Osteoinductive hydroxyapatite-coated titanium implants

Ugo Ripamonti a,*, Laura C. Roden a,b, Louise F. Renton a

a Bone Research Laboratory, School of Physiology, Faculty of Health Sciences, Medical Research Council/University of the Witwatersrand, Johannesburg, 2193 Parktown, South Africa
b Department of Molecular and Cell Biology, University of Cape Town, