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# Osteoinductive Implants: The *Mise-en-scène* for Drug-Bearing Biomimetic Coatings

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**Abstract**—In orthopaedic and dental implantology, novel tools and techniques are being sought to improve the regeneration of bone tissue. Numerous attempts have been made to enhance the osteoconductivity of titanium prostheses, including modifications in their surface properties and coating with layers of calcium phosphate. The technique whereby such layers are produced has recently undergone a revolutionary change, which has had profound consequences for their potential to serve as drug-carrier systems. Hitherto, calcium phosphate layers were deposited upon the surfaces of metal implants under highly unphysiological physical conditions, which precluded the incorporation of proteinaceous osteoinductive drugs. These agents could only be adsorbed, superficially, upon preformed layers. Such superficially adsorbed molecules are released too rapidly within a biological milieu to be effective in their osteoinductive capacity. Now, it is possible to deposit calcium phosphate layers under physiological conditions of temperature and pH by the so-called biomimetic process, during which bioactive agents can be coprecipitated. Since these molecules are integrated into the inorganic latticework, they are released gradually *in vivo* as the layer undergoes degradation. This feature enhances the capacity of these coatings to act as a carrier system for osteogenic agents.

**Keywords**—Biomimetic, Tissue engineering, Implants, Coating, Osteoinductive.

## CLINICAL BACKGROUND AND REQUIREMENTS

Worldwide, more than one million patients need to be treated annually for skeletal problems, which fall within the scope of plastic and reconstructive surgery, orthopaedic surgery, and dental implantology. Handling includes the treatment of bony defects generated traumatically or by the excision of tumors, the reconstruction of congenital skeletal abnormalities, the promotion of fracture healing, the treatment of spinal arthrodesis and the replacement of joints and teeth.[33,37,50] Treatment does not always solve the problem, owing to inadequate local bone conditions and impaired bone healing. Complicated fractures may fail to heal, resulting in so-called delayed unions or non-unions. The excision of bone tumors and the treatment of congenital syndromes frequently involves the creation of large bony defects, which need to be filled with autogenic or allogeneic bone. Autogenic bone is of limited availability for grafting purposes and its excavation is associated with donor site morbidity. Suitable and biocompatible substitutes for bone grafts have therefore been sought.[57,60,73,77] Bone substitutes can be divided into three classes: (1) osteoconductive, (2) directly osteogenic, and (3) osteoinductive. Osteoconductive bone substitutes, such as ceramic materials, do not actively stimulate the bone-formation process, whereas directly osteogenic and osteoinductive bone substitutes do. Osteogenic materials can be produced, for example, by trapping osteogenic cells within a porous scaffold. Osteoinductive materials can be engendered by impregnating such porous scaffolds with osteogenic drugs.

Alloplastic materials are favored for the filling of bone cavities generated traumatically or by the excision of tumors.[9,78,94,100] An ideal candidate is deemed to be one that maintains the volume of the defect during the initial phase of healing and is then resorbed and replaced by bone. However, the compact filling of a defect with alloplastic material, with the intention of barring its invasion by soft tissue, must be balanced against the reduced potential for osseous regeneration from the parietal and marginal surfaces.[16,17]

Of the many materials tested for their potential to serve as bone substitutes, such as ceramics, glass, and various polymers,[12,79] only a few are capable of withstanding the forces operative in load-bearing situations. Bioceramic hydroxyapatite has been widely employed for nearly 20 years. It is relatively cheap, nontoxic, minimally resorbed, of acceptable compressive strength, and attaches well to hard tissues.[19,32] Its most valuable asset is its ability to conduct bone apposition.[38] The major drawback of bioceramic hydroxyapatite is its low tensile strength (brittleness).

If an otherwise suitable bone substitute lacks inherent mechanical strength, it can be coupled with a metallic fixation device which furnishes this property. Furthermore, the osteoconductive material can be rendered osteoinductive by incorporating an osteogenic agent, such as a member of the transforming growth factor beta (TGF- ) superfamily (bone

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morphogenetic proteins (BMPs), growth and differentiation factors (GDFs) and TGF- s). Ideally, the bone substitute should be strong, malleable, osteoconductive, osteoinductive, resorbable, inexpensive, and easy to handle intraoperatively. It should promote cell adhesion, proliferation, and differentiation,[11,37,93] and should also be an effective carrier that protects and predictably releases the entrapped bioactive agent, facilitating tissue ingrowth and establishing a mechanically stable environment that supports bone regeneration[26,36] and patient function.

