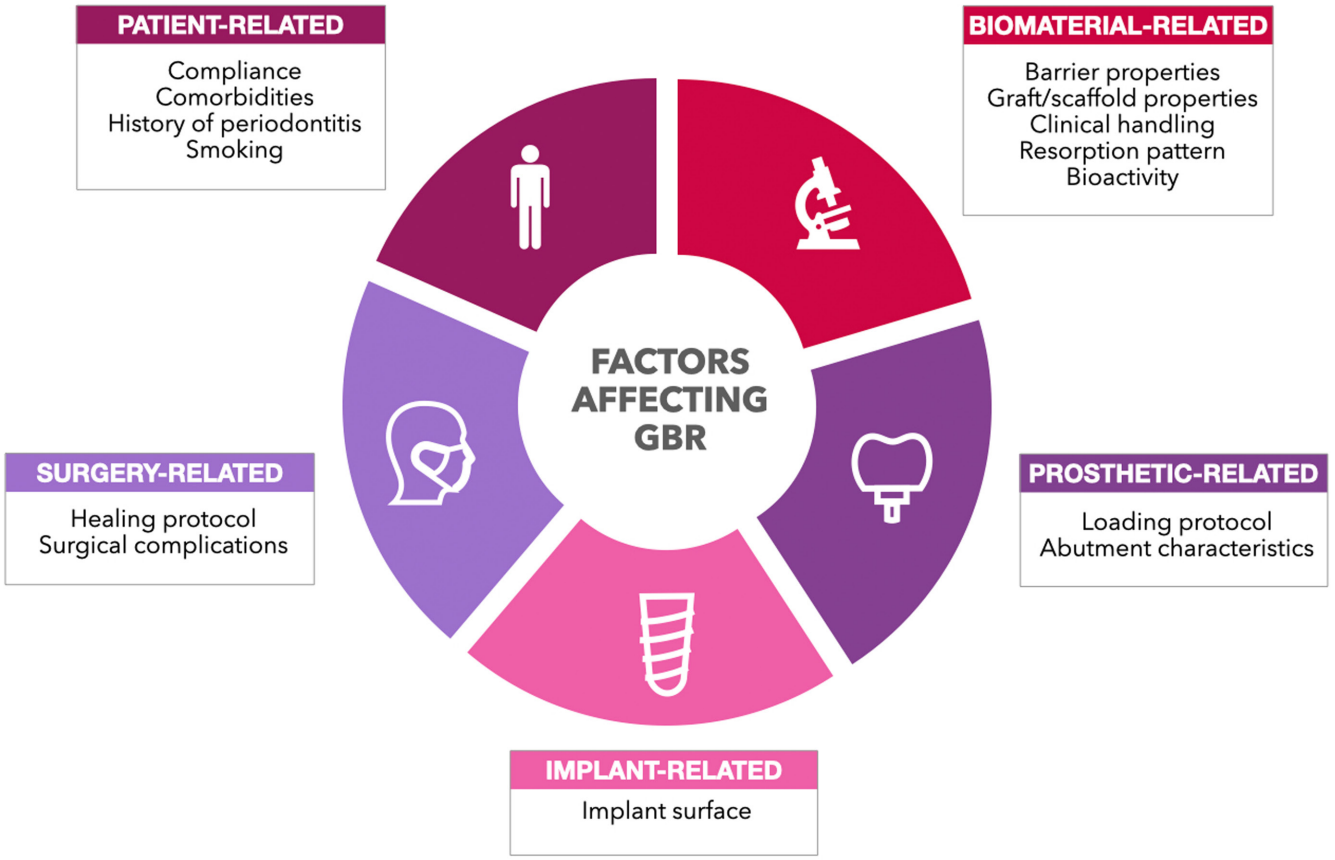

FIGURE 1 Main factors affecting guided bone regeneration.

formation. It is now clear that for bone regeneration to take place it is important to have a source of cells (osteoprogenitor cells and immune-inflammatory cells), a scaffold (the blood clot) that facilitates the deposition of the bone matrix, signaling molecules, as well as an adequate blood supply and mechanical stability to allow the maturation of the immature woven bone into mature lamellar bone (Figure 2).

In the past years, our group has made significant efforts to shed light on the biological events and signaling pathways involved in bone regeneration mainly through a series of pre-clinical studies applying the GBR principle in the critical size defect model in the rat calvarium. Since critical size defects are challenging defects that overcome the physiological threshold of bone regeneration, they have been extensively used in bone regeneration research, where a new biomaterial or technique (such as GBR) is applied in one defect, while an empty defect acts as a control. In this way, the intrinsic regenerative potential of a new material/technique can be easily tested. The calvarial critical size defect, in particular, is extensively utilized for its several advantages, related for example to the inertness of the skull,[7] the easiness of the surgical access, and the enhanced support provided to biomaterials, owing to the presence of the dura mater and overlaying skin.[8,9] Moreover, the structure of the calvarial bone allows creating standardized defects in a uniform and reproducible way,[8] thus making these defects valuable when studying/characterizing biological processes like bone regeneration.

We previously applied microarray technology to compare the gene expression profile at 7 and 14 days of healing of critical size defects treated with an intracranial expanded polytetrafluoroethylene (ePTFE) membrane and an extracranial disk made of polished titanium.[10] We selected 7 and 14 days of healing since this is a critical period in the healing process, being representative of both the early response mechanisms and of the subsequent events involved in the initiation of osteogenesis within the defect. Remarkably, at 7 days of healing, when the regenerative process was still relatively immature and the newly formed tissue scarcely organized, there was an over-representation of genes associated with cell proliferation and mitosis, as well as apoptosis. Moreover, the immune/inflammatory processes were over-represented, with an upregulation of genes associated with leucocyte and T-cell activation. Conversely, at 14 days of healing cell differentiation and a variety of developmental processes, such as anatomical structure and organ development were among the Gene Ontology categories over-represented, thus indicating a maturing wound.

We also compared the gene expression profile during GBR when a polished or micro-rough (SLA) disk was employed as extracranial barrier and e-PTFE was applied as an intracranial barrier in the same critical size defect model.[11] This experimental model could mimic the clinical situation of a dehiscence defect around a titanium implant in need of regeneration. At 7 days of healing a relatively small number of genes were differently regulated

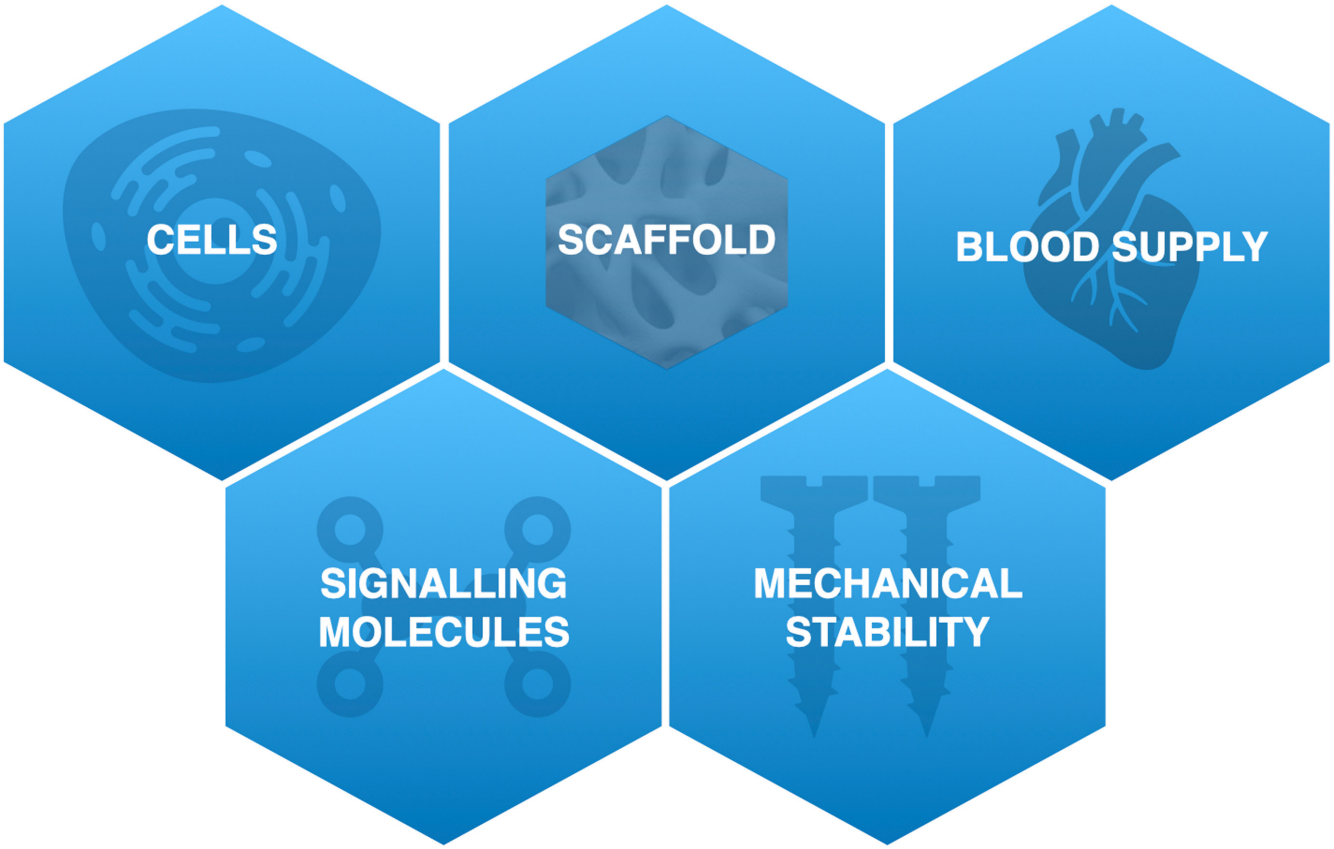

FIGURE 2 Graphical representation of the key elements needed for bone regeneration to take place.

between polished and SLA barriers, with a preponderance of genes involved in cell proliferation and immune-inflammatory processes at this early time point. Conversely, at 14 days of healing, a significant number of genes associated with the biologically relevant processes of regeneration, skeletogenesis, mesenchymal cell differentiation, angiogenesis and neurogenesis were differentially regulated between polished and SLA surfaces. These biological processes are largely controlled through the Wnt pathway, which was, therefore, identified as a key signaling pathway mediating the influence of titanium surface topography on the osseous healing cascade.

More recently, we described in parallel the histology features as well as the genes differentially expressed during the early stages of GBR performed with e-PTFE membranes.[12,13] At 7 days of healing, the defects were filled mainly by a richly vascularized granulation tissue, while at 15 days a significant amount of woven bone could be appreciated extending from the defect margin (28.3%), with a percentage of defect closure of 50.94% ± 9.23%. Similar to what previously described when using titanium discs, a clear upregulation of the immune and inflammatory responses was found at day 7 compared to 15 days, with an upregulation of specific genes such as IL6, IL1-α, IL1-β, and Ccl3. Conversely, at 15 days of healing a more complex cellular activity was evident, with an upregulation of growth factors and hormones involved in bone formation (e.g., BMP3, BMP4, FGFR2) and of Gene Ontology terms related to cell metabolism, ossification, and skeletal development.

We then explored, for the first time, the sequence of proteins and signaling pathways in critical size defects treated with a combination of intracranial and extracranial collagen membranes and deproteinized bovine bone mineral (DBBM) particulate graft.[14] At 7 days of healing the histology features indicated a preponderance of poorly organized granulation tissue associated with a proteome mainly consisting of acute phase proteins and proteins involved in the inflammatory-immune response. At 14 days, the initial maturation of the granulation tissue into woven bone (mainly at the periphery of the defect) coupled with the expression of proteins still belonging to the inflammatory-immune response (mainly proteins of the complement cascade), but we started also identifying proteins involved in angiogenesis, cell proliferation, and osteogenesis and belonging to Rap-1, HIF-1, PI3K-Akt, and TGF-β signaling pathways. At 30 days of healing, 17% of the defects were filled by bone and the proteome identified the presence of proteins involved in extracellular matrix–receptor interaction, cytoskeleton regulation, and energy transduction/ATP synthesis, as well as proteins of the MAPK, HIF-1, VEGF, and PI3K-AKT signaling pathways, thus suggesting a more mature stage of bone formation.

Remarkably, the aforementioned studies also suggested that the type of biomaterials used (type of barriers and grafts), may significantly influence the regenerative outcome by differently modulating the underlying molecular events and signaling pathways.

It is important to highlight that animal models like the critical size defect model in the rat calvarium have important limitations and

should be mainly used for proof of principle studies or to investigate biological processes. Whenever the outcomes of an experimental project have to be translated into the clinical setting, larger animal models or properly designed clinical studies are needed.

3 | THE IMPACT OF SURGERY-RELATED FACTORS

3.1 | Submerged versus non-submerged healing

To minimize the risk of fibrous integration and microbiological contamination due to implant micromovements during osseointegration, a submerged healing protocol was introduced in the early days of implant dentistry.[15] However, in the past decades the development of one-piece implants and non-submerged implant protocols have suggested the possibility of eliminating the second stage surgery, with the aim of preventing also the coronal migration of the mucogingival junction that might be seen in submerged implants.[16] A number of studies indicated minimal differences in short- and moderate-term healing outcomes, as well as crestal bone level changes between submerged versus non-submerged implants placed without GBR.[17–19] Fiorellini et al.[20] demonstrated radiographically that the crestal bone loss was not influenced by that surgical technique (i.e., submerged or non-submerged implants), and similar findings were also reported in a histological analysis by Weber et al.[21] and Hermann et al.[17]

While the aforementioned studies focused on implants placed in pristine bone, different considerations should be made when bone regeneration concomitant to implant placement is required, since non-submerged healing may increase the risk of contamination of the inserted biomaterial due to the inability to attain complete primary closure following surgical implant placement. Moreover, one hypothesis suggests that the implant shoulder in submerged implants might support and stabilize the membrane and overlying flap, leading to better space maintenance, hence improving outcomes.[22] Moreover, in submerged implants the membrane is usually placed over the defect, extending over the implant and across the palatal/lingual surface. This might even result in an enhanced barrier effect function of the membrane.

An early canine study comparing bone regeneration around implants with e-PTFE membranes in case of submerged compared to non-submerged healing showed that implant osseointegration and bone regeneration occurred in both instances.[23] However, due to numerous membrane exposures in the non-submerged implant group, it was recommended that even non-submerged implant designs (i.e., tissue level) should be placed with a submerged protocol when used along with ePTFE membranes.

In a similar study in beagle dogs, posterior teeth were extracted and after a 3-month healing period, standardized buccal dehiscence defects were surgically created following implant site preparation.[22] Implants were then assigned to either the submerged or the non-submerged healing protocol. In histological sections, it was noted that submerged implants had a higher percentage of bone fill, new bone height, and bone-to-implant contact compared to non-submerged implants. Remarkably, submerged implants had higher bone fill in the central aspect of the dehiscence defect area, hence suggesting better space maintenance in this area when following this protocol. Conversely, in a subsequent study in dogs, minimal differences in the healing outcomes were reported for immediate implants placed along with GBR using either the submerged or the non-submerged protocol.[24]

It should be noted that in the majority of the described studies, GBR was performed by combining a particulate DBBM graft with a porcine-resorbable collagen membrane.

If we evaluate the clinical evidence on the impact of the healing protocol on bone regeneration, only limited studies are available in the literature. The initial attempts to promote transmucosal healing in patients undergoing GBR were performed in post-extraction immediate implants and employed non-resorbable ePTFE membranes.[25–27] Hence, despite the fact that implantation and bone regeneration were combined into one surgical procedure, a second surgery was still necessary for the removal of the barrier. Subsequently, Hammerle and Lang[28] were amongst the first to document the successful regeneration of peri-implant bone defects in type II implant placement by applying bioresorbable materials in conjunction with transmucosal healing.

More recently, an RCT compared submerged versus transmucosal implant healing in non-molar cases of lateral GBR simultaneous to immediate implant placement. At up to 5 years of follow-up, similar radiographic crestal bone loss was observed in the two groups.[29] Moreover, peri-implant parameters such as probing depth, clinical attachment level and bleeding on probing were similar in both groups and patient satisfaction was good or excellent for over 90% of the subjects regardless of the groups. Conversely, a controlled clinical trial showed increased PPD and CAL for immediate implants placed in molar extraction sites with buccal dehiscences treated with a graft and a barrier and left for unsubmerged healing, as compared to implants placed in healed sites.[30]

In summary, the majority of the clinical evidence on transmucosal healing is available for immediate implants and it seems to suggest that primary closure of the site of implantation and regeneration is not an absolute prerequisite for successful treatment outcomes. However, studies reporting on transmucosal healing protocols stress the importance of meticulous plaque control during the regeneration period to achieve predictable outcomes.[31] Further research, namely long-term RCTs, is warranted to assess the differences in healing between submerged and non-submerged implant placement protocols associated with bone regeneration and the long-term stability of the regenerated bone.

3.2 | Surgical complications

Despite bone regeneration procedures performed simultaneously to implant placement are predictable and well-documented, they are also relatively technique-sensitive. As such, it is not uncommon

to experience post-surgical complications, which mainly include soft tissue dehiscence, exposure of membranes/biomaterials, and infection. Understanding and minimizing the impact of such complications becomes of crucial importance to ensure predictable regenerative outcomes.

One of the first reviews on lateral augmentation indicated that the most frequently reported complications in controlled studies on GBR for lateral ridge augmentation were per-implant mucosal problems, including redness, hyperplasia, suppuration, pain, and swelling.[2] More recently, a systematic review focusing on GBR-based lateral augmentation procedures performed concomitant or before implant placement and including 15 studies (both prospective and retrospective) indicated that overall the weighted rate of soft tissue complications (including membrane exposure, soft tissue dehiscence, and acute infection) was 16.8%, with no significant differences between resorbable and non-resorbable membranes.[32] When focusing on lateral augmentation simultaneous to implant placement, another systematic review by Thoma et al.[33] indicated a slightly higher mean complication rate of 20.8% at 18 months based on 144 implant sites, but in their analyses they included not only implant exposure, barrier exposure and soft tissue dehiscence, but also peri-implantitis, recurrent bone loss and bone loss >1.5 mm. Interestingly, when looking at longer follow-ups (mean 56.8 months), they indicated a similar complication rate between ePTFE and non-cross-linked collagen membranes (13.9% and 13.6%, respectively), but a higher complication rate for cross-linked collagen membranes (44.4%).

More recently, a systematic review on lateral bone augmentation reported a site-level weighted mean incidence of minor wound dehiscence and minor infections at the augmented sites of 9.9% and 1.5%, respectively.[34] At patient level, the incidence of minor and major complications was 16.1% and 1.6%, respectively. The review also confirmed a non-significant difference in the incidence of complications when dealing with resorbable vs. non-resorbable barriers. Remarkably, sub-group analysis indicated a higher site-level incidence of minor infections (4.2%) for staged GBR compared to simultaneous GBR.

When comparing non-cross-linked to cross-linked membranes for simultaneous augmentation, few studies reported a tendency for higher complications (exposure and risk of infection) with the latter type of membranes.[35–37] One study was also terminated earlier than anticipated because of unacceptable safety issues and severe infections related to the use of a cross-linked membrane, which exposed in 56% of the cases and in 33% of the cases it was associated with infection and therefore needed to be removed.[36]

While it is beside the remit of this review to discuss the management of post-surgical complications (refer to[38]), the impact that such adverse events may have on the regenerative outcome deserves some consideration.

When focusing on peri-implant dehiscence sites, a meta-analysis of five studies indicated a 27% higher defect reduction when membrane exposure did not occur.[39]

This confirms an older review by Machtei[40] based only on two articles on the effect of early membrane exposure on guided tissue and bone regeneration, which indicated a six-time greater difference in bone gain if membrane exposure did not occur during the early healing period. Likewise, another review looking at both simultaneous and staged regeneration indicated that in both scenarios non-exposed sites gained significantly more bone (weighted mean difference of 1.1 and 3.1 mm, respectively).[41]

Obviously, the risk of tissue dehiscence and biomaterial exposure can be more critical when using materials more prone to become contaminated by the oral microbiota.[39,42] For instance, studies have shown that intentionally exposed high-density polytetrafluoroethylene (d-PTFE) membranes for socket preservation[43] and GBR procedures[44,45] may not compromise the regenerative outcomes, as the small pore size (around 0.2 μm) of d-PTFE provides superior resistance to bacterial colonization as compared to the larger pore size that can be found in e-PTFE membranes (around 25 μm). Moreover, exposed e-PTFE barriers showed a more favourable outcome as compared to exposed synthetic resorbable membranes.[33]

A clinical classification of healing complications in GBR procedures performed with e-PTFE membranes was introduced in 2011 to guide clinicians in treatment decisions, with the aim to obtain more predictable outcomes (Table 1).[46] Briefly, the most common and "predictable" complication is premature membrane exposure and, according to the authors, treatment depends on the presence or absence of purulent exudate and the extent of the soft tissue dehiscence. If a ≤3 mm exposure occurs without purulent exudate within the first 2 months, the suggested approach is to leave the membrane in place for a maximum period of 1 month, use topic antiseptics and follow up the patient weekly. As an alternative, the small exposed membrane could be removed and the dehiscence closed with a connective tissue graft or by suturing. In case of large (>3 mm) exposure, the membrane must be removed immediately even if no purulent exudate is present, in order to avoid infection of the regenerating tissues. In case the membrane exposure is associated with a purulent exudate, the barrier must be removed immediately to limit the damage caused by the infection spreading to the underlying regenerating tissue and antibiotic therapy should be prescribed. After removal, a gentle curettage of the graft is also essential to remove the infected particles and inflammatory tissue that could jeopardize the regenerative process. If an abscess is observed, local antibiotic wash and systemic antibiotic should also be considered.

More recently, Vroom et al.[47] adapted this classification to d-PTFE membranes to account for the structural differences between these two barriers (mainly in terms of bacterial permeability), which should reflect also on a different management of surgical complications (Table 1). In summary, the authors suggest controlling with topical antiseptics d-PTFE membrane exposures whenever there is no purulent exudate and edges of the membrane are covered by tissue, while immediate removal and vigorous irrigation to remove any involved graft is recommended when there is membrane exposure with a purulent exudate or when there is an abscess/fistula without membrane exposure.

TABLE 1 Classification of healing complications in GBR procedures performed with e-PTFE membranes[46] or d-PTFE membranes.[47]

Healing complications for GBR procedures performed with e-PTFE membranes	
Healing complications	**Surgical complications**
Class I: small membrane exposure (≤3 mm) without purulent exudate	A. Flap damage
Class II: large membrane exposure (>3 mm) without purulent exudate	B. Neurologic complications
Class III: membrane exposure with purulent exudate	C. Vascular complications
Class IV: abscess formation without membrane exposure	
Healing complications for GBR procedures performed with d-PTFE membranes	
Class I	a = Membrane exposure without purulent exudate
	b = Edges of the membrane covered by tissue (E+) or not (E−)
	c = Time of exposure (*T*) (measured in number of days post-operation)
Class II	a = Membrane exposure with purulent exudate
	b = Time of exposure (*T*) (measured in number of days post-operation)
Class III	a = No membrane exposure but the presence of an abscess and/or fistula
	b = Time of presence of an abscess and or fistula (*T*) (measured in number of days post-operation)

Abbreviations: d-PTFE, high-density polytetrafluoroaethylene; e-PETE, expanded polytetrafluoroaethylene; GBR, guided bone regeneration.

As compared to non-resorbable membranes, resorbable collagen membranes exposed in the oral cavity are usually easier in terms of management and in situ maintenance (which is usually performed with the use of topic antiseptics), however, they rapidly degrade and lose their integrity (even in case of cross-linking), thus inevitably leading to an increased risk of compromised bone regeneration.[48]

Interestingly, out of three studies that performed a histological assessment of the healed bone in cases that had shown graft exposure or soft tissue dehiscence, two indicated no differences in terms of bone quality and no granulocytic infiltration regardless of the barrier (collagen-based or e-PTFE).[49,50] Conversely, in one study the sites presenting membrane exposure (both in the case of cross-linked and non-cross-linked collagen membranes) showed lower remodeling rates, with some sites displaying missing or minimal osteogenesis and the graft material (biphasic calcium phosphate) covered by dense collagen tissue populated by multinucleated cells.[51]

Based on the aforementioned, it can be concluded that healing for primary intention is a crucial aspect for the success of bone regenerative procedures. As such, clinicians should pay particular attention in controlling all those factors that can increase the risk of soft tissue dehiscence, which include tissue inflammation, thickness of the flap, and flap design (including adequate flap release).[38,52] Operator's experience and manual skill, as well as patients' compliance with the pre and postoperative instructions, are other key factors that can significantly impact on the incidence of post-surgical complications.

4 | THE IMPACT OF IMPLANT SURFACE

Over the past years, extensive research has been performed on the development of titanium implants with modified surface properties (such as topography, porosity, wettability, surface charge, and chemistry), essentially with the aim to improve osseointegration and shorten healing times.[53,54] In fact, it has been well documented that surface properties and chemistry of implants directly influence the binding capacity of fibrin and the adhesion, proliferation, and differentiation of cells, thus affecting the overall process of osseointegration.[55–57] In particular, moderately rough, hydrophilic surfaces have shown faster osseointegration in comparison to hydrophobic surfaces both in pre-clinical and clinical studies,[58–60] although after 4 weeks of healing the outcomes are comparable between the two surfaces. Remarkably, titanium surface topography and chemistry have also shown to influence the proteomic profile released by platelets, which can subsequently influence macrophage pro-inflammatory cytokine expression. More specifically, hydrophilic surfaces are able to elicit a macrophage phenotype associated with reduced inflammation and enhanced pro-osteogenic signaling (M2).[61,62]

Different types of modified titanium surfaces have been tested for their ability to promote new bone formation in bone defects created around implants.

Studies in dog models showed that hydroxyapatite-coated implants promoted better bone-to-implant contact (BIC) in the regenerated bone as compared to pure titanium,[63] plasma-sprayed (TPS), and acid-etched surfaces[64] at 4 months of healing. In a study by Lima et al.,[65] when comparing pristine bone to regenerated bone, the fraction of implant-bone integration was always higher in pristine bone, but TPS surfaces positively influenced the fraction of osseointegration in comparison to machined surfaces for both regenerated and pristine bone.

Schwarz et al.[66] analyzed histometrically the healing of untreated (no membrane employed) acute dehiscence defects around submerged micro-rough hydrophilic (SLActive) and hydrophobic (SLA) implants placed in beagle dogs. At 12 weeks of healing, SLActive implants showed significant new bone formation and osseointegration

in the defect area, while around SLA implants there was a predominance of dense connective tissue. In order to assess if this result had been influenced by the submerged healing of the implant, bone regeneration was subsequently tested in standardized dehiscence defects created around SLA and SLActive implants following either a submerged or non-submerged healing protocol.[22] While SLA implants had inferior performance in both protocols, within the SLActive implants the submerged ones showed the highest level of bone regeneration. According to these results, it was concluded that SLActive implants support bone regeneration in acute dehiscence defects and submerged healing patterns, even in the absence of a barrier membrane. A later study by the same group corroborated this finding when comparing acute dehiscence-type defects created around SLActive implants and around implants with dual acid-etched surfaces with a calcium phosphate nanometer particle modification (DCD/CaP). After 2 and 8 weeks of submerged healing, both surfaces promoted similar bone fill, but SLActive implants showed significantly higher new bone height.[67] Our group[68] confirmed that hydrophilic titanium surfaces support significant bone formation in combined chronic and acute dehiscence-type defects, even when no graft/GBR is applied. This study also indicated that loading of SLActive implants inserted in dehiscence sites, treated or not by grafting/GBR, resulted in a tendency toward an increased BIC.[68]

In another preclinical study, our group also showed similar BIC in case hydrophilic implants were immediately placed and immediately loaded or loaded at 4 weeks (delayed loading).[69]

In order to understand the biological mechanisms behind the effect of implant surface on osseous formation, we investigated with different omics technologies (transcriptomics and proteomics) the genes/proteins and signaling pathways differentially regulated by SLA and SLActive surfaces during osseointegration and bone regeneration and we showed that hydrophilic surfaces are able to downregulate the initial inflammatory response and to promote an earlier expression of pathways involved in cell proliferation, osteogenesis, and angiogenesis.[70–72] In particular, when combining gene and protein expression outputs, our data identified 7 days as the most critical time-point accounting for the enhanced pro-osteogenesis properties of hydrophilic compared to hydrophobic surfaces.

When looking at the clinical evidence, it is often difficult to isolate the effect of implant surface on implant outcomes and the stability of peri-implant bone regeneration, since implants do not only differ in surface topography but also implant design, prosthetic connection and loading protocol. A systematic review evaluating the effect of implant surface roughness on long-term bone loss suggested that peri-implant bone loss around minimally rough implant systems was significantly less in comparison to moderately rough and rough implant systems.[73] However, the review did not specify if and in how many cases bone regeneration was performed simultaneously to implant placement, so it is not possible to comment on the impact of implant surface in cases where bone regeneration is performed.

In summary, implant surface-related properties may have a significant impact and directly influence the regeneration of peri-implant bone defects. Fine-tuning implant surface properties is therefore likely to further enhance bone regeneration in the future and might be a particularly valuable option when dealing with challenging clinical scenarios.

5 | THE IMPACT OF BIOMATERIALS

Barrier membranes and bone grafts/substitutes are still the preferred regenerative materials for bone regeneration in implant dentistry.[1] An overview on the impact that their different properties and characteristics can play on bone regenerative outcomes is herein presented.

5.1 | Barrier membranes

The principle of guided bone regeneration (GBR) is based on the use of an occlusive barrier membrane with the aim to create a secluded space around a bone defect and facilitate the recruitment and proliferation of osteoprogenitor cells from the marrow spaces directly into the defect while preventing the downgrowth of the neighboring soft tissues.[74] The composition as well as the physical and mechanical properties of barriers can obviously influence the regenerative outcomes of peri-implant bone defects. Table 2 summarizes the main advantages and disadvantages associated with the different types of membranes available for GBR.

5.1.1 | Occlusiveness/porosity

An ideal level of occlusiveness and porosity enabling the membrane to act as an effective barrier but at the same time allowing the passage of nutrients, fluids, oxygen, and bioactive substances for cell growth has not been clearly defined. Commercially available membranes present a wide variability in the pore size and degree of permeability, ranging from micro-porosity (5–20 μm), which may limit the passage of cells but allows the passage of chemicals, biomolecules and viruses; moderate porosity (non-resorbable materials ≤100 μm) that allows the passage of bacteria, cells and tissue integration/migration; or macro-porosity (non-resorbable materials >100 μm), which allows the unrestricted passage of chemicals, biomolecules, viruses, bacteria, cells and promotes tissue integration and migration.[75]

The impact of membrane porosity on regenerative outcomes has been mainly tested at pre-clinical level through different bone regeneration models.

Non-resorbable barriers

Zellin and Linde[76] evaluated e-PTFE membranes with different degrees of porosity (8, 20–25 and 100 μm) to achieve GBR in calvarial

TABLE 2 Summary of the main advantages and disadvantages associated with the different types of barriers and bone grafts available.

	Advantages	Disadvantages
Barriers		
Native collagen	Biocompatible, biologically active, easier to handle, cell occlusion/porosity, biodegradable (no need for second surgery)	No space maintenance (collapse), resorption time might be fast
Cross-linked collagen		Cross-linking may impair tissue integration and angiogenesis
Polymeric		Inflammatory foreign-body reactions associated with their degradation products
Non-resorbable (e-PTFE, d-PTFE, Ti-d-PTFE; mesh)	Biocompatible, biologically active, space-making, cell-occlusive	Clinical handling, need for a second surgery, increased susceptibility to complications when exposed (mainly e-PTFE and mesh)
Bone grafts		
Autografts	Osteogenic (mainly cancellous bone), osteoinductive, osteoconductive, no immune reaction	Need for additional surgery, increased operative time, limited quantity, donor site morbidity (infection, pain, cosmetic)
Allografts	Osteoinductive (mainly DFDBA), osteoconductive, unlimited quantity, no donor site morbidity, shorter surgical time	Risk of rejection and disease transfer (mainly fresh frozen bone), ethical and religious concerns
Xenografts	Osteoconductive, unlimited quantity, no donor site morbidity, shorter surgical time	Risk of disease transmission, ethical and religious concerns, no osteoinductive properties, may remain in the defect for years
Alloplastic materials	Osteoconductive, unlimited quantity, no donor site morbidity, shorter surgical time	No osteoinductive properties, may remain in the defect for years

Abbreviations: DFDBA, demineralized freeze-dried bone allograft; d-PTFE, high-density polytetrafluoroaethylene; e-PTFE, expanded polytetrafluoroaethylene; Ti-d-PTFE, titanium-reinforced high-density polytetrafluoroaethylene.

defects in rats. Their results at 6 weeks of healing showed that the amount of soft tissue invasion was proportional to the increasing perforation size and that a membrane porosity in the range of 25–100 μm promoted enhanced bone formation in the early phases of bone healing, while the material with the smallest internodal distance did not integrate well with the surrounding tissues.

A couple of years later another study tested the influence of different porosities on GBR using a stiff plastic plate as a solid or occlusive membrane and polyester meshes with different porosities (10, 25, 50, 75, 100 and 300 μm).[77] While a slow rate of bone formation was associated with the totally occlusive barrier, polyester meshes with perforations exceeding 10 μm resulted in a faster rate of bone augmentation as compared with 10-μm meshes.

Our group has recently shown that while in healthy conditions an occlusive membrane (e-PTFE) compared to a perforated membrane enhanced the regeneration of calvarial critical size defects, in uncontrolled diabetic conditions a perforated barrier improved the outcomes as compared to an occlusive membrane.[78] A possible explanation of this unexpected result in the uncontrolled diabetic group might rely on the fact that the perforated membrane allowed the contribution of undifferentiated mesenchymal cells coming from subcutaneous connective tissues, periosteum, and dura mater. This pool of cells promoted and triggered the bone regeneration cascade, thus partially reversing the impaired recruitment and homing of inflammatory and mesenchymal cell populations typical of uncontrolled diabetes.

However, it is also important to point out that the presence of pores with a size of 5–30 μm that we typically find in e-PTFE membranes has been reported to facilitate bacterial contamination.[79,80] This is the reason why a high-density (d)-PTFE with a submicron (0.2 μm) pore size was later developed to avoid the migration of bacteria into the membrane structure.[81,82] However, d-PTFE membranes have the important drawback to show minimal tissue integration as they do not allow fluids and nutrients from the overlying periosteal vessels to pass through, thus creating potential problems for initial clot formation, wound stabilization, and membrane stability.[83,84] As a consequence, when d-PTFE membranes are used it is advisable to perform multiple perforations of the cortical bone in order to enhance blood supply in the augmented site.[85]

Resorbable barriers

Owing to the need of a second-stage surgery and the more complicated management in case of exposure, resorbable barriers have gained increased popularity in the past years.

Amongst them, collagen-based and polymeric membranes are by far the most widely employed. With the aim to improve membrane adaptation and integration to the defect sites and enhance the regenerative outcome, bilayered resorbable membranes (collagenic or polymeric) have been developed, which present one compact layer that is able to prevent infiltration of epithelial cells into the bone defect (facing the bone) and a second, porous, spongy layer that promotes tissue integration (facing the soft tissue).[84]

Interestingly, it has been shown that the surface properties of collagen materials, such as particle size, porosity, and the released ions may be able to modulate the recruitment and polarization of macrophages, which play a pivotal role during bone regeneration based on their polarization into either proinflammatory or anti-inflammatory phenotypes.[86]

Recently, Shim et al.[87] suggested that 3D-printed polycaprolactone barrier membranes with 30% porosity (130 μm pore size) had the best ability to form new bone compared with membranes with 50% or 70% porosity.

In addition to the porosity, the three-dimensional topography of the membrane with its interconnecting pores and channels is also important, as it can modulate cell occlusive properties and the biological response.[88]

Remarkably, with the introduction of more sophisticated manufacturing processes, there is now the possibility to modulate the characteristics of the membranes and create even gradients of porosity. For instance, Oh et al.[89] developed a porous polycaprolactone (PCL)/Pluronic F127 membrane with an asymmetric column-shape pore structure, where the top surface had nanosized pores (100 nm) to prevent infiltration of fibrous tissue but to allow permeation of nutrients, while the bottom surface had microsize pores (100 μm) to improve adhesion to the surrounding bone tissue. By immobilizing BMP-2 in such a membrane and by applying low-intensity pulsed ultrasound, the same authors showed the possibility to significantly enhance the regeneration of critical size defects in rats.[90]

Likewise, a three-dimensional, porous reduced graphene oxide/hydroxyapatite (3D rGO/HA) membrane with two different sides was recently fabricated by a two-step electrochemical method and successfully tested for the regeneration of calvarial defects. The side of this composite membrane facing the bone defect was formed by 3D porous rGO with HA deposited on the frame of its 3D structure to promote osteogenic differentiation of osteoblasts, whereas the other side of the membrane presented a dense 2D rGO surface to prevent the invasion of the gingival epithelium and promote soft tissue growth.[91]

In conclusion, there is no consensus on what is the right balance between membrane porosity and occlusiveness to promote bone regeneration. However, considering the complex milieu in which osseous formation takes place, it is likely that a membrane with a biomimetic porous structure and a porosity gradient, rather than a definite pore size, might have the greatest potential in GBR.

5.1.2 | Stabilization

Stabilization of the blood clot is a prerequisite for bone regeneration to take place. It is known that micromovements between bone and any implanted material prevent bone formation, resulting in the development of fibrous tissue.[92,93] As such, the stability and immobilization of the membrane (and underlying bone graft) becomes of crucial importance, while maintaining the defect space. In order to maximize membrane stability when performing GBR simultaneous to implant placement a variety of stabilization methods have been suggested, including fixation screws,[94] non-resorbable pins[95] or titanium pins,[96] whereas some studies simply indicated that the membranes were tucked under the flaps.[97,98]

Non-resorbable membranes

A clinical controlled trial indicated similar vertical dehiscence and defect width reduction when an e-PTFE membrane alone or combined with allograft particles mixed with tetracycline were employed, thus stressing the importance of space provision for a successful GBR.[99] In particular, in that study space provision was ensured by either inserting the membrane between the bone and the periosteum or by stabilizing the membrane with sutures or with the help of the cover screw.

Remarkably, an RCT comparing a resorbable collagen membrane to an e-PTFE membrane associated with DBBM for the regeneration of peri-implant dehiscence defects concomitant to implant placement clearly indicated that membrane stabilization rather than the type of biomaterials used played a major role in the number of post-operative complications.[100] More specifically, in cases where primary barrier fixation was performed with polylactic acid pins, 63.6% of the sites healed uneventfully, as compared to only 28.6% of sites where the membrane was only secured with the implant cover screw and/or by adapting the membrane beneath the flap.

Resorbable membranes

A pre-clinical study in dogs indicated that the stabilization of poly-l-lactic acid (PLLA) membranes with fixation pins significantly increased the amount of bone regeneration in alveolar ridge defects as compared to PLLA membranes alone.[101]

The fixation of the barrier can also help maintain the underlying graft (particularly particulate grafts) in the desired site and position. An in vitro study on pig mandibles where 20 peri-implant box-shaped defects were treated according to the GBR principle showed that wound closure induces a considerable displacement of DBBM resulting in a partial collapse of the collagen membrane.[102,103] However, the stability of the bone substitute and collagen membrane can be enhanced by the application of fixation pins and by the use of a block bone substitute instead of a particulate graft.

More recently, in a pre-clinical study in box-shaped defects, Park et al.[104] evaluated two different types of collagen membranes applied together with DBBM, which were either unfixed or fixed with six mini screws. While membrane fixation made no difference to the overall volume stability of the grafted sites, the type of collagen membrane employed significantly affected the GBR outcomes, particularly in cases where the membrane was not fixed. More specifically, in the case of a uniform, non-cross-linked collagen membrane derived from porcine tendon, the use of fixation screws improved the augmented tissue width (2.3 ± 0.1 mm vs. 1.57 ± 0.27 mm).

A possible drawback when using certain fixation system is the risk of perforating important anatomical structures (like adjacent teeth, nerves, and sinus membrane), which could be avoided by the use of periosteal sutures.[105]

In conclusion, securing the stability of barrier membranes and the underlying grafting materials/blood clot plays a crucial role in the success of GBR procedures. While there is no guideline on how to reach such an outcome (whether with pins, screws or sutures, or simply adapting the membrane under the flap), clinical experience suggests that the clinician should make a decision based on the defect anatomy, location, and biomechanical properties of the biomaterials used.

5.1.3 | Resorption pattern

An ideal membrane should gradually resorb over time while bone forms and matures and its degradation products should not jeopardize the regeneration process.[75] While there is evidence suggesting that premature exposure of membranes to the oral cavity, as well as premature retrieval or resorption of the membranes, could have a detrimental effect on bone regeneration,[42,106–112] no clear indications on the minimum and maximum resorption time are available, nor on the ideal length the barrier effect should last for.

Despite superior mechanical properties and good compatibility, it is clear that non-resorbable membranes (e-PTFE, d-PTFE, and metal barriers/meshes) present the important drawback of always requiring a second-stage surgery in order to be removed, which extends the overall treatment time, increases patient morbidity and poses risks for biological complications.[79,113,114] As such, resorbable barriers were introduced as second-generation devices, which mainly include collagen-derived and polymeric barriers.

While collagen membranes present excellent biocompatibility, chemotactic properties, and their degradation does not exert any potential deleterious effect on the bone tissue, their lack of rigidity and limited space-making capability often require their combination with a space-making bone graft. Moreover, there is no clear evidence on their degradation time, which may negatively reflect on their occlusive properties. Our group assessed the degradation pattern of a collagen membrane associated with a particulate graft in a pre-clinical model and showed that at 30 days the membranes were significantly reduced in thickness and they presented a diffuse infiltration by vessels and immature woven bone.[115] While in this specific animal model, the early loss of membrane integrity and occlusiveness resulted in the promotion of bone formation, this may not necessarily translate to other animal/human models.

Different methods of chemical cross-linking have been tested to improve the mechanical properties and collagen matrix stability, thus slowing the collagen degradation rate. Although clinical studies support the use of cross-linked membranes for the regeneration of peri-implant bone dehiscence defects with stable long-term outcomes,[116–118] cross-linked membranes have also shown to impair bone-forming cell response and tissue integration[119] and they are associated with a higher incidence of premature exposure, which may impair soft tissue healing or even cause wound infection.[36,120]

A sugar cross-linked collagen membrane was introduced in 2002 for lateral augmentation around implants, with similar outcomes to e-PTFE membranes.[49] Since then, several studies supported its use for lateral bone augmentation in association with different types of grafts.[37,121,122]

A number of membranes based on synthetic polymers, such as polylactic acid (PLA), polyglycolic acid (PGA), or polyethylene glycol (PEG) have also been successfully documented for GBR simultaneous to implant placement.[95,123] However, during their degradation process, these synthetic polymers might elicit a significant inflammatory response that could, in return, negatively influence the regeneration outcomes.[124] Our group suggested to pay particular attention on the manipulation and surgical use of PEG membranes, which could lead to early rupture of the barrier, with a negative impact on the healing outcome.[68]

Overall, when comparing the performance of resorbable vs. non-resorbable membranes for lateral augmentation simultaneous to implant placement, both types of membranes have shown to promote successful regeneration and resolution of the defects, as assessed at re-entry surgeries and in terms of stability of the radiographic regenerated bone.[33,125] In a study by Basler et al.,[126] the use of a resorbable collagen membrane compared to an e-PTFE membrane associated with DBBM led to a slightly higher volume loss at 1 year, but the outcomes were comparable between the two groups at 3 years. Regardless of the membrane adopted, a minimal, but continuous decrease in the buccal contour between the insertion of the final reconstruction and 3 years of follow-up was also observed. These results are in line with previous evidence of non-statistically significant differences in terms of defect resolution when applying a resorbable compared to a non-resorbable membrane.[100,127]

5.1.4 | Bioactivity

In the past years, increasing and convincing evidence has shown that membranes do not simply work as barriers to prevent the migration of undesired cells, but they also behave as bioactive compartments that directly promote the biological events underpinning bone formation.[128] Several pre-clinical studies indicated that both resorbable and non-resorbable membranes are able to promote and direct the regenerative process by virtue of hosting cells that express and secrete pro-osteogenic and bone-promoting factors.[13,115,129]

While research is still at the experimental stage in this respect, it opens stimulating scenarios for the future, with the possibility to direct efforts in manufacturing membranes with different bioactive properties that overcome challenging clinical scenarios and promote bone regeneration also in systemically compromised patients. The incorporation of biological cues and antibacterial agents within the membrane follows this direction and has shown promising in vivo results.[128]

A suggestive field of research relates also to the possibility of developing immune-mediated collagen membranes that can potentially regulate the behavior of macrophages, including the recruitment, polarization, and the cytokines secreted by different phenotypes

during every stage of the healing process.[130,131] In other words, by modifying surface properties, particle size, porosity, and the released ions, an ideal immune-mediated collagen membrane could promote anti-inflammatory M2-type macrophages and the secretion of pro-regenerative cytokines with the ability of preventing migration of the epithelium and maintaining space for bone ingrowth.[86]

5.1.5 | One-layer versus two-layer membranes

The use of a double-layer membrane has been proposed with the aim to further enhance the membrane barrier effect and possibly increase the stability of the underlying graft, particularly in case of resorbable collagen membranes. While one layer of collagen membrane is often sufficient to promote bone regeneration,[132,133] the double layer technique has shown to delay the resorption time, thus prolonging the barrier effect of the material.[134]

The possible clinical advantage of using double-layer membranes for staged ridge augmentation in patients undergoing implant rehabilitations was first suggested by Buser et al.[135,136] In particular, they covered sites horizontally grafted by bone blocks with a DBBM particulate graft and then applied a double-layer collagen membrane.[135] This technique allowed a better protection of the graft and increased the stability of the membrane, leading to a reduction of only 7% of the total width of the graft after 6 months. Cordaro et al.[137] also confirmed that the use of DBBM and a double layer of collagen membrane around and over a mandibular bone block graft placed for lateral ridge augmentation could minimize graft resorption during healing. However, the use of bone substitutes and barrier membranes in combination with block grafts increased the frequency of complications and the difficulty of their management.

Pre-clinical studies also indicated an advantage when either a double-layer collagen membrane or a d-PTFE membrane covered by a collagen membrane was used for the preservation of grafted alveolar ridges.[138,139] On the contrary, a clinical study failed to show differences in terms of preservation of horizontal and vertical dimensions of the alveolar socket when using single- or double-layer collagen membranes.[140]

Only limited studies assessed the use of double-layer collagen membranes for the treatment of peri-implant dehiscences simultaneous to implant placement. In particular, one study compared FDBA to a combination of DBBM and autograft covered by a double layer of collagen membranes and it indicated similar clinical and radiographic outcomes at 1 year post-loading, although in both cases CBCT analysis suggested a continuous reduction in the augmented ridge dimension over time.[141,142]

In summary, since the introduction of GBR more than 30 years ago, barrier membranes have considerably evolved in terms of properties, composition, and biological activity, although a barrier membrane with ideal characteristics is still not available.

Different barrier-related characteristics can modulate the regenerative outcomes when used for the treatment of peri-implant bone defects. Future efforts in the development of GBR membranes should consider fine-tuning of degradation time, controlling the plasticity/rigidity according to the clinical needs, modulation of the inflammatory response, commercial availability in different size and shape to fit different clinical scenarios, promotion of bone regeneration, safety, non-toxicity and non-immunogenicity, predictable thickness and flexibility for the controlled cell/molecule invasion, antimicrobial features and incorporation of biological cues.[75]

Considering the complexity of the wound healing milieu, it is becoming increasingly evident that future efforts should be directed to the development of barriers with different gradient properties. Functionally graded membranes (FGMs) can be designed in such a way that they offer a gradual transition of their components (e.g., microstructure and/or composition) along their structure, thus conferring them different regional features and properties.[143] In the future, FGMs are likely to become an effective strategy to promote bone regeneration also in challenging scenarios, particularly if combined with the controlled release of growth factors/bioactive molecules.

5.2 | Bone grafts

Traditionally, the main purposes for the application of bone grafts or substitutes included enhancement of bone healing by bridging small to large defects, prevention of membrane collapse by maintaining the space beneath the membrane, stabilization of the blood clot and prevention/reduction of bone resorption.

Bone augmentation materials are commonly classified according to their origin into autologous grafts (from the same person), allogenic grafts (from another individual within the same species), xenogenic grafts (from another species), or alloplastic grafts (synthetic materials). They are usually available in the form of blocks or particulate grafts. Table 2 summarizes the main advantages and disadvantages associated with the different types of bone grafts.

The properties of an ideal bone replacement graft have been defined as part of the recent Consensus Report of the 15th European Workshop on Periodontology on Bone Regeneration and they include biocompatibility, porosity, osteoinductivity, osteoconductivity, surface properties adequate for protein adsorption, extracellular matrix deposition, cell adhesion, differentiation and migration, biodegradability, mechanical properties mimicking bone properties, angiogenicity, easiness of handling and manufacturing processes.[75]

While autologous grafts are the only grafts presenting some osteogenic features, the limited availability need for a second surgical site and the associated increased patient morbidity limit their clinical use and has prompted the search for alternatives (Table 2). Allografts are a valid alternative to autologous grafts and are available in different forms: fresh, fresh frozen, freeze-dried, and demineralized freeze-dried. While the first two types of allografts are not recommended for the risk of disease transmission and immune reaction, freeze-dried and demineralized freeze-dried allografts (FDBA and DFDBA) are still successfully used for bone regeneration procedures in the maxillo-facial area. In particular, it has been suggested that the demineralization process exposes the underlying inner bone matrix,

which is rich in bone morphogenic protein and growth factors, such as TGF-β and FGF. These growth factors can stimulate the differentiation of mesenchymal stem cells into osteoblasts, thus conferring an osteoinductive property to this type of allografts.[144,145] However, non-significant clinical differences have been reported when FDBA or DFDBA were used for bone regeneration in the oral cavity.[146,147]

Within the past 30 years, deproteinized bovine bone mineral (DBBM) graft has probably become the most widely applied bone substitute when it comes to peri-implant and periodontal regeneration.[148–151] It consists of deproteinized bovine bone, with an ultrastructure almost identical to human bone and a porosity of 75%–80%. Despite DBBM has been applied with predictable long-term outcomes in association with barrier membranes for peri-implant bone regeneration, it should be noted that the use of DBBM does not enhance per se the capacity of the membrane to promote bone formation. However, it demonstrated osteoconductive properties both in pre-clinical and clinical studies, since its peculiar porous network promotes the migration and attachment of osteoblasts as well as angiogenesis, thus offering a scaffold that facilitates bone matrix deposition.[152–155] Nevertheless, a general delay in new bone formation has been histologically documented when GBR performed with a bovine-derived xenograft is compared to GBR alone for bone augmentation in the calvaria/mandible.[155–159] Interestingly, pre-clinical studies seemed to suggest that this xenograft is beneficial in promoting bone formation in the early stages (1 month), while at later healing periods, it might delay the osseous healing process.[157,158]

With the aim to transfer osteoinductive properties to osteoconductive materials, the use of a bone-conditioned medium has been proposed.[160,161] Although a standardized clinical protocol has not been established yet, bone-conditioned medium obtained from human autologous bone chips may be able to enhance bone grafting procedures performed with DBBM by influencing the cellular viability and the release of growth factors.[162]

Beside xenografts, synthetic bone substitutes, including for instance hydroxyapatite, β-tricalcium phosphate, and biphasic calcium phosphate have also attracted increasing interest. Synthetic grafts come in different forms, such as mouldable, pellets, injectable, and 3D printed. One of the main advantages of such grafts is that they can be manufactured with specific tailored properties and this is why they are used as scaffolds in tissue engineering, usually in association with cells and osteoinductive signals.[163] Composite bone substitute materials have also been proposed to improve the mechanical properties of different synthetic materials, such as bioglass and polymers by combining their osteoconductive properties.[164]

5.2.1 | What is the minimum defect size that requires the use of a graft?

The regeneration of peri-implant bony defects usually requires the combined use of bone grafts and/or substitutes in order to provide adequate mechanical support and for their reported synergistic effects on regenerative outcomes.[2]

The minimum peri-implant defect size that would require bone augmentation has been questioned in a recent RCT.[165] Twenty-two patients having small peri-implant bone dehiscence defects (≤5 mm) around posterior implants were either left to heal spontaneously or treated with GBR (DBBM and collagen membrane). Clinical and radiological findings at 18 months demonstrated 100% survival rates in both groups. However, spontaneously healed defects revealed an elevated marginal bone loss compared to defects treated with GBR at the 18-month follow-up, as well as an increased buccal vertical bone loss at 6 months after implant placement. As such, the study concluded that GBR procedures improve the stability of the buccal bone of implants presenting bony dehiscence defects. At a follow-up of 7.5 years, CBCT analysis showed a residual vertical defect depth that was higher in the group left for spontaneous healing, while the GBR-treated implants showed a higher buccal bone thickness. Nevertheless, all implants survived and similar interproximal marginal bone levels and peri-implant clinical parameters were reported in the two groups,[166] thus questioning the clinical need for GBR in ≤5 mm dehiscences.

While there is no consensus on what is the minimum defect that requires regeneration, it is important to note that dehiscence-like bone defects resulting from previous unsuccessful regenerative procedures or during implant placement in pristine alveolar bone[166] may lead to instability of the soft and hard peri-implant tissues, with a greater risk of developing biological complications. As a matter of fact, a thin buccal bone thickness, often due to a buccal implant position[167] has been shown to increase the risk of peri-implant bone resorption during initial healing, thus resulting in a greater susceptibility to develop unfavorable peri-implant conditions, including mucosal recession and peri-implantitis.[168–170]

5.2.2 | Micro and macro architecture

Microstructure of grafts

The microarchitecture of a grafting material, including its relative cortical and cancellous composition, together with its embryogenic origin influences its resorption rate and degree of angiogenesis, which in turn can affect volume maintenance over time.[1,5,171]

The superiority of intramembranous (e.g., calvarial) versus endochondral (e.g., iliac) autogenous bone grafts when combined or not with a membrane has been questioned and studies suggest that bone graft's survival is determined primarily by its relative cortical and cancellous composition rather than its embryologic origin.[172,173]

Regardless of the embryologic origin, pre-clinical findings suggested better long-term volumetric stability for both autogenous endochondral and intramembranous onlay grafts whenever they were covered with a barrier membrane, thus emphasizing the importance of GBR and the creation of a secluded space.[174] Remarkably, in a study comparing different harvesting sites of autologous bone to treat peri-implant dehiscence defects, it was shown that the mandibular symphysis led to the highest mean bone growth, followed by the mandibular ramus, while the tuberosity produced the poorest results.[98]

Proximity of the elastic modules of bone grafts to the human structure is another important factor when dealing with bone regeneration procedures. While bovine xenografts are close to human bone, synthetic materials might have elevated elastic modules compared to ideal conditions.[175]

Remarkably, it was shown that the surface area of the graft influences the growth factor release potential with a positive trend, meaning that the higher the absolute surface area of the autograft, the more the osteoconductive and osteoinductive potentials are.[176] Likewise, increasing the surface area by diminishing the particle size of a synthetic beta-tricalcium phosphate graft to a nano-seized level was shown to improve bone formation and enhance the circulation of body fluids and micronutrients.[177]

Finally, the graft structure should have an ideal pore size (i.e., interconnecting pore size >300 μm in diameter[178]) that enables turnover of the required nutrients, signaling factors, proteins, chemicals, and end-products within the wound milieu. In general, bone pores should be wide enough to allow vascular ingrowth (≥100 μm) and neovascularization within the bone particles. Studies conducted with different bone graft materials suggested a greater degree and faster rate of bone penetration as the macroporosity of a scaffold increases (where macroporosity can be considered as pores >50 μm in size).[179–181] In contrast, increased levels of microporosity/strut porosity (pores <50 μm in size) also appear to promote osteogenesis and a faster apposition of a greater volume of new bone.[180,182,183] Similarly, the degree of structural interconnectivity between the pores of the graft material was shown to influence the speed and extent of the development of the vascular network essential for new bone formation.[179]

Macrostructure of grafts

In terms of macrostructure, the size/dimension of the graft to be employed may play a role in bone regeneration. For instance, one study indicated superior dehiscence resolution when a block DBBM rather than a particulate DBBM was applied in combination with a non-cross-linked collagen membrane, with 11 out of 12 (91.7%) block sites and 3 out of 12 (25%) particulate sites showing a complete vertical defect fill at re-entry.[184] On the contrary, the use of a particulate versus soft-type block of biphasic calcium phosphate associated with a cross-linked collagen membrane did not differ in terms of vertical dehiscence resolution at re-entry.[118] However, the authors highlighted that the morphology of the defect played a significant role in the regenerative outcome, with non-containing defects showing incomplete vertical defect fill in 61.9% of the cases, regardless of the graft used.

5.2.3 | Resorption pattern

The resorption rate of a grafting material depends on its physical and chemical properties.[185] It should be gradual and timely in order to facilitate its progressive replacement with newly formed bone.[75] When it is faster than ideal, the graft might resorb before the formation and/or maturation of new bone, with the risk of jeopardizing the final regenerative outcome. On the contrary, delayed resorbing or non-resorbing materials may prevent maturation and remodeling of the newly formed bone by limiting natural stresses that should be directed to them without any interferences.

Autologous grafts

Autologous bone grafts are incorporated into the surrounding bone through a process called "creeping substitution".[186–188] In case of cortical bone, the regeneration process is mainly preceded by resorption, while in cancellous bone the osseous formation is initiated directly in the marrow spaces, by the differentiation of graft mesenchymal cells into osteoblasts.[189] In a series of pre-clinical studies, Donos et al. tested the volume stability of autologous onlay bone grafts applied alone or together with an e-PTFE or a resorbable copolymer membrane in the lower border of the mandible or maxillary alveolar ridge. The studies showed predictable regeneration outcomes and stability of the autogenous bone graft provided that the membranes were properly adapted and kept covered during healing.[42,110–112] On the contrary, membrane exposure led to an increased risk of infection and compromised regenerative outcomes.

In another clinical study we also clearly demonstrated that membrane removal leads to some resorption of the underlying bone graft, although the overall bulk of newly formed bone is maintained.[174] This is in line with other experimental studies that demonstrated that following membrane removal resorption of the newly formed bone occurs up to a certain extent, but at the same time, bone becomes also more mature and trabecular.[190–192]

Besides the use of a barrier, in order to slow down resorption and enhance volume maintenance of autologous grafts, different strategies have been proposed, including the combination with a slow-resorption particulate graft[137,193,194] or the combination with bioactive factors.[195]

Allogenic grafts

Allograft incorporation follows a similar sequence of events but vascular penetration, bone formation, and remodeling are slower and reduced as compared to autografts.[196]

Remarkably, allografts are never completely replaced by new bone, and allograft particles can remain embedded within newly formed bone for years.

Xenogenic grafts

As already mentioned, DBBM is by far the most applied xenograft for the regeneration of peri-implant defects. The resorption pattern of DBBM is still debatable. Several studies reported osteoclast-like multinucleated cell activity on the surface of DBBM particles and scalloped edges of the particles.[149,154,155,197] This suggests that, although delayed, the resorption of the graft occurs and eventually it may be replaced by bone. However, non-resorbed, inert DBBM particles embedded into bone and marrow have been histologically and radiographically documented up to more than 10 years from their placement, with no or only little signs of resorption, thus raising doubts on the actual possibility to clear off the graft.[198–201]

Considering the slow-resorption characteristics of DBBM, it has been proposed to combine it with autogenous bone particles and a barrier membrane in GBR. The rationale behind this is that, while autogenous particles undergo a rapid creeping substitution, DBBM particles would keep the space and volume for the bone regeneration process and would act as osteoconductive materials.[202,203] Following this principle, the "sandwich bone augmentation technique" has been proposed whenever GBR is applied for buccal dehiscences around implants.[204–207] According to this technique, autogenous bone should be used to cover the exposed implant threads, followed by a layer of particulate cortical bone (either human demineralized cortical bone or bovine hydroxyapatite). A collagen membrane should ultimately cover and protect the grafts.[207] The aim is to mimic the composition of the native bone, since the inner layer of autogenous bone should undergo creeping substitution and promote early osseointegration, while the outer slow-resorption cortical graft should maintain the space and have osteoconductive properties.[208] Long-term clinical success of this approach has been confirmed by clinical and radiographic data.[209,210]

Synthetic grafts

The combination of different chemically processed alloplastic bone substitutes mixed in different ratios has been tested with the aim to obtain optimal properties, including fine-tuning their degradation. As a matter of fact, an important clinical limitation of certain synthetic bone substitutes is their fast substitution rate, which limits their space-maintenance properties during bone remodeling. Bioactivity and resorption of biphasic calcium phosphates can vary depending on the HA/β-TCP ratio and the crystallinity of the ceramic.[211]

In summary, fine tuning of graft resorption plays an important role in guiding the bone regenerative process. While research is still needed to clarify how to optimize graft resorption to enhance the regenerative outcomes, we are witnessing a change in mindset, as instead of applying slow- or non-resorbing grafts, the trend is becoming to use faster resorbing grafts that ensure resorption during the tissue remodeling process and avoid delays in osseous healing.[211]

5.2.4 | Space maintenance

Together with primary wound closure, angiogenesis, and clot stability, space maintenance is one of the key factors for successful new bone formation following GBR procedures.[212]

A favorable correlation has been observed between space protection and the level of new bone formation.[213] Studies employing rigid capsules/domes in animal models have even shown the possibility of regenerating bone beyond the genetically determined skeletal profile (neo-osteogenesis). When a secluded space/volume was created and maintained with rigid capsules applied to the mandibular ramus, a substantial amount of osseous formation occurred, which could exceed 5–6 times the original skeletal profile.[190,214]

Both resorbable and to a lesser extent non-resorbable membranes have limitations for maintaining the architecture of the defect due to their limited rigidity. Risk of membrane collapse and early degradation has been reported particularly when resorbable membranes are applied.[215] As such, whenever the risk of membrane collapse is high, it is suggested to apply a bone filler with a low substitution rate to avoid an early collapse and to maintain the augmented bone volume.[216] The space-maintenance capability of a bone filler would ensure an ideal microenvironment for the revascularization of the augmented volume and enable tissue to form in a preserved and protected space. Therefore, underneath the secluded space created by the membrane, an ideal bone filler should promote the recruitment, adhesion, proliferation, and differentiation of osteoprogenitor cells, which will secrete the bone matrix that will eventually mineralize to form mature bone. Mechanical properties (load bearing capability) significantly influence the space-maintaining ability of the grafts,[217] as well as manufacturing methods. For instance, freeze-drying of allografts can reduce up to one fifth the graft strength,[218] while the demineralization process reduces its mineral content.

Although bone grafts play a crucial role in space maintenance, when a biomaterial is present (especially a slow-resorption one), it can also delay the osseous formation process by occupying the space where the newly formed tissue should form. As a matter of fact, a delay in new bone formation has been documented when GBR associated with a xenograft is compared to GBR alone for bone augmentation in the calvaria/mandible.[155–159]

It has been demonstrated that the displacement of particulate grafting material, especially in the coronal portion of the augmented site, may lead to a partial collapse of the collagen membrane, with the risk of jeopardizing the regenerative outcomes.[103] In order to overcome this problem, fixation of the collagen membrane has been proposed to prevent graft displacement (see section 5.1.2). A recent pre-clinical study corroborated the importance of space provision through collagen membrane fixation and the use of membrane-supporting materials in standardized calvarial defects, by documenting improved new bone and mineralized tissue formation as compared to the use of unfixed-collagen membranes.[219] Moreover, bone regeneration-related gene expression (BMP-2, FGF-2, VEGF, and osteocalcin) was enhanced when space provision was provided, while collapsing membranes were highly correlated with reduced bone formation. These findings support the well-established positive effect of space maintenance philosophy on the wound microenvironment via signaling factors and osteogenesis.[220]

On the contrary, non-resorbable membranes present with better mechanical properties as well as space-provision properties. As such, the use of a graft might not always be necessary. As a matter of fact, Mattout et al.[99] indicated similar regeneration of peri-implant dehiscence defects treated either with an e-PTFE membrane alone or in association with an allograft.

Finally, peri-implant defect characteristics and defect morphology (i.e., defect extension, wall number) and defect size (i.e., length of the dehiscence, defect depth) are also important determinants to take into consideration in terms of space provision. The fact that a defect is well-contained rather than non-containing plays a key role in determining the bone housing ability and should be considered as

a local factor which in turn influences the blood clot stability as well as the success of osseous regeneration.[166]

In summary, the role of bone grafts in space provision seems to be particularly relevant when there is a high tendency of membrane collapse due to the characteristics of the defects, or to the mechanical properties of the barrier, or a combination of both factors. Loss of the augmented space due to the compression of the augmented material, or to the migration of the material underneath the barrier, or to the fast resorption of the membrane might compromise the dimensional stability of the augmented site and the regenerative outcome. However, it is also important to acknowledge that using a bone graft for space maintenance, especially when it has a slow resorption rate, may delay the osseous formation process.

6 | THE IMPACT OF PROSTHETIC FACTORS

6.1 | Implant loading

Traditionally, dental implants have been prosthetically loaded following a healing phase from 3 to 6 months after stage I implant surgery. However, in order to shorten the treatment time and to meet the requests of patients unwilling to wait months before the rehabilitation of edentulous areas (particularly in the aesthetic zone), immediate and early prosthetic loading of implants have been introduced. Currently, the different times for implant loading are defined as follows[221]:

- Immediate loading, when an implant-retained prosthesis is connected within 1 week following implant placement.
- Early loading, when an implant-retained prosthesis is connected between 1 week and 2 months following implant placement.
- Conventional loading, when implants are allowed to heal for more than 2 months after placement, without connecting a prosthesis.

Buser et al.[191] were the first to provide histological evidence that implants placed in entirely regenerated bone (through GBR) can successfully osseointegrate and that regenerated bone can sustain functional load like pristine bone. According to their pre-clinical study, functional vs non-functional loading did not influence bone remodeling, whereas in the control regenerated sites where no implants were placed, they observed bone atrophy with a rarefied bone structure and a thin cortical layer. They, therefore, concluded that implant placement into regenerated bone was able to stimulate bone maturation and remodeling.

However, in another study, assessing the effect of loading on the outcome of GBR in peri-implant dehiscence defects, a significant decrease in bone fill was observed at augmented sites subjected to loading between the 3- and 9-month healing period, whereas no change was observed at non-loaded sites.[222]

Our group has also pre-clinically investigated the impact of loading and we showed that bone regeneration and osseointegration can be achieved in dehiscence defects at implants with a hydrophilic surface treated with or without GBR and grafting, and that regeneration is not impaired by functional loading.[68]

Extensive clinical evidence is available on the success of conventionally loaded implants with simultaneous GBR,[123,223,224] with a survival and success rate ranging from 95% to 100% over a 5-year follow-up.[225] Predictable soft tissue aesthetics can also be attained in conventionally loaded implants, and the procedure is relatively less technique-sensitive.[226] Remarkably, conventional loading provides predictable results also in implants placed with simultaneous GBR and lacking adequate primary stability.[224]

Immediate loading was introduced in the early 1990s, and today it has >20 years of clinical and histologic evidence, with the anterior mandible being the most documented area.[227-229] Although immediate and early loading protocols provide the advantage of rapid rehabilitation, meticulous occlusal schemes are recommended to minimize non-axial forces on the implant[230] (for review on the impact of loading see[231]). The concept of immediate loading carries some potential issues related to the fact that micromotion and implant instability during the early healing days might result in fibrous encapsulation rather than osseointegration of the implant.[232] Also, an adequate implant primary stability (30–35 N/cm) is a pre-requisite for immediate loading,[233] which may sometimes be difficult to reach in the upper jaw, where the bone tends to be more porous and in patients with alveolar bone resorption requiring simultaneous bone regeneration along with implant placement.[232,234]

Since immediate or early loading is generally performed in aesthetically demanding areas, most of the available studies relate to implants placed in fresh extraction sockets (Type I implant placement). For instance, in an RCT where 60 patients were randomly allocated to receive either immediate- or conventionally-loaded implants with GBR and then followed up over a period of 24 months, similar bone gain and soft tissue aesthetic outcomes were reported.[235] In a similar single-arm prospective study, a 100% implant survival at 1-year follow-up, with maintenance of bone levels was noted for immediately loaded implants with simultaneous GBR.[236] However, in a retrospective study involving post-extraction compromised sites (thinner than 1 mm, dehiscenced or fenestrated, or combination of 2 of those defects) due to previous periodontal disease, periapical pathologies or traumatic extraction, immediately restored implants combined with GBR and a connective tissue graft were frequently associated with recession and incomplete papilla.[237]

Only limited data are available on the effect of immediate loading on GBR around implants placed in healed ridges. In two RCTs on single implants in the anterior maxilla, similar radiographic and aesthetic outcomes were obtained at 12[238] and 18 months[239] for immediate non-occusally loaded as compared to conventionally loaded implants placed in concomitance (as needed) with GBR.

Our group compared conventionally loaded and immediately provisionalized implants with non-occluding crowns. In both groups, GBR was performed with a collagen membrane and particulate graft whenever a fenestration/dehiscence occurred.

We showed that immediately provisionalized implants had a higher interproximal bone loss (−0.44 mm) at 24 months of function,[240] which was also confirmed at 36 months (−0.32 mm), but at 5 years bone levels stabilized and reached comparable values.[241] Interestingly, data on aesthetic scores suggested that, by placing a crown within the first 48 h after implant placement, it might be possible to condition the peri-implant soft tissues earlier, particularly in terms of papilla shape, level of soft tissue margin, and soft tissue contour, while the papilla fill might require a longer time to be achieved. However, in the long term (5 years), comparable results could be achieved with non-functional immediate loading and conventional loading.

Taking the aforementioned studies together, it can be concluded that overall loading does not seem to have a negative impact in case of bone regeneration, however careful considerations need to be made in terms of load distribution, patient, and site selection. It would be interesting in the future to test whether immediate provisionalization or conventional loading of implants with different surface characteristics and design might result in different outcomes, as no data are available in the literature in this respect. Moreover, there is currently a lack of understanding of the molecular mechanisms taking place in association with different loading protocols when GBR is performed or not. In this respect, the use of biomarker analyses (of the peri-implant crevicular fluid) and of new imaging systems that can clarify and objectively monitor peri-implant tissue morphometric and vascularization changes during the healing process might be particularly useful.

6.2 | Abutment characteristics

Guided bone regeneration is often performed to allow the correct prosthetic positioning of implants, which ultimately influences their long-term clinical success. Morphologically, the implant supracrestal complex extends from the coronal aspect of the peri-implant mucosa to the marginal peri-implant bone level, thus encompassing the implant-abutment-prosthesis junction.[242] In implants placed with GBR and immediately loaded, various prosthetic parameters such as abutment design, position, or implant-abutment-prosthesis junction may possibly influence the regenerative outcome.

Using optically scanned 3D images, Benic et al.[243] compared the soft tissue contours around implants placed with or without simultaneous GBR. It was noted that the implant-abutment connection increased the buccal contour of the marginal mucosa at the augmented sites. In another study, metal temporary abutments were hand-tightened onto immediately placed maxillary anterior implants.[244] The labial osseous defects were classified as U-, V,- or UU-shaped, and were simultaneously grafted using autologous and xenogenic bone. The provisional restorations were cemented on the abutment and were kept out of occlusion in centric and eccentric movements. While a 100% survival rate for the implants was reported, few cases of gingival recession were seen for the U-shaped defects.

To the best of our knowledge, no evidence is available on the influence of abutment materials on the regenerative outcome around dental implants. However, a systematic review by Linkevicius and Vaitelis[245] indicated no significant differences between titanium and zirconia implant abutments when evaluating probing pocket depth, bleeding on probing, marginal bone levels, and mucosal recessions. Zirconia abutments were associated with more biological complications but demonstrated superiority in terms of achieving natural soft tissue color.

In summary, although current literature suggests a minimal influence of prosthetic parameters on the outcomes of peri-implant bone regeneration, robust conclusions cannot be drawn due to the paucity of available RCTs. Future studies should consider evaluating the impact of the labial contour and emergence profile of the temporary implant-supported prosthesis on the results of GBR procedures, and the stability of the gingival margin contour. Moreover, the influence of the nano-, micro-, and macro-structures, as well as the cleaning (chemical composition of the surface) of the trans-mucosal components would deserve investigation in relation to their potential influence on peri-implant tissue stability both in cases where bone regeneration is performed and in cases where it is not performed.

7 | THE IMPACT OF PATIENT-RELATED FACTORS

Although overall bone regeneration modalities can be considered predictable and effective,[246] treatment outcomes can potentially be challenged by multiple patient-related factors. A recent retrospective analysis on 5404 implants placed simultaneously with GBR indicated that among patient-related factors, gender (i.e., male patient), periodontal status (i.e., periodontitis), maxillary posterior region, as well as age at the time of implant placement are risk factors for implant loss.[247]

7.1 | History of periodontitis, compliance and oral hygiene

As clearly indicated in the recent European Federation of Periodontology S3-level treatment guidelines, clinicians should not perform periodontal as well as implant surgeries in patients not practicing and maintaining adequate levels of self-performed oral hygiene.[248,249] As a matter of fact, step 1 of periodontal therapy, which aims at guiding behavior change by motivating the patient to undertake successful removal of supragingival dental biofilm and risk factor control is a prerequisite before undertaking any further step of therapy. Inadequate plaque control may negatively affect any type of oral surgery, as bacterial plaque induces gingival inflammation, which can interfere with osseous formation and increase the risk of biomaterial infection.

There is almost no literature investigating whether history of periodontitis can directly influence the outcomes of bone

regeneration simultaneous to dental implant placement. One RCT specifically focused on studying the effect of loading (immediate vs. conventional) on implants placed in concomitance with bone regeneration performed with an allograft and a collagen membrane in patients previously treated for periodontitis.[235] The study showed successful implant stability, radiographic bone gain and high survival and aesthetic outcomes with both loading protocols in this category of patients. Another prospective study reported on the outcomes of implants placed into bone regenerated through titanium-reinforced e-PTFE membranes (staged regeneration) in patients previously treated for generalized aggressive periodontitis.[250] While a 100% implant survival rate was documented at 3 years, these patients showed a higher attachment loss and bone loss when compared to healthy patients receiving dental implants. Nevertheless, the 10–20-year follow-up of this study showed encouraging outcomes, with no implant loss and only a small percentage of implants that developed peri-mucositis (28%), probably owing to the strict supportive care regime followed by the patients.[251]

While it is likely that bone regenerative procedures in patients with a history of periodontitis can be as successful as in patients without a history of periodontitis, there is a large body of evidence that implants placed in patients treated for periodontal disease are associated with a higher incidence of biological complications and lower long-term success and survival rates than those placed in periodontally healthy patients.[252,253] Moreover, periodontitis-associated alveolar bone resorption may lead to significant alveolar atrophy and potentially to more challenging defects that may not always allow the placement of dental implants simultaneous to bone regeneration, but may require a staged implant placement.

Considering the aforementioned, the long-term stability of bone regeneration simultaneous to implant placement in patients previously treated for periodontitis may not be so obvious. In these patients, the adherence to supportive care is crucial to enhance the long-term outcomes of implant therapy, including the stability of peri-implant regenerative procedures.

As recommended in the EFP S3 level clinical practice guideline for the prevention and treatment of peri-implant diseases,[254] it is recommended that a patient-centered supportive peri-implant care protocol is implemented, which should include the following components:

- Interview (medical, social, and oral history update, risk assessment, patient feedback).
- Assessment of oral situation, including peri-implant tissue health, prosthetic components and patient competence to undertake oral hygiene.
- Reinforce risk factor control (e.g., smoking, oral dryness, glycaemic control).
- Professional intervention: individualized oral healthcare plan, including oral hygiene coaching and professional mechanical plaque removal of the entire dentition/implants.
- Determination of next recall interval tailored according to patient-, implant-, and restoration-based risk factors.

7.2 | Systemic conditions and smoking

Besides underlying medical conditions (e.g., serious cardiovascular diseases or cancer) that can place the patients at risk during surgery irrespectively of the nature of the intervention, different diseases that have a direct/indirect impact on bone and soft tissue healing may potentially affect the outcomes of bone regenerative procedures performed as part of implant rehabilitations. The increasing demand for implant-based treatments together with the demographic shift toward an ageing population have resulted in a growing body of literature dealing with the impact of systemic conditions on the success/survival of implant rehabilitations.[255] While the majority of the studies have focused on the impact of systemic conditions on osseointegration and implant loss, very limited studies have focused on the impact that the underlying medical conditions may have when regenerative procedures are performed together with implant placement.

7.2.1 | Diabetes mellitus

Diabetes mellitus has been associated with the occurrence of a series of complications on the skeletal system collectively referred to as “diabetic bone disease” or “diabetic osteopathy”.[256] The hyperglycaemic wound healing milieu and the accumulation of advanced glycation end-products (AGEs) have been directly implicated in the impaired osteogenic potential of diabetic bone.[257]

Pre-clinical studies suggested that GBR treatment allows the regeneration of critical size defects, as well as de novo bone formation even in the presence of uncontrolled diabetes, although less predictably compared with the healthy status or controlled diabetes.[12,258] Impaired peri-implant bone formation and mineralization, as well as impaired regeneration of peri-implant dehiscence defects were also reported in streptozicin-treated diabetic pigs.[259,260] With the help of microarray gene expression analysis our group tried to shed light on the molecular mechanisms behind the negative impact of hyperglycemia on bone regeneration. Remarkably, we showed that uncontrolled diabetes is associated with a delayed and prolonged inflammatory response and with a downregulation of key genes (e.g., bone morphogenetic protein 4, latent transforming growth factor beta binding protein 4, thyroid hormone receptor alpha and CD276 antigen) and pathways (e.g., Wnt) implicated in the osteogenesis process.[12] This is in agreement with another pre-clinical study that suggested an increased expression of pro-inflammatory cytokines in diabetic rats during osseous healing.[261] Interestingly, this study and a subsequent study from the same group[262] also showed that by using hydrophilic micro-rough titanium surfaces it was possible to successfully compensate for the compromised M2 macrophage function in type 1 and 2 diabetes by attenuating the pro-inflammatory response and restoring macrophage homeostasis. This is in line with another experimental study that showed that substantial de novo bone formation can be achieved underneath micro-rough titanium domes with a hydrophobic and hydrophilic surface also in case of uncontrolled diabetes.[263]

Only limited clinical studies have investigated the impact of diabetes on bone regenerative procedures performed during implant rehabilitations.

A prospective study following up for 1–12 years on 45 patients with type 2 diabetes mellitus showed similar survival rates of implants placed following conventional protocols or advanced protocols involving different types of regenerative procedures (including sinus augmentation, immediate loading, and GBR).[264] Remarkably, HbA1c was the only multivariate independent factor affecting the complication rate. Likewise, a recent retrospective study also suggested that simultaneous horizontal GBR is not associated with increased failure rates when it is performed in patients with controlled type 2 diabetes mellitus.[265]

7.2.2 | Osteoporosis

While it is still controversial whether osteoporosis has a detrimental effect on the jawbones, growing evidence from pre-clinical and clinical studies seems to suggest a correlation between bone density measured at different systemic skeletal sites and at the jawbones,[266–275] and that osteoporosis is associated with a reduced bone quality and increased cortical porosity in the jaws.[276–281] As such, a review has suggested that osteoporotic bone should be regarded as equivalent to Type IV according to Lekholm and Zarb[282] classification and that clinicians may consider a longer healing period for implant osseointegration before prostheses insertion in patients with osteoporosis.

Despite pre-clinical studies overall suggest a lower osseointegration rate and reduced mechanical properties in osteoporotic bone,[283] clinical evidence is far less robust.[284] The efficacy of dental implants in osteoporotic patients has been assessed in prospective case–control studies and overall they support the applicability of implants in osteoporotic patients, even for immediate loading.[285] Therefore, nowadays a diagnosis of osteopenia or osteoporosis is not considered an absolute contraindication to dental implants.[286–291] Tadinada et al.[292] also showed successful buccal bone regeneration 9 months following grafting of peri-implant dehiscence defects (with DBBM) in 10 osteoporotic women with the use of CBCT scans.

Nevertheless, there are data coming mainly from retrospective studies speculating that osteoporosis may negatively impact on large bone reconstructions, such as in pre-prosthetic graft surgeries or sinus augmentation.[284,293–298]

Pre-clinical studies also suggested that osteoporosis might negatively impact on bone regeneration. In particular, few studies reported reduced regeneration of calvarial critical size defects treated with different grafts, together with a reduced expression of osteoblast-specific genes (such as RUNX 2, Col I and OC) and an altered expression of estrogen receptors and adipogenic markers.[299,300] Moreover, in a study evaluating the healing of autologous bone grafts fixed to the mandibular ramus of healthy and osteoporotic-like rats, histological analysis showed differences in bone quality, since osteoporotic rats showed larger quantities of medullary spaces both in the regenerated bone and receptor bed.[301] Likewise, Li et al.[302] investigated the influence of osteoporosis on autologous iliac crest grafts around dental implants in rabbits. Although osteoporosis did not delay osseointegration, it was associated with more graft resorption, decreased cancellous bone volume, trabecular thickness, trabecular number, and BIC, as assessed by micro-CT. These results were confirmed in the same model in another study, which suggested that experimental osteoporosis not only induced resorption of host bone, as demonstrated by bone mineral density and histology observations in the rabbit femurs, but also accelerated resorption of the autologous graft and delayed its healing.[303]

In a study in osteoporotic rabbits, our group also indicated that antiresorptive medications can negatively impact on the bone regeneration process, as we should anticipate approximately 21% and 19% less BIC in bisphosphonate-treated osteoporotic animals as compared to healthy and untreated osteoporotic animals, respectively.[304] On the contrary, few pre-clinical studies demonstrated that implant topography and hydrophilicity can compensate the deleterious impact of osteoporosis on early osseointegration.[276,304,305]

Our group was the first to assess from a molecular (proteomic) point of view the proteins and signaling pathways expressed during bone regeneration in condition of health and osteoporosis and we showed that osteoporosis is associated with a tendency for an enhanced inflammatory and stress response and a delayed expression of pathways involved in osteoblast differentiation and osteogenesis.[14] Since hydrophilic micro rough (SLActive) titanium surfaces are able to modulate inflammatory and osteogeneis-related pathways, it is possible to speculate that such surfaces might be particularly beneficial in osteoporotic patients, where the same pathways are negatively affected.[306]

Finally, if osteoporotic patients are treated with antiresorptive drugs, then clinicians should also be aware of the potential risk of triggering a medication-related osteonecrosis of the jaw (MRONJ) through the implant surgery and associated bone regenerative procedure.[307] It is, therefore, important that osteoporotic patients taking antiresorptive medications who are undergoing invasive surgical procedures, including implant placement associated with bone regeneration, are adequately informed of the risk, albeit small, of developing MRONJ and that clinicians take all necessary precautions to make the surgeries less invasive as possible and promote healing for primary closure.[308]

7.2.3 | Smoking

It is well established that smoking has a detrimental effect on wound and bone healing owing to its local and systemic action. Locally, the thermal trauma is the principal consequence of cigarette smoking. Heat can cause modifications of the membrane integrity, thus altering the cellular osmotic balance and causing edema and activation of the inflammatory process.[309] Besides the thermal injury, many irritants, toxins, and carcinogens found in cigarette smoke can also cause intraoral pH changes, mucosal drying,

nicotinic stomatitis and keratosis. At a systemic level, smoke and its principal toxic component nicotine have an immunosuppressive effect on both the innate and adaptive immune responses. They are able to develop a constitutive inflammatory status, reduce tissue perfusion, impair the proliferation and differentiation of osteoblasts, fibroblasts, and other cells involved in the healing process, and can also modulate the expression of genes that are crucial for bone metabolism.[310–314]

Remarkably, smoking has also a harmful effect on vascularization. Nicotine increases platelet adhesiveness and this can lead to the formation of microclots, to the reduction of microvascular perfusion, and eventually ischemia.[315–317] As angiogenesis and an ample blood supply are mandatory for graft revascularization and integration and eventually for the long-term success of bone regeneration, it is reasonable to assume that smoking should be considered a risk factor for bone regenerative procedures, with heavy smokers (more than 10 cigarettes a day) carrying the highest risk for surgical complications.

Few systematic reviews clearly indicated that the rates of implant failure, postoperative infections, and peri-implant crestal bone loss are significantly higher in smokers compared with non-smokers.[318,319]

In a prospective controlled study with 22–24 years of follow-up, Jung et al.[320] compared the performance of implants placed with simultaneous GBR using resorbable or non-resorbable membranes to implants placed in pristine bone without bone regeneration. The study showed that smoking significantly impaired implant survival rates. Few studies on staged and simultaneous bone augmentation have also suggested an increased risk of complications (e.g., membrane exposure, signs of local inflammation) in smokers[321,322] and a 5-year retrospective study indicated that smoking significantly increased the risk of implant failure both in implants placed with GBR and in implants placed in pristine bone.[323]

In summary, although the patient-related factors described above are not considered as an absolute contraindication for implant-associated bone regeneration procedures, it is clear that they can play a role on the overall treatment success and risk of complications. Hence, patient selection, control of underlying medical conditions and concomitant risk factors and, whenever necessary, consultation with the patient's physician are recommended.

8 | RISK OF PERIIMPLANTITIS IN GBR-TREATED SITES

Despite the fact that dental implants in conjunction with augmentation procedures are well-established, there is limited knowledge on the incidence of peri-implantitis in regenerated compared to pristine sites. However, it might be speculated that regenerated bone (particularly when bone replacement grafts are used) might offer a locus minoris resistentiae to the dysbiotic biofilm involved in the development of peri-implantitis.

While a review by Salvi et al.[324] failed to identify differences in the occurrence of biological complications in pristine and augmented bone sites, a recent pre-clinical study in beagle dogs where a ligature-induced peri-implantitis model was applied on implants placed in pristine bone and on implants placed with simultaneous bone regeneration (DBBM combined with a collagen membrane) showed small differences in bone loss between the two sites.[325] In particular, the size and vertical dimension of the peri-implantitis lesions were larger at augmented sites than at pristine sites. Remarkably, implants with non-modified (turned) surfaces exhibited smaller amounts of bone loss and smaller dimensions of peri-implantitis lesions than implants with modified surfaces (hydrophobic or hydrophilic). Conversely, in another study in dogs, Sato et al.[326] suggested a similar degree of bone resorption when peri-implantitis was induced in implants that had previously been grafted with either an autograft or DBBM as compared to implants placed in pristine bone. While these pre-clinical studies provide useful proof of principle data on the incidence of peri-implantitis in regenerated bone, their findings may not fully reflect the complexity and variability of outcomes in human patients, hence their results should be interpreted with caution.

In a 4-year prospective study, Schwarz et al.[327] investigated the impact of residual defect height following bone augmentation using DBBM and a collagen membrane in dehiscence-type defects on the stability of peri-implant health. The study suggested that implants exhibiting a residual defect height >1 mm following GBR simultaneous to implant placement are at a higher risk of developing peri-implant disease.

According to the 6th ITI Consensus Conference and based on 1 RCT, 1 case–control study and 4 case series studies, patients with implants placed in pristine sites have a prevalence of peri-implant mucositis of 22.4% (95% CI: 6%–38%) compared with a prevalence of 19.6% (95% CI: 0%–40%) for patients with implants in augmented sites.[328] It was also highlighted that for patients presenting implants in augmented sites, the prevalence of peri-implantitis and implant loss is overall low over the medium to long term. However, it is of utmost importance for these patients (as well as for patients receiving implants in pristine sites) to be enrolled in regular supportive care progras.[254]

Special consideration should be given to periodontally susceptible patients with implants placed in augmented sites.[252]

9 | CONCLUSION

The success and predictability of bone regeneration procedures associated with dental implants are related to the presence of osteoprogenitor cells, creation/maintenance of space with/without a scaffold, adequate blood supply, mechanical stability, and signaling molecules that guide the maturation of the deposited bone matrix (Figure 2). Despite adhering to these key principles, a certain variability in the regenerative outcome can be observed among different patients.

In the past years, pre-clinical and clinical studies have started to unravel which are the main biomaterial-related, implant-related, surgery-related, prosthesis-related, and patient-related

factors that can play a role on the success and long-term stability of bone regenerative procedures (Figure 1). Nevertheless, our knowledge is still limited in this respect and future studies are needed for the optimization and fine-tuning of osseous regeneration, particularly in challenging scenarios. Ultimately the goal will be to optimize case selection and at the same time to tailor the regenerative procedure based on the different specific local and systemic factors.

The continuous evolution in terms of implant design and surfaces, together with the refinement of less invasive surgical techniques are helping make bone regenerative procedures more predictable. At the same time, considering the complexity of the bone regeneration process, combined therapies that incorporate cells, signals, and a scaffold are gaining increasing attention, as compared to single biomaterials. In this respect, it is likely that in the future bone tissue engineering will dramatically change our approach to bone regeneration in implant dentistry by progressively replacing traditional barriers and grafts with osteoinductive scaffolds containing osteogenic cells and osteoinductive factors with adequate mechanical properties to support the organization and maturation of newly formed bone, as well as to promote vascularisation. The tissue engineering field is progressing at an incredibly fast rate and a promising research line is related for instance to the development of biomaterials that can modulate various events during bone tissue regeneration such as immune response, osteogenesis/osteoclastogenesis, and infection or inflammation.[329] Another interesting possibility is to modify the synthetic biomaterial substrates with biological properties, such as anti-inflammatory drugs or cytokines, or to modify the biomaterial surface chemistry so it can influence the protein adsorption and further downstream signaling processes with the immune cells, including macrophages.[329]

Despite the progresses made so far, research is still needed to address fundamental challenges related for instance to the selection of the most effective cells, scaffolds, and signaling molecules, and to address important gaps on our current knowledge of the cascade of events taking place during bone formation. Moreover, an unsolved challenge currently faced by tissue engineering is a better understanding of the role that the microenvironment plays on regenerative outcomes.[330] In this respect, a close interplay between immune and skeletal systems has emerged (osteoimmunology), therefore it is crucial to understand how the biomaterial-immune cell interactions can contribute/influence bone regeneration. In the future, the goal for engineered regenerative constructs should be not only to form bone, but to make it function in an explicit manner in the patient-specific microenvironment.

In order to reach the ultimate goal of optimizing bone regeneration and controlling for factors that can have a negative impact on it, it is clear that the collaborative and joint efforts of scientists, engineers, and surgeons is needed.

Advances in "omics" technologies offer new perspectives to prompt our understanding of the biology underpinning bone regeneration in health and medically compromised conditions and in the future, they will likely help identify new therapeutic targets to improve regenerative outcomes (for instance by using smart biomaterials) also in compromised conditions.[306]

Since the limited available studies do not allow to make robust conclusions regarding the possible impact of biomaterials and implant surfaces on the risk of peri-implantitis occurrence and progression,[331] future studies are warranted to shed light on these aspects, with the aim to promote the use of biomaterials and implants that can actively counteract the risk of developing biological complications.

It is also important that future clinical studies on implant-associated regenerative procedures will include a detailed report of adverse events, complications and patient-reported outcome measures (PROMs) together with clinical and radiographic measures, since patient perception and satisfaction about the therapy, as well as risk of complications are important factors that need to be taken into account when selecting between different regenerative procedures.[332]

Finally, it is important to highlight that regardless of the continuous progresses in terms of biomaterials, implant surface and surgical technique, patients are required to adhere to strict supportive care programs and comply with a high standard of oral hygiene (for review see,[333]), as this is a prerequisite for the long-term success of any regenerative procedure).

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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REFERENCES

1. Retzepi M, Donos N. Guided bone regeneration: biological principle and therapeutic applications. *Clin Oral Implants Res*. 2010;21:567-576.
2. Donos N, Mardas N, Chadha V. Clinical outcomes of implants following lateral bone augmentation: systematic assessment of available options (barrier membranes, bone grafts, split osteotomy). *J Clin Periodontol*. 2008;35:173-202.
3. Chiapasco M, Casentini P, Zaniboni M. Bone augmentation procedures in implant dentistry. *Int J Oral Maxillofac Implants*. 2009;24(Suppl):237-259.
4. Buser D, Sennerby L, De Bruyn H. Modern implant dentistry based on osseointegration: 50 years of progress, current trends and open questions. *Periodontology 2000*. 2017;73:7-21.
5. Donos N, Dereka X, Mardas N. Experimental models for guided bone regeneration in healthy and medically compromised conditions. *Periodontology 2000*. 2015;68:99-121.
6. Schenk RK, Buser D, Hardwick WR, Dahlin C. Healing pattern of bone regeneration in membrane-protected defects: a histologic study in the canine mandible. *Int J Oral Maxillofac Implants*. 1994;9:13-29.
7. Prolo DJ, Gutierrez RV, DeVine JS, Oklund SA. Clinical utility of allogeneic skull discs in human craniotomy. *Neurosurgery*. 1984;14:183-186.

8. Gomes PS, Fernandes MH. Rodent models in bone-related research: the relevance of calvarial defects in the assessment of bone regeneration strategies. *Lab Anim*. 2011;45:14-24.
9. Vajgel A, Mardas N, Farias BC, Petrie A, Cimoes R, Donos N. A systematic review on the critical size defect model. *Clin Oral Implants Res*. 2014;25:879-893.
10. Ivanovski S, Hamlet S, Retzepi M, Wall I, Donos N. Transcriptional profiling of "guided bone regeneration" in a critical-size calvarial defect. *Clin Oral Implants Res*. 2011;22:382-389.
11. Donos N, Retzepi M, Wall I, Hamlet S, Ivanovski S. In vivo gene expression profile of guided bone regeneration associated with a microrough titanium surface. *Clin Oral Implants Res*. 2011;22:390-398.
12. Retzepi M, Calciolari E, Wall I, Lewis MP, Donos N. The effect of experimental diabetes and glycaemic control on guided bone regeneration: histology and gene expression analyses. *Clin Oral Implants Res*. 2018;29:139-154.
13. Al-Kattan R, Retzepi M, Calciolari E, Donos N. Microarray gene expression during early healing of GBR-treated calvarial critical size defects. *Clin Oral Implants Res*. 2017;28:1248-1257.
14. Calciolari E, Mardas N, Dereka X, Anagnostopoulos AK, Tsangaris GT, Donos N. The effect of experimental osteoporosis on bone regeneration: part 2, proteomics results. *Clin Oral Implants Res*. 2017;28:e135-e145.
15. Frost HM. A 2003 update of bone physiology and Wolff's law for clinicians. *Angle Orthod*. 2004;74:3-15.
16. Torkzaban P, Arabi SR, Roshanaei G, Rostami M, Soheilifar S. A comparative study of clinical parameters in submerged and non submerged implants. *J Clin Diagn Res*. 2015;9:ZC26-ZC29.
17. Hermann JS, Buser D, Schenk RK, Cochran DL. Crestal bone changes around titanium implants. A histometric evaluation of unloaded non-submerged and submerged implants in the canine mandible. *J Periodontol*. 2000;71:1412-1424.
18. Cordaro L, Torsello F, Roccuzzo M. Clinical outcome of submerged vs. non-submerged implants placed in fresh extraction sockets. *Clin Oral Implants Res*. 2009;20:1307-1313.
19. Astrand P, Engquist B, Anzen B, et al. Nonsubmerged and submerged implants in the treatment of the partially edentulous maxilla. *Clin Implant Dent Relat Res*. 2002;4:115-127.
20. Fiorellini JP, Buser D, Paquette DW, Williams RC, Haghighi D, Weber HP. A radiographic evaluation of bone healing around submerged and non-submerged dental implants in beagle dogs. *J Periodontol*. 1999;70:248-254.
21. Weber HP, Buser D, Donath K, et al. Comparison of healed tissues adjacent to submerged and non-submerged unloaded titanium dental implants. A histometric study in beagle dogs. *Clin Oral Implants Res*. 1996;7:11-19.
22. Schwarz F, Sager M, Ferrari D, Herten M, Wieland M, Becker J. Bone regeneration in dehiscence-type defects at non-submerged and submerged chemically modified (SLActive) and conventional SLA titanium implants: an immunohistochemical study in dogs. *J Clin Periodontol*. 2008;35:64-75.
23. Fiorellini JP, Engebretson SP, Donath K, Weber HP. Guided bone regeneration utilizing expanded polytetrafluoroethylene membranes in combination with submerged and nonsubmerged dental implants in beagle dogs. *J Periodontol*. 1998;69:528-535.
24. Mellati E, Chen S, Davies H, Fitzgerald W, Darby I. Healing of Bio-Oss® grafted marginal gaps at implants placed into fresh extraction sockets of incisor teeth in dogs: a study on the effect of submerged vs. non-submerged healing. *Clin Oral Implants Res*. 2015;26:553-562.
25. Cochran DL, Douglas HB. Augmentation of osseous tissue around nonsubmerged endosseous dental implants. *Int J Periodontics Restorative Dent*. 1993;13:506-519.
26. Lang NP, Bragger U, Hammerle CH, Sutter F. Immediate transmucosal implants using the principle of guided tissue regeneration. I. Rationale, clinical procedures and 30-month results. *Clin Oral Implants Res*. 1994;5:154-163.
27. Hammerle CH, Bragger U, Schmid B, Lang NP. Successful bone formation at immediate transmucosal implants: a clinical report. *Int J Oral Maxillofac Implants*. 1998;13:522-530.
28. Hammerle CH, Lang NP. Single stage surgery combining transmucosal implant placement with guided bone regeneration and bioresorbable materials. *Clin Oral Implants Res*. 2001;12:9-18.
29. Flores-Guillen J, Alvarez-Novoa C, Barbieri G, Martin C, Sanz M. Five-year outcomes of a randomized clinical trial comparing bone-level implants with either submerged or transmucosal healing. *J Clin Periodontol*. 2018;45:125-135.
30. Siciliano VI, Salvi GE, Matarasso S, Cafiero C, Blasi A, Lang NP. Soft tissues healing at immediate transmucosal implants placed into molar extraction sites with buccal self-contained dehiscences. A 12-month controlled clinical trial. *Clin Oral Implants Res*. 2009;20:482-488.
31. Hammerle CH, Jung RE. Bone augmentation by means of barrier membranes. *Periodontology 2000*. 2003;33:36-53.
32. Lim G, Lin GH, Monje A, Chan HL, Wang HL. Wound healing complications following guided bone regeneration for ridge augmentation: a systematic review and meta-analysis. *Int J Oral Maxillofac Implants*. 2018;33:41-50.
33. Thoma DS, Bienz SP, Figuero E, Jung RE, Sanz-Martin I. Efficacy of lateral bone augmentation performed simultaneously with dental implant placement. A systematic review and meta-analysis. *J Clin Periodontol*. 2019;46:257-276.
34. Tay JRH, Lu XJ, Lai WMC, Fu JH. Clinical and histological sequelae of surgical complications in horizontal guided bone regeneration: a systematic review and proposal for management. *Int J Implant Dent*. 2020;6:76.
35. Becker J, Al-Nawas B, Klein MO, Schliephake H, Terheyden H, Schwarz F. Use of a new cross-linked collagen membrane for the treatment of dehiscence-type defects at titanium implants: a prospective, randomized-controlled double-blinded clinical multicenter study. *Clin Oral Implants Res*. 2009;20:742-749.
36. Annen BM, Ramel CF, Hammerle CH, Jung RE. Use of a new cross-linked collagen membrane for the treatment of peri-implant dehiscence defects: a randomised controlled double-blinded clinical trial. *Eur J Oral Implantol*. 2011;4:87-100.
37. Lee D-W, Kim K-T, Joo Y-S, Yoo M-K, Yu J-A, Ryu J-J. The role of two different collagen membranes for dehiscence defect around implants in humans. *J Oral Implantol*. 2015;41:445-448.
38. Sanz-Sanchez I, Sanz-Martin I, Ortiz-Vigon A, Molina A, Sanz M. Complications in bone-grafting procedures: classification and management. *Periodontology 2000*. 2022;88:86-102.
39. Garcia J, Dodge A, Luepke P, Wang HL, Kapila Y, Lin GH. Effect of membrane exposure on guided bone regeneration: a systematic review and meta-analysis. *Clin Oral Implants Res*. 2018;29:328-338.
40. Machtei EE. The effect of membrane exposure on the outcome of regenerative procedures in humans: a meta-analysis. *J Periodontol*. 2001;72:512-516.
41. Sanz-Sanchez I, Ortiz-Vigon A, Sanz-Martin I, Figuero E, Sanz M. Effectiveness of lateral bone augmentation on the alveolar crest dimension: a systematic review and meta-analysis. *J Dent Res*. 2015;94:128S-142S.
42. Donos N, Kostopoulos L, Karring T. Alveolar ridge augmentation by combining autogenous mandibular bone grafts and nonresorbable membranes. *Clin Oral Implants Res*. 2002;13:185-191.
43. Greenstein G, Carpentieri JR. Utilization of d-PTFE barriers for post-extraction bone regeneration in preparation for dental implants. *Compend Contin Educ Dent*. 2015;36:465-473.
44. Hoffmann O, Bartee BK, Beaumont C, Kasaj A, Deli G, Zafiropoulos GG. Alveolar bone preservation in extraction sockets using nonresorbable dPTFE membranes: a retrospective non-randomized study. *J Periodontol*. 2008;79:1355-1369.