## BIOMIMETICS AND TISSUE ENGINEERING

Biomimetics and tissue engineering are new fields that go hand-in-hand in the pursuit of an old objective, namely, the repair or replacement of bodily parts. Biomimetics, which literally means the mimicry of biology, is a branch of science in which biologists and engineers jointly endeavor to produce "bioinspired" materials that can be used for tissue engineering. This broad new field has ancient roots. The replacement of bodily parts dates back at least 2500 years, to the time when the Etruscans substituted missing teeth with artificial ones carved from the bones of oxen. The first recorded use of dental amalgam to repair decayed teeth was in China in the year A.D. 659.[5] As human life expectancy continues to increase, the need for better coping with diseased, damaged, or destroyed bodily tissues or parts has heightened. And it is within the framework of molecular biology, engineering, and materials science that biomimetics and tissue engineering are emerging as invaluable tools.

Tissue engineering is the art of reconstituting mammalian tissues, both structurally and functionally. Such reconstruction processes can be conducted either entirely *in vitro* or partially *in vitro* and then completed *in vivo*. Success in this technology would obviate the need for tissue transplantation. And if the appropriate precursor cell pools could be obtained from embryonic, foetal, or adult allogeneic sources, then the numerous problems associated with the use of donor tissue would be avoided. Autogenic stem or precursor cells could also be employed, and these would indeed be more osteogenic than allogeneic ones. But the enterprise would be more arduous, costly, and risky for the patient. It would involve two surgical interventions: one to remove tissue from a suitable donor site and another to implant the harvested cells at the defect site. Tissue excised during the first surgical intervention would need to be transported (using a costly courier service) to a central laboratory for the isolation and expansion of cells, which would then have to be shipped back for implantation. Allogeneic cells could be isolated, expanded, and stored centrally in readiness to meet a demand as it arises.

Three key constituents usually form the basis of a tissue engineering approach,[35] namely, a matrix scaffold, cells, and growth factors. In conjunction with the biomimetic implant coating process, osteogenic growth factors could be coprecipitated to yield a matrix with osteoinductive as well as osteoconductive properties.[21,55,56] Cells could also be incorporated into this system.[18,54,84–87] In one such setup, mesenchymal cells have been isolated from bone-marrow biopsies, expanded *in vitro* and then cultured on the surfaces of implants bearing a biomimetically coprecipitated layer of calcium phosphate and BMP-2. Thus cultured, these osteoprogenitor cells were triggered to form bone tissue. By adopting this strategy, bone healing *in vivo* could be enhanced. Such a bone tissue engineering approach could be implemented in conjunction not only with implant coatings but also with porous bone fillers, for the treatment of large osseous defects.

## CARRIER MATERIALS

Various materials have been tested for their potential to serve as carriers for the delivery of cells and/or growth factors to bony sites. These include both organic and inorganic substances.[34,43,59,65,81] Organic materials can be further subdivided into biological and synthetic compounds. The former category includes demineralized, inactivated, or insoluble bone matrix, autolyzed antigen-extracted allogeneic bone, collagen types I and IV in various forms, such as sponges, gels, or membranes, noncollagenous proteins and fibrin. Synthetic organic materials include mainly poly -hydroxyacids, such as polylactic acid, polyglycolic acid, or a combination of the two. Inorganic materials include metals, such as titanium, and calcium-phosphate-based substances. Composites of two carrier materials have also been employed, the classical example being titanium implants coated with a layer of calcium phosphate.

None of the carrier materials thus far tested have proved to be entirely satisfactory. Indeed, it is probably overly optimistic to conceive of any single material embodying all of the desired properties, which include biodegradability, biocompatibility, cohesiveness, elasticity, volume stability, and cell-adhesiveness, to name but a few. Natural bone matrix, for example, carries the risk of disease transmission and immune rejection, whilst synthetic hydroxyapatite prepared under the usual high-temperature conditions is poorly biodegradable. However, the new bone laid down in association with this latter material merges relatively well, although to a variable degree, with native osseous tissue. It appears that the pore size and conformation of the hydroxyapatite scaffolding are crucial in determining the efficiency of bone formation.[34,51,66,67]