45. Waasdorp J, Feldman S. Bone regeneration around immediate implants utilizing a dense polytetrafluoroethylene membrane without primary closure: a report of 3 cases. *J Oral Implantol*. 2013;39:355-361.
46. Fontana F, Maschera E, Rocchietta I, Simion M. Clinical classification of complications in guided bone regeneration procedures by means of a nonresorbable membrane. *Int J Periodontics Restorative Dent*. 2011;31:265-273.
47. Vroom MG, Grundemann LJ, Gallo P. Clinical classification of healing complications and management in guided bone regeneration procedures with a nonresorbable d-PTFE membrane. *Int J Periodontics Restorative Dent*. 2022;42:419-427.
48. Tal H, Kozlovsky A, Artzi Z, Nemcovsky CE, Moses O. Cross-linked and non-cross-linked collagen barrier membranes disintegrate following surgical exposure to the oral environment: a histological study in the cat. *Clin Oral Implants Res*. 2008;19:760-766.
49. Friedmann A, Strietzel FP, Maretzki B, Pitaru S, Bernimoulin JP. Histological assessment of augmented jaw bone utilizing a new collagen barrier membrane compared to a standard barrier membrane to protect a granular bone substitute material. *Clin Oral Implants Res*. 2002;13:587-594.
50. Friedmann A, Strietzel FP, Maretzki B, Pitaru S, Bernimoulin JP. Observations on a new collagen barrier membrane in 16 consecutively treated patients. Clinical and hhistological ffindings. *J Periodontol*. 2001;72:1616-1623.
51. Friedmann A, Gissel K, Konermann A, Gotz W. Tissue reactions after simultaneous alveolar ridge augmentation with biphasic calcium phosphate and implant insertion—histological and immunohistochemical evaluation in humans. *Clin Oral Investig*. 2015;19:1595-1603.
52. Park SH, Wang HL. Clinical significance of incision location on guided bone regeneration: human study. *J Periodontol*. 2007;78:47-51.
53. Jemat A, Ghazali MJ, Razali M, Otsuka Y. Surface modifications and their effects on titanium dental implants. *Biomed Res Int*. 2015;2015:791725.
54. Salvi GE, Bosshardt DD, Lang NP, et al. Temporal sequence of hard and soft tissue healing around titanium dental implants. *Periodontology 2000*. 2015;68:135-152.
55. Puleo DA, Nanci A. Understanding and controlling the bone-implant interface. *Biomaterials*. 1999;20:2311-2321.
56. Khan MR, Donos N, Salih V, Brett PM. The enhanced modulation of key bone matrix components by modified titanium implant surfaces. *Bone*. 2012;50:1-8.
57. Wall I, Donos N, Carlqvist K, Jones F, Brett P. Modified titanium surfaces promote accelerated osteogenic differentiation of mesenchymal stromal cells in vitro. *Bone*. 2009;45:17-26.
58. Bornstein MM, Valderrama P, Jones AA, Wilson TG, Seibl R, Cochran DL. Bone apposition around two different sand-blasted and acid-etched titanium implant surfaces: a histomorphometric study in canine mandibles. *Clin Oral Implants Res*. 2008;19:233-241.
59. Buser D, Broggini N, Wieland M, et al. Enhanced bone apposition to a chemically modified SLA titanium surface. *J Dent Res*. 2004;83:529-533.
60. Lang NP, Salvi GE, Huynh-Ba G, Ivanovski S, Donos N, Bosshardt DD. Early osseointegration to hydrophilic and hydrophobic implant surfaces in humans. *Clin Oral Implants Res*. 2011;22:349-356.
61. Hamlet SM, Lee RSB, Moon HJ, Alfarsi MA, Ivanovski S. Hydrophilic titanium surface-induced macrophage modulation promotes pro-osteogenic signalling. *Clin Oral Implants Res*. 2019;30:1085-1096.
62. Alfarsi MA, Hamlet SM, Ivanovski S. The effect of platelet proteins released in response to titanium implant surfaces on macrophage pro-inflammatory cytokine gene expression. *Clin Implant Dent Relat Res*. 2015;17:1036-1047.
63. Stentz WC, Mealey BL, Gunsolley JC, Waldrop TC. Effects of guided bone regeneration around commercially pure titanium and hydroxyapatite-coated dental implants. II. Histologic aanalysis. *J Periodontol*. 1997;68:933-949.
64. Conner KA, Sabatini R, Mealey BL, Takacs VJ, Mills MP, Cochran DL. Guided bone regeneration around titanium plasma-sprayed, acid-etched, and hydroxyapatite-coated implants in the canine model. *J Periodontol*. 2003;74:658-668.
65. Lima LA, Fuchs-Wehrle AM, Lang NP, et al. Surface characteristics of implants influence their bone integration after simultaneous placement of implant and GBR membrane. *Clin Oral Implants Res*. 2003;14:669-679.
66. Schwarz F, Ferrari D, Herten M, et al. Effects of surface hydrophilicity and microtopography on early stages of soft and hard tissue integration at non-submerged titanium implants: an immunohistochemical study in dogs. *J Periodontol*. 2007;78:2171-2184.
67. Schwarz F, Sager M, Kadelka I, Ferrari D, Becker J. Influence of titanium implant surface characteristics on bone regeneration in dehiscence-type defects: an experimental study in dogs. *J Clin Periodontol*. 2010;37:466-473.
68. Zambon R, Mardas N, Horvath A, Petrie A, Dard M, Donos N. The effect of loading in regenerated bone in dehiscence defects following a combined approach of bone grafting and GBR. *Clin Oral Implants Res*. 2012;23:591-601.
69. Linares A, Mardas N, Dard M, Donos N. Effect of immediate or delayed loading following immediate placement of implants with a modified surface. *Clin Oral Implants Res*. 2011;22:38-46.
70. Calciolari E, Mardas N, Dereka X, Anagnostopoulos AK, Tsangaris GT, Donos N. Protein expression during early stages of bone regeneration under hydrophobic and hydrophilic titanium domes. A pilot study. *J Periodontal Res*. 2018;53:174-187.
71. Calciolari E, Hamlet S, Ivanovski S, Donos N. Pro-osteogenic properties of hydrophilic and hydrophobic titanium surfaces: crosstalk between signalling pathways in in vivo models. *J Periodontal Res*. 2018;53:598-609.
72. Donos N, Hamlet S, Lang NP, et al. Gene expression profile of osseointegration of a hydrophilic compared with a hydrophobic microrough implant surface. *Clin Oral Implants Res*. 2011;22:365-372.
73. Doornewaard R, Christiaens V, De Bruyn H, et al. Long-term effect of surface roughness and patients' factors on crestal bone loss at dental implants. A systematic review and meta-analysis. *Clin Implant Dent Relat Res*. 2017;19:372-399.
74. Dahlin C, Linde A, Gottlow J, Nyman S. Healing of bone defects by guided tissue regeneration. *Plast Reconstr Surg*. 1988;81:672-676.
75. Sanz M, Dahlin C, Apatzidou D, et al. Biomaterials and regenerative technologies used in bone regeneration in the craniomaxillofacial region: consensus report of group 2 of the 15th European Workshop on Periodontology on Bone Regeneration. *J Clin Periodontol*. 2019;46(Suppl 21):82-91.
76. Zellin G, Linde A. Effects of different osteopromotive membrane porosities on experimental bone neogenesis in rats. *Biomaterials*. 1996;17:695-702.
77. Lundgren A, Lundgren D, Taylor A. Influence of barrier occlusiveness on guided bone augmentation. An experimental study in the rat. *Clin Oral Implants Res*. 1998;9:251-260.
78. Aristodemou E, Retzepi M, Calciolari E, Donos N. The effect of experimental diabetes and membrane occlusiveness on guided bone regeneration: a proof of principle study. *Clin Oral Investig*. 2022;26:5223-5235.
79. Simion M, Trisi P, Maglione M, Piattelli A. A preliminary report on a method for studying the permeability of expanded polytetrafluoroethylene membrane to bacteria in vitro: a scanning electron microscopic and histological study. *J Periodontol*. 1994;65:755-761.
80. Rossa ML, Lima LA, Pustiglioni FE, et al. SEM analyses of bacterial contamination of e-PTFE membranes and GTR clinical results. *J Int Acad Periodontol*. 2006;8:115-124.

81. Bartee BK. Evaluation of a new polytetrafluoroethylene guided tissue regeneration membrane in healing extraction sites. *Compend Contin Educ Dent*. 1998;19:1256-1258, 1260, 1262–4.
82. Bartee BK, Carr JA. Evaluation of a high-density polytetrafluoroethylene (n-PTFE) membrane as a barrier material to facilitate guided bone regeneration in the rat mandible. *J Oral Implantol*. 1995;21:88-95.
83. Carbonell JM, Martin IS, Santos A, Pujol A, Sanz-Moliner JD, Nart J. High-density polytetrafluoroethylene membranes in guided bone and tissue regeneration procedures: a literature review. *Int J Oral Maxillofac Surg*. 2014;43:75-84.
84. Elgali I, Omar O, Dahlin C, Thomsen P. Guided bone regeneration: materials and biological mechanisms revisited. *Eur J Oral Sci*. 2017;125:315-337.
85. Nishimura I, Shimizu Y, Ooya K. Effects of cortical bone perforation on experimental guided bone regeneration. *Clin Oral Implants Res*. 2004;15:293-300.
86. Chu C, Deng J, Sun X, Qu Y, Man Y. Collagen membrane and immune response in guided bone regeneration: recent progress and perspectives. *Tissue Eng Part B Rev*. 2017;23:421-435.
87. Shim JH, Jeong JH, Won JY, et al. Porosity effect of 3D-printed polycaprolactone membranes on calvarial defect model for guided bone regeneration. *Biomed Mater*. 2017;13:015014.
88. de Santana RB, de Mattos CM, Francischone CE, Van Dyke T. Superficial topography and porosity of an absorbable barrier membrane impacts soft tissue response in guided bone regeneration. *J Periodontol*. 2010;81:926-933.
89. Oh SH, Kim TH, Chun SY, Park EK, Lee JH. Enhanced guided bone regeneration by asymmetrically porous PCL/pluronic F127 membrane and ultrasound stimulation. *J Biomater Sci Polym Ed*. 2012;23:1673-1686.
90. Kim TH, Oh SH, Na SY, Chun SY, Lee JH. Effect of biological/physical stimulation on guided bone regeneration through asymmetrically porous membrane. *J Biomed Mater Res A*. 2012;100:1512-1520.
91. Liu W, Dong X, Qin H, Sui L, Wang J. Three-dimensional porous reduced graphene oxide/hydroxyapatite membrane for guided bone regeneration. *Colloids Surf B Biointerfaces*. 2021;208:112102.
92. Heck DA, Nakajima I, Kelly PJ, Chao EY. The effect of load alteration on the biological and biomechanical performance of a titanium fiber-metal segmental prosthesis. *J Bone Joint Surg Am*. 1986;68:118-126.
93. Ducheyne P, De Meester P, Aernoudt E. Influence of a functional dynamic loading on bone ingrowth into surface pores of orthopedic implants. *J Biomed Mater Res*. 1977;11:811-838.
94. Simion M, Misitano U, Gionso L, Salvato A. Treatment of dehiscences and fenestrations around dental implants using resorbable and nonresorbable membranes associated with bone autografts: a comparative clinical study. *Int J Oral Maxillofac Implants*. 1997;12:159-167.
95. Schneider D, Weber FE, Grunder U, Andreoni C, Burkhardt R, Jung RE. A randomized controlled clinical multicenter trial comparing the clinical and histological performance of a new, modified polylactide-co-glycolide acid membrane to an expanded polytetrafluorethylene membrane in guided bone regeneration procedures. *Clin Oral Implants Res*. 2014;25:150-158.
96. Naenni N, Schneider D, Jung RE, Husler J, Hammerle CHF, Thoma DS. Randomized clinical study assessing two membranes for guided bone regeneration of peri-implant bone defects: clinical and histological outcomes at 6 months. *Clin Oral Implants Res*. 2017;28:1309-1317.
97. Moses O, Pitaru S, Artzi Z, Nemcovsky CE. Healing of dehiscence-type defects in implants placed together with different barrier membranes: a comparative clinical study. *Clin Oral Implants Res*. 2005;16:210-219.
98. Veis AA, Tsirlis AT, Parisis NA. Effect of autogenous harvest site location on the outcome of ridge augmentation for implant dehiscences. *Int J Periodontics Restorative Dent*. 2004;24:155-163.
99. Mattout P, Nowzari H, Mattout C. Clinical evaluation of guided bone regeneration at exposed parts of Branemark dental implants with and without bone allograft. *Clin Oral Implants Res*. 1995;6:189-195.
100. Carpio L, Loza J, Lynch S, Genco R. Guided bone regeneration around endosseous implants with anorganic bovine bone mineral. A randomized controlled trial comparing bioabsorbable versus non-resorbable barriers. *J Periodontol*. 2000;71:1743-1749.
101. Amano Y, Ota M, Sekiguchi K, Shibukawa Y, Yamada S. Evaluation of a poly-l-lactic acid membrane and membrane fixing pin for guided tissue regeneration on bone defects in dogs. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2004;97:155-163.
102. Mir-Mari J, Benic GI, Valmaseda-Castellon E, Hammerle CHF, Jung RE. Influence of wound closure on the volume stability of particulate and non-particulate GBR materials: an in vitro cone-beam computed tomographic examination. Part II. *Clin Oral Implants Res*. 2017;28:631-639.
103. Mir-Mari J, Wui H, Jung RE, Hammerle CH, Benic GI. Influence of blinded wound closure on the volume stability of different GBR materials: an in vitro cone-beam computed tomographic examination. *Clin Oral Implants Res*. 2016;27:258-265.
104. Park JY, Song YW, Ko KA, Strauss FJ, Thoma DS, Lee JS. Effect of collagen membrane fixation on ridge volume stability and new bone formation following guided bone regeneration. *J Clin Periodontol*. 2022;49:684-693.
105. Urban IA, Lozada JL, Wessing B, Suarez-Lopez del Amo F, Wang HL. Vertical bone grafting and periosteal vertical mattress suture for the fixation of resorbable membranes and stabilization of particulate grafts in horizontal guided bone regeneration to achieve more predictable results: a technical report. *Int J Periodontics Restorative Dent*. 2016;36:153-159.
106. Jovanovic SA, Spiekermann H, Richter EJ. Bone regeneration around titanium dental implants in dehisced defect sites: a clinical study. *Int J Oral Maxillofac Implants*. 1992;7:233-245.
107. Lekholm U, Becker W, Dahlin C, Becker B, Donath K, Morrison E. The role of early versus late removal of GTAM membranes on bone formation at oral implants placed into immediate extraction sockets. An experimental study in dogs. *Clin Oral Implants Res*. 1993;4:121-129.
108. Simion M, Baldoni M, Rossi P, Zaffe D. A comparative study of the effectiveness of e-PTFE membranes with and without early exposure during the healing period. *Int J Periodontics Restorative Dent*. 1994;14:166-180.
109. Gher ME, Quintero G, Assad D, Monaco E, Richardson AC. Bone grafting and guided bone regeneration for immediate dental implants in humans. *J Periodontol*. 1994;65:881-891.
110. Donos N, Kostopoulos L, Karring T. Alveolar ridge augmentation using a resorbable copolymer membrane and autogenous bone grafts. An experimental study in the rat. *Clin Oral Implants Res*. 2002;13:203-213.
111. Donos N, Kostopoulos L, Karring T. Augmentation of the rat jaw with autogeneic cortico-cancellous bone grafts and guided tissue regeneration. *Clin Oral Implants Res*. 2002;13:192-202.
112. Donos N, Kostopoulos L, Karring T. Augmentation of the mandible with GTR and onlay cortical bone grafting. An experimental study in the rat. *Clin Oral Implants Res*. 2002;13:175-184.
113. Nowzari H, Slots J. Microorganisms in polytetrafluoroethylene barrier membranes for guided tissue regeneration. *J Clin Periodontol*. 1994;21:203-210.
114. Nowzari H, Slots J. Microbiologic and clinical study of polytetrafluoroethylene membranes for guided bone regeneration around implants. *Int J Oral Maxillofac Implants*. 1995;10:67-73.

115. Calciolari E, Ravanetti F, Strange A, et al. Degradation pattern of a porcine collagen membrane in an in vivo model of guided bone regeneration. *J Periodontal Res*. 2018;53:430-439.
116. Schwarz F, Schmucker A, Becker J. Long-term outcomes of simultaneous guided bone regeneration using native and cross-linked collagen membranes after 8 years. *Clin Oral Implants Res*. 2017;28:779-784.
117. Lee J, Park S, Kim D, Jung U. Assessment of clinical and radiographic outcomes of guided bone regeneration with dehydrothermally cross-linked collagen membrane around peri-implant dehiscence defects: results from a 3-year randomized clinical trial. *Oral Biol Res*. 2019;43:8-16.
118. Benic GI, Bienz SP, Song YW, et al. Randomized controlled clinical trial comparing guided bone regeneration of peri-implant defects with soft-type block versus particulate bone substitutes: six-month results of hard-tissue changes. *J Clin Periodontol*. 2022;49:480-495.
119. Rothamel D, Schwarz F, Sager M, Herten M, Sculean A, Becker J. Biodegradation of differently cross-linked collagen membranes: an experimental study in the rat. *Clin Oral Implants Res*. 2005;16:369-378.
120. Scheyer ET, McGuire MK. Evaluation of premature membrane exposure and early healing in guided bone regeneration of peri-implant dehiscence and fenestration defects with a slowly resorbing porcine collagen ribose cross-linked membrane: a consecutive case series. *Clin Adv Periodontics*. 2015;5:165-170.
121. Friedmann A, Gissel K, Soudan M, Kleber BM, Pitaru S, Dietrich T. Randomized controlled trial on lateral augmentation using two collagen membranes: morphometric results on mineralized tissue compound. *J Clin Periodontol*. 2011;38:677-685.
122. Kim YK, Kim SG, Lim SC, Lee HJ, Yun PY. A clinical study on bone formation using a demineralized bone matrix and resorbable membrane. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;109:e6-e11.
123. Jung RE, Benic GI, Scherrer D, Hammerle CH. Cone beam computed tomography evaluation of regenerated buccal bone 5 years after simultaneous implant placement and guided bone regeneration procedures—a randomized, controlled clinical trial. *Clin Oral Implants Res*. 2015;26:28-34.
124. Gentile P, Chiono V, Tonda-Turo C, Ferreira AM, Ciardelli G. Polymeric membranes for guided bone regeneration. *Biotechnol J*. 2011;6:1187-1197.
125. Sanz-Sanchez I, Carrillo de Albornoz A, Figuero E, et al. Effects of lateral bone augmentation procedures on peri-implant health or disease: a systematic review and meta-analysis. *Clin Oral Implants Res*. 2018;29(Suppl 15):18-31.
126. Basler T, Naenni N, Schneider D, Hammerle CHF, Jung RE, Thoma DS. Randomized controlled clinical study assessing two membranes for guided bone regeneration of peri-implant bone defects: 3-year results. *Clin Oral Implants Res*. 2018;29:499-507.
127. Zitzmann NU, Naef R, Scharer P. Resorbable versus nonresorbable membranes in combination with Bio-Oss for guided bone regeneration. *Int J Oral Maxillofac Implants*. 1997;12:844-852.
128. Omar O, Elgali I, Dahlin C, Thomsen P. Barrier membranes: more than the barrier effect? *J Clin Periodontol*. 2019;46(Suppl 21):103-123.
129. Elgali I, Turri A, Xia W, et al. Guided bone regeneration using resorbable membrane and different bone substitutes: early histological and molecular events. *Acta Biomater*. 2016;29:409-423.
130. Garg K, Pullen NA, Oskeritzian CA, Ryan JJ, Bowlin GL. Macrophage functional polarization (M1/M2) in response to varying fiber and pore dimensions of electrospun scaffolds. *Biomaterials*. 2013;34:4439-4451.
131. Spiller KL, Nassiri S, Witherel CE, et al. Sequential delivery of immunomodulatory cytokines to facilitate the M1-to-M2 transition of macrophages and enhance vascularization of bone scaffolds. *Biomaterials*. 2015;37:194-207.
132. Abou Fadel R, Samarani R, Chakar C. Guided bone regeneration in calvarial critical size bony defect using a double-layer resorbable collagen membrane covering a xenograft: a histological and histomorphometric study in rats. *Oral Maxillofac Surg*. 2018;22:203-213.
133. Calciolari E, Mardas N, Dereka X, Kostomitsopoulos N, Petrie A, Donos N. The effect of experimental osteoporosis on bone regeneration: part 1, histology findings. *Clin Oral Implants Res*. 2017;28:e101-e110.
134. Kozlovsky A, Aboodi G, Moses O, et al. Bio-degradation of a resorbable collagen membrane (Bio-Gide) applied in a double-layer technique in rats. *Clin Oral Implants Res*. 2009;20:1116-1123.
135. von Arx T, Buser D. Horizontal ridge augmentation using autogenous block grafts and the guided bone regeneration technique with collagen membranes: a clinical study with 42 patients. *Clin Oral Implants Res*. 2006;17:359-366.
136. Buser D, Martin W, Belser UC. Optimizing esthetics for implant restorations in the anterior maxilla: anatomic and surgical considerations. *Int J Oral Maxillofac Implants*. 2004;19(Suppl):43-61.
137. Cordaro L, Torsello F, Morcavallo S, di Torresanto VM. Effect of bovine bone and collagen membranes on healing of mandibular bone blocks: a prospective randomized controlled study. *Clin Oral Implants Res*. 2011;22:1145-1150.
138. Al-Hezaimi K, Rudek I, Al-Hamdan KS, Javed F, Nooh N, Wang HL. Efficacy of using a dual layer of membrane (dPTFE placed over collagen) for ridge preservation in fresh extraction sites: a micro-computed tomographic study in dogs. *Clin Oral Implants Res*. 2013;24:1152-1157.
139. Lee J, Lim YJ, Kim B, Koo KT, Lee YM. Biphasic calcium phosphate sphere graft combined with a double-layer non-crosslinked collagen membrane technique for ridge preservation: a randomized controlled animal study. *Materials (Basel)*. 2019;13:13.
140. Choi HK, Cho HY, Lee SJ, et al. Alveolar ridge preservation with an open-healing approach using single-layer or double-layer coverage with collagen membranes. *J Periodontal Implant Sci*. 2017;47:372-380.
141. Mau JL, Grodin E, Lin JJ, Chen MCJ, Ho CH, Cochran D. A comparative, randomized, prospective, two-center clinical study to evaluate the clinical and esthetic outcomes of two different bone grafting techniques in early implant placement. *J Periodontol*. 2019;90:247-255.
142. Tsai YL, Tsao JP, Wang CL, et al. Stability of contour augmentation of implant-supported single crowns in the esthetic zone: one-year cone-beam computed tomography results of a comparative, randomized, prospective, two-center clinical study using two different bone grafting techniques in early implant placement. *J Periodontol*. 2022;93:1661-1670.
143. Claussen KU, Giesa R, Scheibel T, Schmidt HW. Learning from nature: synthesis and characterization of longitudinal polymer gradient materials inspired by mussel byssus threads. *Macromol Rapid Commun*. 2012;33:206-211.
144. Urist MR, Strates BS. Bone formation in implants of partially and wholly demineralized bone matrix. Including observations on acetone-fixed intra and extracellular proteins. *Clin Orthop Relat Res*. 1970;71:271-278.
145. Vaziri S, Vahabi S, Torshabi M, Hematzadeh S. In vitro assay for osteoinductive activity of different demineralized freeze-dried bone allograft. *J Periodontal Implant Sci*. 2012;42:224-230.
146. Cammack GV 2nd, Nevins M, Clem DS 3rd, Hatch JP, Mellonig JT. Histologic evaluation of mineralized and demineralized freeze-dried bone allograft for ridge and sinus augmentations. *Int J Periodontics Restorative Dent*. 2005;25:231-237.
147. Gothi R, Bansal M, Kaushik M, Khattak BP, Sood N, Taneja V. A comparative evaluation of freeze dried bone allograft and