Hydrogels represent a new generation of carrier material. The novel concept underlying their use is that the "fluid" polymer, carrying progenitor cells and growth factors, can be injected into the implantation site without extensive surgical intervention. The compound is photopolymerized *in situ*, thereby yielding a solid structure to support bone formation. This approach is being currently explored for the repair of craniofacial structures and for the treatment of

dental defects generated by poor bonding between teeth and the underlying jawbone.[74,80]

## SURFACE CHARACTERISTICS OF METAL IMPLANTS

Metal-based implants or endoprostheses have been used for many decades in clinical dentistry and orthopaedic surgery. Titanium and its alloys are especially popular due to their excellent mechanical properties and ease of handling during surgery. Furthermore, they are highly biocompatible with the bony tissue compartment.[2,61] In orthopaedics, such materials are used not only for prosthetic devices[23,70] but also for internal fixation during fracture healing.[24]

The microtopographic profile of a metal implant surface is known to influence its osteoconductivity.[10,13,98] Modifications in surface geometry have been effected by blasting with corundum or sand,[13,29,68,69] or by etching with acid.[47] Such treatment generates small pits within the metal surface which correspond in size to the resorption pits excavated by osteoclasts in bone. This pitted microtopographic profile is conducive to osteogenesis. It also enhances osseointegration, by facilitating interlocking between the implant substrate and ongrown bone, thereby improving the long-term mechanical stability of the prosthesis.[20,63,73,92,94]

Investigators soon realized that if an implant surface could be coated with a layer whose characteristics mimicked those of bone matrix, particularly the mineralized components, then its osteoconductive features could be still further enhanced. And such was found to be the case. Metal implants have been coated (by plasma spraying or other methodologies) with layers of hydroxyapatite,[18,21] calcium phosphate[20,90] or mixtures of the two.[44–46] These coated implants are, moreover, characterized by a rough surface profile, which further improves osteoconduction and osseointegration.

## CALCIUM PHOSPHATE LAYERS (NONBIOMIMETIC COATING PROCEDURES)

Until recently, layers of calcium phosphate were deposited upon the surfaces of metal implants under physical conditions that were highly unphysiological. The methods employed have been various and include plasma spraying, high-velocity oxygen-fuel spraying, electrophoretic deposition, sol–gel deposition, hot isostatic pressing, frit enamelling, ion-assisted deposition, pulsed laser deposition, electrochemical deposition, and sputter coating.[95–97] Hydroxyapatite is the most important representative of the bioactive calcium phosphate ceramics. There is abundant evidence in the literature that sintered hydroxyapatite is well incorporated into living bone and that it does not undergo any significant biodegradation once it has become bonded to it (a feature that may be disadvantageous as well as advantageous). Although the static mechanical strength of sintered hydroxyapatite is comparable to that of cortical bone, this material is prone to fatigue failure under conditions of high-tensile loading, which renders it unsuitable for applications in load-bearing situations.

Although these calcium phosphate coatings improve the osteoconductivity of metal implants, they do not render them osteoinductive, a feature that is required to expedite the osteogenic process and to accelerate implant integration. This property can be conferred by introducing an osteogenic growth factor into the system.[1,30,39,66,72] But herein lies a difficulty. The aforementioned methods used for depositing layers of calcium phosphate upon metal implant surfaces employ such unphysiological conditions (with temperatures sometimes in the order of several thousand degrees celsius) as preclude the incorporation of proteinaceous signalling substances. Hence, these agents can be deposited only superficially upon preformed coatings, either by adsorption,[29,66,67,88] by binding to biofunctional proteins,[28] or by chemical treatment.[40] The disadvantage of this mode of attachment is that the biologically active molecules are released rapidly upon exposure to a physiological environment.[55,58] Consequently, their osteogenic effects[14,15] are of short range and short-lived.

## THE BIOMIMETIC COATING PROCEDURE

A few years ago, attempts were made to coat metal implants with layers of calcium phosphate under more physiological or "biomimetic" conditions of temperature and pH,[20,22,48,49,56,91] primarily to improve their biocompatibility and biodegradability. The mineral layers generated by existing methods, being composed of large, partially molten hydroxyapatite particles, were not only prone to delamination but also poorly degraded in a biological milieu.[62] An additional advantage of the biomimetic method is that biologically active molecules, such as osteogenic agents, can be coprecipitated with the inorganic components. As a consequence, the proteins are truly incorporated into the crystal latticeworks and not merely deposited upon their surfaces. In forming an integral part of the calcium phosphate coatings, the protein molecules are liberated not in a single burst (as when superficially adsorbed), but gradually, which bodes well for an enduring osteogenic effect at the implantation site. The biomimetic coating technique (Fig. 1) involves the nucleation and growth of bone-like crystals (Fig. 2) upon a pretreated substrate by immersing this in a supersaturated solution of calcium phosphate under physiological conditions of temperature (37°C) and pH (7.4). The method, originally developed by Kokubo in 1990,[48,49] has since undergone improvement and refinement by several groups of investigators.[5–8,25,41,56,81,89] It is simple to perform, is cost-effective and may be applied even to heat-sensitive, nonconductive and porous materials of large dimensions and with complex surface geometries.

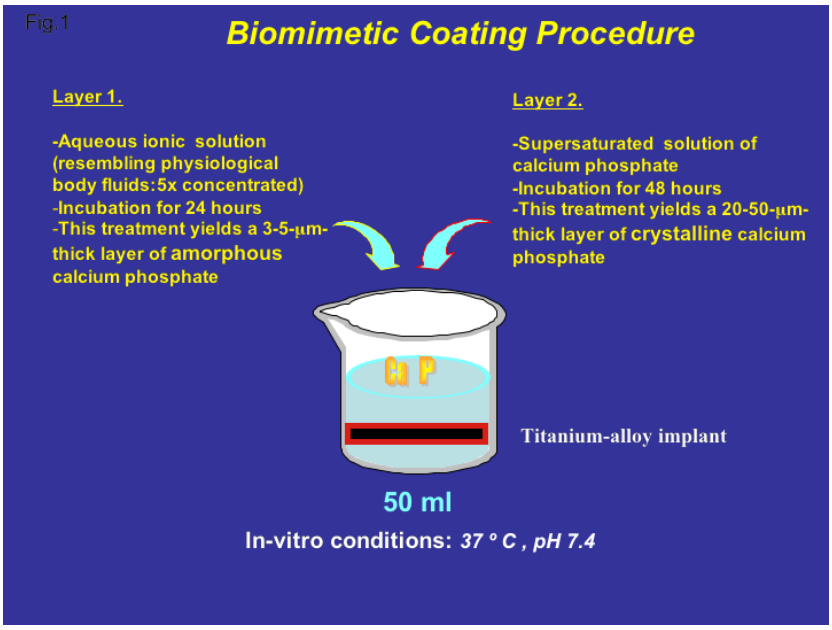


**FIGURE 1. Scheme summarizing the biomimetic coating procedure.**

Briefly, the implant is first immersed within five-times concentrated simulated body fluid under high-nucleation conditions, i.e., in the presence of $Mg^{2+}$ ions [(7.5 mM) see Table 1], to inhibit crystal growth, for 24 h at 37°C. A thin (<3- m thick), dense, and amorphous layer of calcium phosphate is thereby deposited uniformly upon the implant surface, which serves as a seeding substratum for the subsequent growth of a substantial (30- to 50- m thick) crystalline latticework. The latter is prepared by immersing the coated implant within a supersaturated solution of calcium phosphate [(pH 7.4) Table 1], either in the absence or presence of the drug of interest, for 48 hours at 37°C.[56]

## BIOMIMETIC CALCIUM PHOSPHATE COATINGS AS A DELIVERY VEHICLE FOR OSTEOGENIC DRUGS

The advent of the biomimetic coating technique has broadened the potential of calcium phosphate layers to serve as a carrier system for osteogenic drugs, thereby rendering them osteoinductive as well as osteoconductive. Granted this facility, investigators must select an appropriate osteogenic agent.