decalcified freeze dried bone allograft in the treatment of intrabony defects: a clinical and radiographic study. *J Indian Soc Periodontol*. 2015;19:411-415.
148. Chen S. Experimental study on recombined xenogeneic bone transplants in the mandible. *Zhonghua Kou Qiang Yi Xue Za Zhi*. 1987;22:261-264, 310, 221.
149. Pinholt EM, Bang G, Haanaes HR. Alveolar ridge augmentation in rats by Bio-Oss. *Scand J Dent Res*. 1991;99:154-161.
150. Ma WG. An experimental study on ceramic xenogenic bone in reconstructing defects of the mandible in rabbits. *Zhonghua Kou Qiang Yi Xue Za Zhi*. 1989;24:212-214, 255.
151. Smiler DG, Johnson PW, Lozada JL, et al. Sinus lift grafts and endosseous implants. Treatment of the atrophic posterior maxilla. *Dent Clin N Am*. 1992;36:151-186; discussion 187-158.
152. Berglundh T, Lindhe J. Healing around implants placed in bone defects treated with Bio-Oss. An experimental study in the dog. *Clin Oral Implants Res*. 1997;8:117-124.
153. Orsini G, Traini T, Scarano A, et al. Maxillary sinus augmentation with Bio-Oss particles: a light, scanning, and transmission electron microscopy study in man. *J Biomed Mater Res B Appl Biomater*. 2005;74:448-457.
154. Tapety FI, Amizuka N, Uoshima K, Nomura S, Maeda T. A histological evaluation of the involvement of Bio-Oss in osteoblastic differentiation and matrix synthesis. *Clin Oral Implants Res*. 2004;15:315-324.
155. Hammerle CH, Olah AJ, Schmid J, et al. The biological effect of natural bone mineral on bone neoformation on the rabbit skull. *Clin Oral Implants Res*. 1997;8:198-207.
156. Donos N, Bosshardt D, Lang N, et al. Bone formation by enamel matrix proteins and xenografts: an experimental study in the rat ramus. *Clin Oral Implants Res*. 2005;16:140-146.
157. Stavropoulos A, Kostopoulos L, Mardas N, Nyengaard JR, Karring T. Deproteinized bovine bone used as an adjunct to guided bone augmentation: an experimental study in the rat. *Clin Implant Dent Relat Res*. 2001;3:156-165.
158. Schmid J, Hammerle CH, Fluckiger L, et al. Blood-filled spaces with and without filler materials in guided bone regeneration. A comparative experimental study in the rabbit using bioresorbable membranes. *Clin Oral Implants Res*. 1997;8:75-81.
159. Slotte C, Lundgren D. Augmentation of calvarial tissue using non-permeable silicone domes and bovine bone mineral. An experimental study in the rat. *Clin Oral Implants Res*. 1999;10:468-476.
160. Caballé-Serrano J, Bosshardt DD, Buser D, Gruber R. Proteomic analysis of porcine bone-conditioned medium. *Int J Oral Maxillofac Implants*. 2014;29:1208-1215d.
161. Caballe-Serrano J, Fujioka-Kobayashi M, Bosshardt DD, Gruber R, Buser D, Miron RJ. Pre-coating deproteinized bovine bone mineral (DBBM) with bone-conditioned medium (BCM) improves osteoblast migration, adhesion, and differentiation in vitro. *Clin Oral Investig*. 2016;20:2507-2513.
162. Parisi L, Buser D, Chappuis V, Asparuhova MB. Cellular responses to deproteinized bovine bone mineral biofunctionalized with bone-conditioned medium. *Clin Oral Investig*. 2021;25:2159-2173.
163. Neto AS, Ferreira JMF. Synthetic and marine-derived porous scaffolds for bone tissue engineering. *Materials (Basel)*. 2018;11:1702.
164. Kolk A, Handschel J, Drescher W, et al. Current trends and future perspectives of bone substitute materials – from space holders to innovative biomaterials. *J Craniomaxillofac Surg*. 2012;40:706-718.
165. Jung RE, Herzog M, Wolleb K, Ramel CF, Thoma DS, Hammerle CH. A randomized controlled clinical trial comparing small buccal dehiscence defects around dental implants treated with guided bone regeneration or left for spontaneous healing. *Clin Oral Implants Res*. 2017;28:348-354.
166. Waller T, Herzog M, Thoma DS, Hüsler J, Hämmerle CHF, Jung RE. Long-term clinical and radiographic results after treatment or no treatment of small buccal bone dehiscences at posterior dental implants: a randomized, controlled clinical trial. *Clin Oral Implants Res*. 2020;31:517-525.
167. Sanz-Martin I, Regidor E, Cosyn J, Wiedemeier DB, Thoma DS. Buccal soft tissue dehiscence defects at dental implants-associated factors and frequency of occurrence: a systematic review and meta-analysis. *Clin Oral Implants Res*. 2022;33(Suppl 23):109-124.
168. Monje A, Roccuzzo A, Buser D, Wang HL. Influence of buccal bone wall thickness on the peri-implant hard and soft tissue dimensional changes: a systematic review. *Clin Oral Implants Res*. 2023;34:157-176.
169. Monje A, Chappuis V, Monje F, et al. The critical peri-implant buccal bone wall thickness revisited: an experimental study in the beagle dog. *Int J Oral Maxillofac Implants*. 2019;34:1328-1336.
170. Farronato D, Pasini PM, Orsina AA, Manfredini M, Azzi L, Farronato M. Correlation between buccal bone thickness at implant placement in healed sites and buccal soft tissue maturation pattern: a prospective three-year study. *Materials (Basel)*. 2020;13:511.
171. De Santis E, Lang NP, Favero G, Beolchini M, Morelli F, Botticelli D. Healing at mandibular block-grafted sites. An experimental study in dogs. *Clin Oral Implants Res*. 2015;26:516-522.
172. Ozaki W, Buchman SR, Goldstein SA, Fyhrie DP. A comparative analysis of the microarchitecture of cortical membranous and cortical endochondral onlay bone grafts in the craniofacial skeleton. *Plast Reconstr Surg*. 1999;104:139-147.
173. Ozaki W, Buchman SR. Volume maintenance of onlay bone grafts in the craniofacial skeleton: micro-architecture versus embryologic origin. *Plast Reconstr Surg*. 1998;102:291-299.
174. Donos N, Kostopoulos L, Tonetti M, Karring T. Long-term stability of autogenous bone grafts following combined application with guided bone regeneration. *Clin Oral Implants Res*. 2005;16:133-139.
175. Liang L, Rulis P, Ching WY. Mechanical properties, electronic structure and bonding of alpha- and beta-tricalcium phosphates with surface characterization. *Acta Biomater*. 2010;6:3763-3771.
176. Pallesen L, Schou S, Aaboe M, Hjørting-Hansen E, Nattestad A, Melsen F. Influence of particle size of autogenous bone grafts on the early stages of bone regeneration: a histologic and stereologic study in rabbit calvarium. *Int J Oral Maxillofac Implants*. 2002;17:498-506.
177. Lee DS, Pai Y, Chang S, Kim DH. Microstructure, physical properties, and bone regeneration effect of the nano-sized beta-tricalcium phosphate granules. *Mater Sci Eng C Mater Biol Appl*. 2016;58:971-976.
178. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials*. 2005;26:5474-5491.
179. Eggli PS, Muller W, Schenk RK. Porous hydroxyapatite and tricalcium phosphate cylinders with two different pore size ranges implanted in the cancellous bone of rabbits. A comparative histomorphometric and histologic study of bony ingrowth and implant substitution. *Clin Orthop Relat Res*. 1988;232:127-138.
180. Hing KA, Best SM, Tanner KE, Bonfield W, Revell PA. Mediation of bone ingrowth in porous hydroxyapatite bone graft substitutes. *J Biomed Mater Res A*. 2004;68:187-200.
181. Kuhne JH, Bartl R, Frisch B, Hammer C, Jansson V, Zimmer M. Bone formation in coralline hydroxyapatite. Effects of pore size studied in rabbits. *Acta Orthop Scand*. 1994;65:246-252.
182. Hing KA, Annaz B, Saeed S, Revell PA, Buckland T. Microporosity enhances bioactivity of synthetic bone graft substitutes. *J Mater Sci Mater Med*. 2005;16:467-475.
183. Campion CR, Chander C, Buckland T, Hing K. Increasing strut porosity in silicate-substituted calcium-phosphate bone graft substitutes enhances osteogenesis. *J Biomed Mater Res B Appl Biomater*. 2011;97:245-254.
184. Benic GI, Eisner BM, Jung RE, Basler T, Schneider D, Hammerle CHF. Hard tissue changes after guided bone regeneration of peri-implant defects comparing block versus particulate bone

substitutes: 6-month results of a randomized controlled clinical trial. *Clin Oral Implants Res*. 2019;30:1016-1026.
185. Riachi F, Naaman N, Tabarani C, et al. Influence of material properties on rate of resorption of two bone graft materials after sinus lift using radiographic assessment. *Int J Dent*. 2012;2012:737262.
186. Dinopoulos H, Dimitriou R, Giannoudis PV. Bone graft substitutes: what are the options? *The Surgeon*. 2012;10:230-239.
187. Burchardt H, Enneking WF. Transplantation of bone. *Surg Clin North Am*. 1978;58:403-427.
188. Prolo DJ, Rodrigo JJ. Contemporary bone graft physiology and surgery. *Clin Orthop Relat Res*. 1985;200:322-342.
189. Bauer TW, Muschler GF. Bone graft materials. An overview of the basic science. *Clin Orthop Relat Res*. 2000;371:10-27.
190. Lioubavina N, Kostopoulos L, Wenzel A, Karring T. Long-term stability of jaw bone tuberosities formed by "guided tissue regeneration". *Clin Oral Implants Res*. 1999;10:477-486.
191. Buser D, Ruskin J, Higginbottom F, Hardwick R, Dahlin C, Schenk RK. Osseointegration of titanium implants in bone regenerated in membrane-protected defects: a histologic study in the canine mandible. *Int J Oral Maxillofac Implants*. 1995;10:666-681.
192. Gordh M, Alberius P, Johnell O, Lindberg L, Linde A. Osteopromotive membranes enhance onlay integration and maintenance in the adult rat skull. *Int J Oral Maxillofac Surg*. 1998;27:67-73.
193. Maiorana C, Beretta M, Battista Grossi G, et al. Histomorphometric evaluation of anorganic bovine bone coverage to reduce autogenous grafts resorption: preliminary results. *Open Dent J*. 2011;5:71-78.
194. Maiorana C, Beretta M, Salina S, Santoro F. Reduction of autogenous bone graft resorption by means of Bio-Oss coverage: a prospective study. *Int J Periodontics Restorative Dent*. 2005;25:19-25.
195. Batista JD, Justino Oliveira Limirio PH, Rocha FS, Gomes Moura CC, Zanetta-Barbosa D, Dechichi P. Influence of platelet-poor plasma on angiogenesis and maintenance of volume in autogenous bone grafts. *J Oral Maxillofac Surg*. 2018;76:2097-2102.
196. Khan SN, Cammisa FP Jr, Sandhu HS, Diwan AD, Girardi FP, Lane JM. The biology of bone grafting. *J Am Acad Orthop Surg*. 2005;13:77-86.
197. Hammerle CH, Chiantella GC, Karring T, Lang NP. The effect of a deproteinized bovine bone mineral on bone regeneration around titanium dental implants. *Clin Oral Implants Res*. 1998;9:151-162.
198. Sartori S, Silvestri M, Forni F, Icaro Cornaglia A, Tesei P, Cattaneo V. Ten-year follow-up in a maxillary sinus augmentation using anorganic bovine bone (Bio-Oss). A case report with histomorphometric evaluation. *Clin Oral Implants Res*. 2003;14:369-372.
199. Ayna M, Acil Y, Gulses A. Fate of a bovine-derived xenograft in maxillary sinus floor elevation after 14 years: histologic and radiologic analysis. *Int J Periodontics Restorative Dent*. 2015;35:541-547.
200. Schlegel AK, Donath K. BIO-OSS—a resorbable bone substitute? *J Long Term Eff Med Implants*. 1998;8:201-209.
201. Mordenfeld A, Hallman M, Johansson CB, Albrektsson T. Histological and histomorphometrical analyses of biopsies harvested 11 years after maxillary sinus floor augmentation with deproteinized bovine and autogenous bone. *Clin Oral Implants Res*. 2010;21:961-970.
202. Urban IA, Nagursky H, Lozada JL, Nagy K. Horizontal ridge augmentation with a collagen membrane and a combination of particulated autogenous bone and anorganic bovine bone-derived mineral: a prospective case series in 25 patients. *Int J Periodontics Restorative Dent*. 2013;33:299-307.
203. Simion M, Fontana F, Rasperini G, Maiorana C. Vertical ridge augmentation by expanded-polytetrafluoroethylene membrane and a combination of intraoral autogenous bone graft and deproteinized anorganic bovine bone (Bio Oss). *Clin Oral Implants Res*. 2007;18:620-629.
204. Park SH, Lee KW, Oh TJ, Misch CE, Shotwell J, Wang HL. Effect of absorbable membranes on sandwich bone augmentation. *Clin Oral Implants Res*. 2008;19:32-41.
205. Fu JH, Oh TJ, Benavides E, Rudek I, Wang HL. A randomized clinical trial evaluating the efficacy of the sandwich bone augmentation technique in increasing buccal bone thickness during implant placement surgery: I. Clinical and radiographic parameters. *Clin Oral Implants Res*. 2014;25:458-467.
206. Garaicoa C, Suarez F, Fu JH, et al. Using cone beam computed tomography angle for predicting the outcome of horizontal bone augmentation. *Clin Implant Dent Relat Res*. 2015;17:717-723.
207. Lee A, Brown D, Wang HL. Sandwich bone augmentation for predictable horizontal bone augmentation. *Implant Dent*. 2009;18:282-290.
208. Wang HL, Misch C, Neiva RF. "Sandwich" bone augmentation technique: rationale and report of pilot cases. *Int J Periodontics Restorative Dent*. 2004;24:232-245.
209. Buser D, Chappuis V, Bornstein MM, Wittneben JG, Frei M, Belser UC. Long-term stability of contour augmentation with early implant placement following single tooth extraction in the esthetic zone: a prospective, cross-sectional study in 41 patients with a 5- to 9-year follow-up. *J Periodontol*. 2013;84:1517-1527.
210. Jensen SS, Bosshardt DD, Gruber R, Buser D. Long-term stability of contour augmentation in the esthetic zone: histologic and histomorphometric evaluation of 12 human biopsies 14 to 80 months after augmentation. *J Periodontol*. 2014;85:1549-1556.
211. Dahlin C, Obrecht M, Dard M, Donos N. Bone tissue modelling and remodelling following guided bone regeneration in combination with biphasic calcium phosphate materials presenting different microporosity. *Clin Oral Implants Res*. 2015;26:814-822.
212. Wang HL, Boyapati L. "PASS" principles for predictable bone regeneration. *Implant Dent*. 2006;15:8-17.
213. Polimeni G, Albandar JM, Wikesjö UM. Prognostic factors for alveolar regeneration: effect of space provision. *J Clin Periodontol*. 2005;32:951-954.
214. Kostopoulos L, Karring T, Uraguchi R. Formation of jawbone tuberosities by guided tissue regeneration. An experimental study in the rat. *Clin Oral Implants Res*. 1994;5:245-253.
215. Wessing B, Urban I, Montero E, et al. A multicenter randomized controlled clinical trial using a new resorbable non-cross-linked collagen membrane for guided bone regeneration at dehisced single implant sites: interim results of a bone augmentation procedure. *Clin Oral Implants Res*. 2017;28:e218-e226.
216. Hürzeler MB, Quiñones CR, Schüpbach P. Guided bone regeneration around dental implants in the atrophic alveolar ridge using a bioresorbable barrier. An experimental study in the monkey. *Clin Oral Implants Res*. 1997;8:323-331.
217. Yamada M, Egusa H. Current bone substitutes for implant dentistry. *J Prosthodont Res*. 2018;62:152-161.
218. Cornu O, Boquet J, Nonclercq O, et al. Synergetic effect of freeze-drying and gamma irradiation on the mechanical properties of human cancellous bone. *Cell Tissue Bank*. 2011;12:281-288.
219. An YZ, Strauss FJ, Park JY, Shen YQ, Thoma DS, Lee JS. Membrane fixation enhances guided bone regeneration in standardized calvarial defects: a pre-clinical study. *J Clin Periodontol*. 2022;49:177-187.
220. Hämmerle CH, Schmid J, Lang NP, Olah AJ. Temporal dynamics of healing in rabbit cranial defects using guided bone regeneration. *J Oral Maxillofac Surg*. 1995;53:167-174.
221. Gallucci GO, Hamilton A, Zhou W, Buser D, Chen S. Implant placement and loading protocols in partially edentulous patients: a systematic review. *Clin Oral Implants Res*. 2018;29(Suppl 16):106-134.
222. Becker W, Lekholm U, Dahlin C, Becker BE, Donath K. The effect of clinical loading on bone regenerated by gtam barriers: a study in dogs. *J Oral Maxillofac Implants*. 1994;9:305-313.

223. Arunjaroensuk S, Panmekiate S, Pimkhaokham A. The stability of augmented bone between two different membranes used for guided bone regeneration simultaneous with dental implant placement in the esthetic zone. *Int J Oral Maxillofac Implants*. 2018;33:206-216.
224. Wessing B, Lettner S, Zechner W. Guided bone regeneration with collagen membranes and particulate graft materials: a systematic review and meta-analysis. *Int J Oral Maxillofac Implants*. 2018;33:87-100.
225. Clementini M, Morlupi A, Canullo L, Agrestini C, Barlattani A. Success rate of dental implants inserted in horizontal and vertical guided bone regenerated areas: a systematic review. *Int J Oral Maxillofac Surg*. 2012;41:847-852.
226. Buser D, Bornstein MM, Weber HP, Grutter L, Schmid B, Belser UC. Early implant placement with simultaneous guided bone regeneration following single-tooth extraction in the esthetic zone: a cross-sectional, retrospective study in 45 subjects with a 2- to 4-year follow-up. *J Periodontol*. 2008;79:1773-1781.
227. Schnitman PA, Wohrle PS, Rubenstein JE. Immediate fixed interim prostheses supported by two-stage threaded implants: methodology and results. *J Oral Implantol*. 1990;16:96-105.
228. Ericsson I, Nilson H, Lindh T, Nilner K, Randow K. Immediate functional loading of Branemark single tooth implants. An 18 months' clinical pilot follow-up study. *Clin Oral Implants Res*. 2000;11:26-33.
229. Cooper LF, Rahman A, Moriarty J, Chaffee N, Sacco D. Immediate mandibular rehabilitation with endosseous implants: simultaneous extraction, implant placement, and loading. *Int J Oral Maxillofac Implants*. 2002;17:517-525.
230. Margossian P, Mariani P, Stephan G, Margerit J, Jorgensen C. Immediate loading of mandibular dental implants in partially edentulous patients: a prospective randomized comparative study. *Int J Periodontics Restorative Dent*. 2012;32:e51-e58.
231. Donos N, Asche NV, Akbar AN, et al. Impact of timing of dental implant placement and loading: summary and consensus statements of group 1-the 6th EAO consensus conference 2021. *Clin Oral Implants Res*. 2021;32(Suppl 21):85-92.
232. Trisi P, Perfetti G, Baldoni E, Berardi D, Colagiovanni M, Scogna G. Implant micromotion is related to peak insertion torque and bone density. *Clin Oral Implants Res*. 2009;20:467-471.
233. Javed F, Romanos GE. The role of primary stability for successful immediate loading of dental implants. A literature review. *J Dent*. 2010;38:612-620.
234. Javed F, Ahmed HB, Crespi R, Romanos GE. Role of primary stability for successful osseointegration of dental implants: factors of influence and evaluation. *Interv Med Appl Sci*. 2013;5:162-167.
235. Shibly O, Patel N, Albandar JM, Kutkut A. Bone regeneration around implants in periodontally compromised patients: a randomized clinical trial of the effect of immediate implant with immediate loading. *J Periodontol*. 2010;81:1743-1751.
236. Levin BP, Wilk BL. Immediate provisionalization of immediate implants in the esthetic zone: a prospective case series evaluating implant survival, esthetics, and bone maintenance. *Compend Contin Educ Dent*. 2013;34:352-361.
237. Kolerman R, Nissan J, Mijiritsky E, Hamoudi N, Mangano C, Tal H. Esthetic assessment of immediately restored implants combined with GBR and free connective tissue graft. *Clin Oral Implants Res*. 2016;27:1414-1422.
238. Hall JA, Payne AG, Purton DG, Torr B, Duncan WJ, De Silva RK. Immediately restored, single-tapered implants in the anterior maxilla: prosthodontic and aesthetic outcomes after 1 year. *Clin Implant Dent Relat Res*. 2007;9:34-45.
239. den Hartog L, Raghoebar GM, Stellingsma K, Vissink A, Meijer HJ. Immediate non-occlusal loading of single implants in the aesthetic zone: a randomized clinical trial. *J Clin Periodontol*. 2011;38:186-194.
240. Donos N, Horvath A, Mezzomo LA, Dedi D, Calciolari E, Mardas N. The role of immediate provisional restorations on implants with a hydrophilic surface: a randomised, single-blind controlled clinical trial. *Clin Oral Implants Res*. 2018;29:55-66.
241. Donos N, Horvath A, Calciolari E, Mardas N. Immediate provisionalization of bone level implants with a hydrophilic surface. A five-year follow-up of a randomized controlled clinical trial. *Clin Oral Implants Res*. 2019;30:139-149.
242. Mattheos N, Vergoullis I, Janda M, Miseli A. The implant supracrestal complex and its significance for long-term successful clinical outcomes. *Int J Prosthodont*. 2021;34:88-100.
243. Benic GI, Ge Y, Gallucci GO, Jung RE, Schneider D, Hammerle CH. Guided bone regeneration and abutment connection augment the buccal soft tissue contour: 3-year results of a prospective comparative clinical study. *Clin Oral Implants Res*. 2017;28:219-225.
244. Kan JY, Rungcharassaeng K, Sclar A, Lozada JL. Effects of the facial osseous defect morphology on gingival dynamics after immediate tooth replacement and guided bone regeneration: 1-year results. *J Oral Maxillofac Surg*. 2007;65:13-19.
245. Linkevicius T, Vaitelis J. The effect of zirconia or titanium as abutment material on soft peri-implant tissues: a systematic review and meta-analysis. *Clin Oral Implants Res*. 2015;26(Suppl 11):139-147.
246. Chappuis V, Rahman L, Buser R, Janner SFM, Belser UC, Buser D. Effectiveness of contour augmentation with guided bone regeneration: 10-year results. *J Dent Res*. 2018;97:266-274.
247. Shen X, Yang S, Xu Y, Xu J, Feng Y, He F. Analysis of implant loss risk factors after simultaneous guided bone regeneration: a retrospective study of 5404 dental implants. *Clin Implant Dent Relat Res*. 2022;24:276-286.
248. Sanz M, Herrera D, Kebschull M, et al. Treatment of stage I–III periodontitis-the EFP S3 level clinical practice guideline. *J Clin Periodontol*. 2020;47(Suppl 22):4-60.
249. Herrera D, Sanz M, Kebschull M, et al. Treatment of stage IV periodontitis: the EFP S3 level clinical practice guideline. *J Clin Periodontol*. 2022;49(Suppl 24):4-71.
250. Mengel R, Flores-de-Jacoby L. Implants in regenerated bone in patients treated for generalized aggressive periodontitis: a prospective longitudinal study. *Int J Periodontics Restorative Dent*. 2005;25:331-341.
251. Mengel R, Eckert A, Greene B, Thone-Muhling M. Implants in GBR-augmented sites in patients treated for generalized aggressive periodontitis: a 10- to 20-year prospective case series. *Int J Periodontics Restorative Dent*. 2022;42:243-250.
252. Sousa V, Mardas N, Farias B, et al. A systematic review of implant outcomes in treated periodontitis patients. *Clin Oral Implants Res*. 2016;27:787-844.
253. Roccuzzo M, De Angelis N, Bonino L, Aglietta M. Ten-year results of a three-arm prospective cohort study on implants in periodontally compromised patients. Part 1: implant loss and radiographic bone loss. *Clin Oral Implants Res*. 2010;21:490-496.
254. Herrera D, Berglundh T, Schwarz F, et al. Prevention and treatment of peri-implant diseases-the EFP S3 level clinical practice guideline. *J Clin Periodontol*. 2023;50:4-76.
255. Schliephake H. The role of systemic diseases and local conditions as risk factors. *Periodontology 2000*. 2022;88:36-51.
256. Bouillon R. Diabetic bone disease. *Calcif Tissue Int*. 1991;49:155-160.
257. Retzepi M, Donos N. The effect of diabetes mellitus on osseous healing. *Clin Oral Implants Res*. 2010;21:673-681.
258. Retzepi M, Lewis MP, Donos N. Effect of diabetes and metabolic control on de novo bone formation following guided bone regeneration. *Clin Oral Implants Res*. 2010;21:71-79.
259. von Wilmowsky C, Schlegel KA, Baran C, Nkenke E, Neukam FW, Moest T. Peri-implant defect regeneration in the diabetic pig: a preclinical study. *J Craniomaxillofac Surg*. 2016;44:827-834.

260. von Wilmowsky C, Stockmann P, Harsch I, et al. Diabetes mellitus negatively affects peri-implant bone formation in the diabetic domestic pig. *J Clin Periodontol*. 2011;38:771-779.
261. Lee RSB, Hamlet SM, Ivanovski S. The influence of titanium surface characteristics on macrophage phenotype polarization during osseous healing in type I diabetic rats: a pilot study. *Clin Oral Implants Res*. 2017;28:e159-e168.
262. Lee RSB, Hamlet SM, Moon HJ, Ivanovski S. Re-establishment of macrophage homeostasis by titanium surface modification in type II diabetes promotes osseous healing. *Biomaterials*. 2021;267:120464.
263. Lee SB, Retzepi M, Petrie A, Hakimi AR, Schwarz F, Donos N. The effect of diabetes on bone formation following application of the GBR principle with the use of titanium domes. *Clin Oral Implants Res*. 2013;24:28-35.
264. Tawil G, Younan R, Azar P, Sleilati G. Conventional and advanced implant treatment in the type II diabetic patient: surgical protocol and long-term clinical results. *Int J Oral Maxillofac Implants*. 2008;23:744-752.
265. De Angelis P, Manicone PF, Gasparini G, et al. The effect of controlled diabetes and hyperglycemia on implant placement with simultaneous horizontal guided bone regeneration: a clinical retrospective analysis. *Biomed Res Int*. 2021;2021:9931505.
266. Erdogan O, Incki KK, Benlidayi ME, Seydaoglu G, Kelekci S. Dental and radiographic findings as predictors of osteoporosis in postmenopausal women. *Geriatr Gerontol Int*. 2009;9:155-164.
267. Drozdzowska B, Pluskiewicz W, Tarnawska B. Panoramic-based mandibular indices in relation to mandibular bone mineral density and skeletal status assessed by dual energy X-ray absorptiometry and quantitative ultrasound. *Dentomaxillofac Radiol*. 2002;31:361-367.
268. Jonasson G, Bankvall G, Kiliaridis S. Estimation of skeletal bone mineral density by means of the trabecular pattern of the alveolar bone, its interdental thickness, and the bone mass of the mandible. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2001;92:346-352.
269. Makker A, Singh MM, Mishra G, Singh BP, Jain GK, Jadhav S. Relationship between bone turnover biomarkers, mandibular bone mineral density, and systemic skeletal bone mineral density in premenopausal and postmenopausal Indian women. *Menopause*. 2012;19:642-649.
270. Takaishi Y, Okamoto Y, Ikeo T, et al. Correlations between periodontitis and loss of mandibular bone in relation to systemic bone changes in postmenopausal Japanese women. *Osteoporosis Int*. 2005;16:1875-1882.
271. Vishwanath SB, Kumar V, Kumar S, Shashikumar P, Shashikumar Y, Patel PV. Correlation of periodontal status and bone mineral density in postmenopausal women: a digital radiographic and quantitative ultrasound study. *Indian J Dent Res*. 2011;22:270-276.
272. Horner K, Devlin H, Alsop CW, Hodgkinson IM, Adams JE. Mandibular bone mineral density as a predictor of skeletal osteoporosis. *Br J Radiol*. 1996;69:1019-1025.
273. Esfahanizadeh N, Davaie S, Rokn AR, et al. Correlation between bone mineral density of jaws and skeletal sites in an Iranian population using dual X-ray energy absorptiometry. *Dent Res J (Isfahan)*. 2013;10:460-466.
274. Anwar RB, Tanaka M, Kohno S, et al. Relationship between porotic changes in alveolar bone and spinal osteoporosis. *J Dent Res*. 2007;86:52-57.
275. Calciolari E, Donos N, Park JC, Petrie A, Mardas N. A systematic review on the correlation between skeletal and jawbone mineral density in osteoporotic subjects. *Clin Oral Implants Res*. 2016;27:433-442.
276. Du Z, Xiao Y, Hashimi S, Hamlet SM, Ivanovski S. The effects of implant topography on osseointegration under estrogen deficiency induced osteoporotic conditions: histomorphometric, transcriptional and ultrastructural analysis. *Acta Biomater*. 2016;42:351-363.
277. Kosugi K, Yonezu H, Kawashima S, Honda K, Arai Y, Shibahara T. A longitudinal study of the effect of experimental osteoporosis on bone trabecular structure in the rat mandibular condyle. *Cranio*. 2013;31:140-150.
278. Kuroda S, Mukohyama H, Kondo H, et al. Bone mineral density of the mandible in ovariectomized rats: analyses using dual energy X-ray absorptiometry and peripheral quantitative computed tomography. *Oral Dis*. 2003;9:24-28.
279. Dvorak G, Reich KM, Tangl S, Goldhahn J, Haas R, Gruber R. Cortical porosity of the mandible in an osteoporotic sheep model. *Clin Oral Implants Res*. 2011;22:500-505.
280. Hirai T, Ishijima T, Hashikawa Y, Yajima T. Osteoporosis and reduction of residual ridge in edentulous patients. *J Prosthet Dent*. 1993;69:49-56.
281. Singhal S, Chand P, Singh BP, et al. The effect of osteoporosis on residual ridge resorption and masticatory performance in denture wearers. *Gerodontology*. 2012;29:e1059-e1066.
282. Gaetti-Jardim EC, Santiago-Junior JF, Goiato MC, Pellizer EP, Magro-Filho O, Jardim Junior EG. Dental implants in patients with osteoporosis: a clinical reality? *J Craniofac Surg*. 2011;22:1111-1113.
283. Dereka X, Calciolari E, Donos N, Mardas N. Osseointegration in osteoporotic-like condition: a systematic review of preclinical studies. *J Periodontal Res*. 2018;53:933-940.
284. Donos N, Calciolari E. Dental implants in patients affected by systemic diseases. *Br Dent J*. 2014;217:425-430.
285. Degidi M, Piattelli A. Immediately loaded bar-connected implants with an anodized surface inserted in the anterior mandible in a patient treated with diphosphonates for osteoporosis: a case report with a 12-month follow-up. *Clin Implant Dent Relat Res*. 2003;5:269-272.
286. Tsolaki IN, Madianos PN, Vrotsos JA. Outcomes of dental implants in osteoporotic patients. A literature review. *J Prosthodont*. 2009;18:309-323.
287. Holahan CM, Koka S, Kennel KA, et al. Effect of osteoporotic status on the survival of titanium dental implants. *Int J Oral Maxillofac Implants*. 2008;23:905-910.
288. Amorim MA, Takayama L, Jorgetti V, Pereira RM. Comparative study of axial and femoral bone mineral density and parameters of mandibular bone quality in patients receiving dental implants. *Osteoporos Int*. 2007;18:703-709.
289. Dao TT, Anderson JD, Zarb GA. Is osteoporosis a risk factor for osseointegration of dental implants? *Int J Oral Maxillofac Implants*. 1993;8:137-144.
290. Holahan CM, Wiens JL, Weaver A, Assad D, Koka S. Relationship between systemic bone mineral density and local bone quality as effectors of dental implant survival. *Clin Implant Dent Relat Res*. 2011;13:29-33.
291. Friberg B, Ekestubbe A, Mellstrom D, Sennerby L. Branemark implants and osteoporosis: a clinical exploratory study. *Clin Implant Dent Relat Res*. 2001;3:50-56.
292. Tadinada A, Ortiz D, Taxel P, et al. CBCT evaluation of buccal bone regeneration in postmenopausal women with and without osteopenia or osteoporosis undergoing dental implant therapy. *J Prosthet Dent*. 2015;114:498-505.
293. Hildebrand H, Ehrenfeld M, Schneider F, et al. [Possibilities for preoperative determination of transplant quality using an osteoporosis index]. Moglichkeiten der praoperativen Bestimmung der Transplantatqualitat mit Hilfe eines Osteoporose-indexes. *Fortschr Kiefer Gesichtschir*. 1994;39:34-39.
294. Blomqvist JE, Alberius P, Isaksson S, Linde A, Hansson BG. Factors in implant integration failure after bone grafting: an osteometric and endocrinologic matched analysis. *Int J Oral Maxillofac Surg*. 1996;25:63-68.

295. Toffler M. Osteotome-mediated sinus floor elevation: a clinical report. *Int J Oral Maxillofac Implants*. 2004;19:266-273.
296. Kramer FJ, Schliephake H, Wichmann M. Endosseous implants for functional masticatory rehabilitation in the extremely atrophied edentulous maxilla. *Mund Kiefer Gesichtschir*. 1999;3(Suppl 1):S19-S23.
297. Schliephake H, Neukam FW, Wichmann M. Survival analysis of endosseous implants in bone grafts used for the treatment of severe alveolar ridge atrophy. *J Oral Maxillofac Surg*. 1997;55:1227-1233; discussion 1233-1224.
298. Pinholt EM. Branemark and ITI dental implants in the human bone-grafted maxilla: a comparative evaluation. *Clin Oral Implants Res*. 2003;14:584-592.
299. Durao SF, Gomes PS, Colaco BJ, et al. The biomaterial-mediated healing of critical size bone defects in the ovariectomized rat. *Osteoporos Int*. 2014;25:1535-1545.
300. Kim S-Y, Kim S-G, Lim S-C, Bae C-S. Effects on bone formation in ovariectomized rats after implantation of tooth ash and plaster of Paris mixture. *J Oral Maxillofac Surg*. 2004;62:852-857.
301. Nascimento RD, Cardoso PE, De Marco AC, de Lima LAPA, Jardini MAN. Influence of osteopenia in autogenous bone graft healing with or without expanded polytetrafluoroethylene membranes: histologic and histomorphometric study in rats. *Int J Oral Maxillofac Implants*. 2009;24:1074-1082.
302. Li JP, Zhang WQ, Yu J, Qi MC, Hu J, Wang DS. The effect of experimental osteoporosis on bone healing of autologous iliac crest graft around implants. *Hua Xi Kou Qiang Yi Xue Za Zhi*. 2010;28:435-438.
303. Qi M, Hu J, Li J, et al. Effect of zoledronate acid treatment on osseointegration and fixation of implants in autologous iliac bone grafts in ovariectomized rabbits. *Bone*. 2012;50:119-127.
304. Mardas N, Schwarz F, Petrie A, Hakimi A-R, Donos N. The effect of SLActive surface in guided bone formation in osteoporotic-like conditions. *Clin Oral Implants Res*. 2011;22:406-415.
305. Siqueira R, Ferreira JA, Rizzante FAP, et al. Hydrophilic titanium surface modulates early stages of osseointegration in osteoporosis. *J Periodontal Res*. 2021;56:351-362.
306. Calciolari E, Donos N. Proteomic and transcriptomic approaches for studying bone regeneration in health and systemically compromised conditions. *Proteomics Clin Appl*. 2020;14:e1900084.
307. Stavropoulos A, Bertl K, Pietschmann P, Pandis N, Schiodt M, Klinge B. The effect of antiresorptive drugs on implant therapy: systematic review and meta-analysis. *Clin Oral Implants Res*. 2018;29(Suppl 18):54-92.
308. American Dental Association Council on Scientific A. Dental management of patients receiving oral bisphosphonate therapy: expert panel recommendations. *J Am Dent Assoc*. 2006;137:1144-1150.
309. Quigley LF Jr, Cobb CM, Hunt EE Jr. Measurement of oral and burning zone temperatures during conventional and reverse cigarette smoking. *Arch Oral Biol*. 1965;10:35-44.
310. Fatemi K, Radvar M, Rezaee A, et al. Comparison of relative TLR-2 and TLR-4 expression level of disease and healthy gingival tissue of smoking and non-smoking patients and periodontally healthy control patients. *Aust Dent J*. 2013;58:315-320.
311. Wu L, Zhou Y, Zhou Z, et al. Nicotine induces the production of IL-1beta and IL-8 via the alpha7 nAChR/NF-kappaB pathway in human periodontal ligament cells: an in vitro study. *Cell Physiol Biochem*. 2014;34:423-431.
312. Johnson JD, Houchens DP, Kluwe WM, Craig DK, Fisher GL. Effects of mainstream and environmental tobacco smoke on the immune system in animals and humans: a review. *Crit Rev Toxicol*. 1990;20:369-395.
313. Giorgetti AP, Cesar Neto JB, Ruiz KG, Casati MZ, Sallum EA, Nociti FH Jr. Cigarette smoke inhalation modulates gene expression in sites of bone healing: a study in rats. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;110:447-452.
314. Palmer RM, Wilson RF, Hasan AS, Scott DA. Mechanisms of action of environmental factors–tobacco smoking. *J Clin Periodontol*. 2005;32(Suppl 6):180-195.
315. Javed F, Rahman I, Romanos GE. Tobacco-product usage as a risk factor for dental implants. *Periodontology 2000*. 2019;81:48-56.
316. Huang YS, Li A, Yang ZC. Effect of smoke inhalation injury on thromboxane levels and platelet counts. *Burns Incl Therm Inj*. 1988;14:440-446.
317. Pretorius E. Ultrastructural changes in platelet membranes due to cigarette smoking. *Ultrastruct Pathol*. 2012;36:239-243.
318. Keenan JR, Veitz-Keenan A. The impact of smoking on failure rates, postoperative infection and marginal bone loss of dental implants. *Evid Based Dent*. 2016;17:4-5.
319. Alfadda SA. Current evidence on dental implants outcomes in smokers and nonsmokers: a systematic review and meta-analysis. *J Oral Implantol*. 2018;44:390-399.
320. Jung RE, Brügger LV, Bienz SP, Hüsler J, Hämmerle CHF, Zitzmann NU. Clinical and radiographical performance of implants placed with simultaneous guided bone regeneration using resorbable and nonresorbable membranes after 22–24 years, a prospective, controlled clinical trial. *Clin Oral Implants Res*. 2021;32:1455-1465.
321. Lindfors LT, Tervonen EA, Sandor GK, Ylikontiola LP. Guided bone regeneration using a titanium-reinforced ePTFE membrane and particulate autogenous bone: the effect of smoking and membrane exposure. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;109:825-830.
322. Meloni SM, Jovanovic SA, Urban I, Baldoni E, Pisano M, Tallarico M. Horizontal ridge augmentation using GBR with a native collagen membrane and 1:1 ratio of particulate xenograft and autologous bone: a 3-year after final loading prospective clinical study. *Clin Implant Dent Relat Res*. 2019;21:669-677.
323. Hong JY, Shin EY, Herr Y, Chung JH, Lim HC, Shin SI. Implant survival and risk factor analysis in regenerated bone: results from a 5-year retrospective study. *J Periodontal Implant Sci*. 2020;50:379-391.
324. Salvi GE, Monje A, Tomasi C. Long-term biological complications of dental implants placed either in pristine or in augmented sites: a systematic review and meta-analysis. *Clin Oral Implants Res*. 2018;29(Suppl 16):294-310.
325. Carcuac O, Abrahamsson I, Derks J, Petzold M, Berglundh T. Spontaneous progression of experimental peri-implantitis in augmented and pristine bone: a pre-clinical in vivo study. *Clin Oral Implants Res*. 2020;31:192-200.
326. Sato R, Matsuura T, Akizuki T, et al. Influence of the bone graft materials used for guided bone regeneration on subsequent peri-implant inflammation: an experimental ligature-induced peri-implantitis model in beagle dogs. *Int J Implant Dent*. 2022;8:3.
327. Schwarz F, Sahm N, Becker J. Impact of the outcome of guided bone regeneration in dehiscence-type defects on the long-term stability of peri-implant health: clinical observations at 4 years. *Clin Oral Implants Res*. 2012;23:191-196.
328. Heitz-Mayfield LJ, Aaboe M, Araujo M, et al. Group 4 ITI consensus report: risks and biologic complications associated with implant dentistry. *Clin Oral Implants Res*. 2018;29(Suppl 16):351-358.
329. Lee J, Byun H, Madhurakkat Perikamana SK, Lee S, Shin H. Current advances in immunomodulatory biomaterials for bone regeneration. *Adv Healthc Mater*. 2019;8:e1801106.
330. Ho-Shui-Ling A, Bolander J, Rustom LE, Johnson AW, Luyten FP, Picart C. Bone regeneration strategies: engineered scaffolds, bioactive molecules and stem cells current stage and future perspectives. *Biomaterials*. 2018;180:143-162.
331. Stavropoulos A, Bertl K, Winning L, Polyzois I. What is the influence of implant surface characteristics and/or implant material on the incidence and progression of peri-implantitis? A

systematic literature review. *Clin Oral Implants Res*. 2021;32(Suppl 21):203-229.
332. Thoma DS, Cosyn J, Fickl S, et al. Soft tissue management at implants: summary and consensus statements of group 2. The 6th EAO consensus conference 2021. *Clin Oral Implants Res*. 2021;32(Suppl 21):174-180.
333. Donos N, Laurell L, Mardas N. Hierarchical decisions on teeth vs. implants in the periodontitis-susceptible patient: the modern dilemma. *Periodontology 2000*. 2012;59:89-110.

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