Members of the TGF- superfamily, such as certain TGF- s and GDFs, and especially BMPs, probably represent the most promising candidates for this purpose. Interest in this latter group of agents dates back to 1964. In this year, Urist[83] demonstrated that demineralized, dried, and pulverized bone could stimulate the formation of osseous tissue at an ectopic site (muscle) in rabbits. The osteogenic fraction of the bone matrix was later isolated and subjected to amino-acid sequencing. This analysis revealed BMPs to be structurally similar to TGF- s, and they are now classed as a subgroup of this superfamily.[4,27,52,82] More than 15 of the 30 odd members have been isolated and synthesized by recombinant DNA technology.[31,71,99]

Interest in BMPs obviously preceeded the advent of the biomimetic coating technology, and various materials have thus already served as their carriers. These include collagen, synthetic and natural ceramics, demineralized bone matrix, and polyglycolic acid.[34,42,43,51,75,80,82] Human recombinant BMP-2 used in conjunction with each of these carrier systems is released in two kinetically distinct phases: an initial rapid one of a few hours' duration and a second slower one spanning several weeks. Collagen retains the largest fraction of BMP-2 during the initial phase and synthetic hydroxyapatite particles the smallest (10%). The other carriers retain between 30 and 50% of their load during this phase. With the exception of synthetic hydroxyapatite, the mineral-based carriers retain the largest fraction of BMP-2 during the second phase, which reflects their high affinity for this agent. However, none of the materials serve as an optimal drug-delivery system.

The potential of biomimetic calcium phosphate coatings to serve as a carrier system for BMP-2 is now being investigated by our group.[53–55] The drug has been successfully coprecipitated with the inorganic components and, thus incorporated, retains its biological activity *in vitro*.[54,55] Preliminary experiments using an ectopic (subcutaneous) model for bone formation in rats have revealed BMP-2 thus borne to be osteogenically potent. Furthermore, bone-forming activities are sustained for a considerable period of time, which indicates that BMP-2 is indeed released

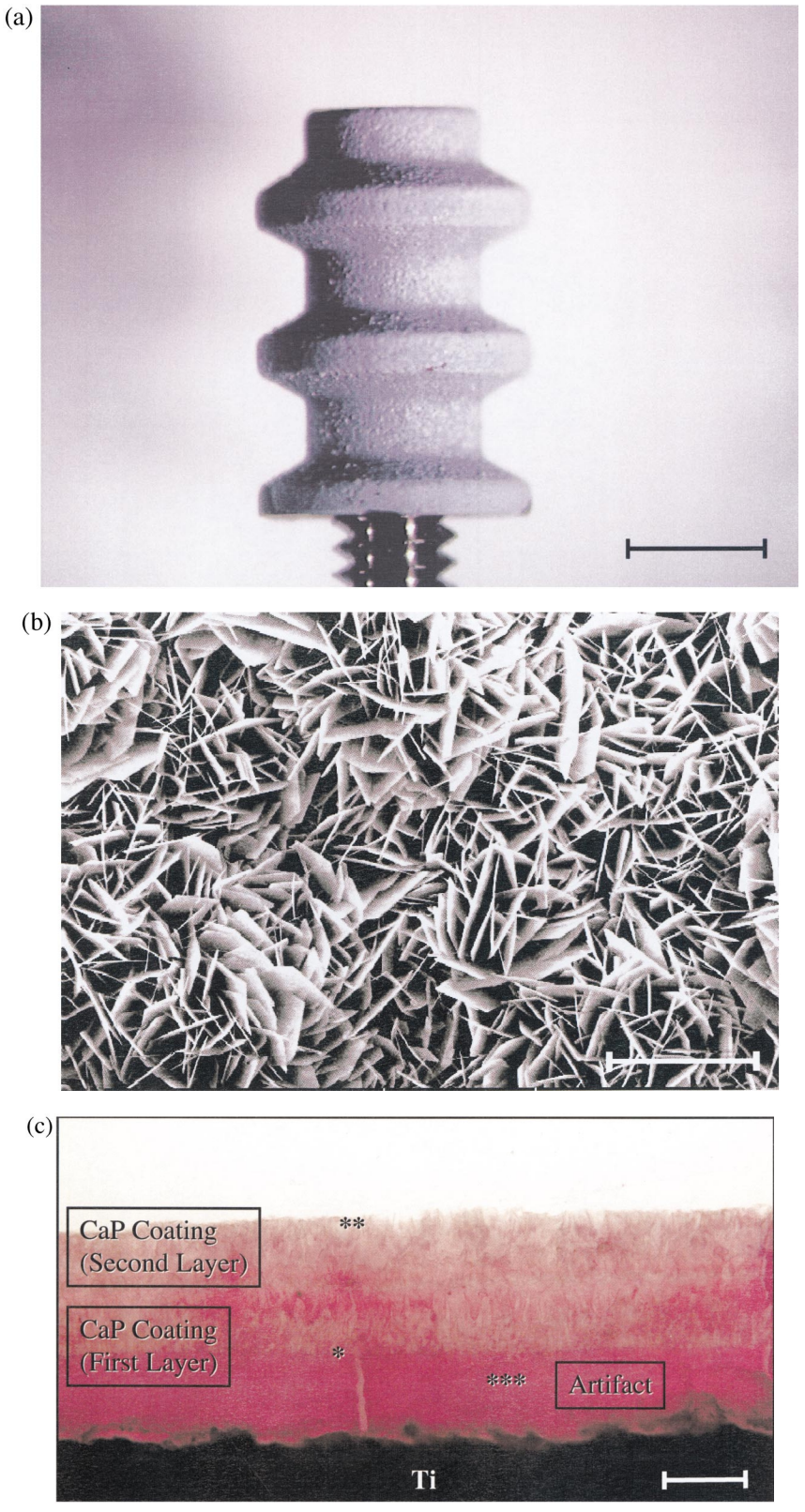


**FIGURE 2. (a) Experimental (titanium-alloy) dental implant bearing a biomimetic calcium phosphate coating. Magnification bar = 2.1 mm. (b) Surface view of a biomimetic calcium phosphate coating. Magnification bar = 20 $\mu$m. (c) Cross-section of a titanium–alloy implant bearing a biomimetic calcium phosphate coating. Magnification bar = 25 $\mu$m. Abbreviations: (*) Amorphous layer of calcium phosphate; ** Crystalline layer of calcium phosphate; (***) Artifactual space generated by shrinkage during processing; and (Ti) Titanium-alloy implant.**

**TABLE 1. Inorganic ion composition of five-times concentrated simulated body fluid (SBF × 5) and of supersaturated calcium phosphate (SCP) solution; that of human blood plasma (HBP) is included for comparative purposes.**

| | Ionic concentration (mM) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Solutions | $Na^+$ | $K^+$ | $Mg^{2+}$ | $Ca^{2+}$ | $Cl^-$ | $HPO_4^{2-}$ | $SO_4^{2-}$ | $HCO_3^{2-}$ | Buffer |
| SBF × 5 | 733.5 | — | 7.5 | 12.5 | 720.0 | 5.0 | 2.5 | 21.0 | $CO_2$ |
| SCP | 140.0 | — | — | 4.0 | 144.0 | 2.0 | — | — | Tris |
| HBP | 142.0 | 5.0 | 1.5 | 2.5 | 147.8 | 1.0 | 0.5 | 4.2 | — |

gradually (as anticipated) and not in a single burst.[53] This system now needs to be refined for testing at an orthotopic site.

Instead of incorporating BMP-2 itself into a carrier system, the drug could be substituted for a plasmid containing the gene for BMP-2. The rationale behind such an undertaking would be that whilst a protein is ultimately degraded, a gene would become incorporated into a host cell and remain active for a longer period.[64,65,76] The gene for BMP-2 has been delivered to orthotopic sites in rats and dogs via viral and nonviral carriers;[3,31] but it has not yet been incorporated into biomimetic implant coatings.

## CONCLUSION

Historically, the osteoconductivity of titaniuim-alloy implants used in dental and orthopaedic surgery was first improved by modifying their surfaces either chemically or physically. Later, a further improvement in osteoconductivity was achieved by coating these implants with a layer of calcium phosphate. For many years, these inorganic coatings were prepared under highly unphysiological conditions, which precluded the incorporation of proteinaceous osteogenic agents. Hence, they could be rendered osteoinductive only after their formation, by the superficial adsorption of osteogenic growth factors upon their surfaces, which limited the effects of these drugs *in vivo*, both spatially and temporally. With the advent of the biomimetic coating process, which is effected under physiological conditions of temperature (37°C) and pH (7.4), proteins could be coprecipitated with the inorganic elements. By this means, titanium-alloy implants have been rendered osteoinductive as well as osteoconductive by incorporating BMP-2 into the crystal latticework of coatings. Using an ectopic ossification model in rats, titanium-alloy implants bearing a coprecipitated layer of BMP-2 and calcium phosphate have been shown not only to induce bone formation in the periimplant region but also to sustain this process for a considerable period of time. The sustainment of osteogenic activity is essential for the osseointegration of implants. We are thus on the road to achieving this aim and have the means at hand to accelerate this process and thus expedite the reestablishment of full functionality at the implantation site.

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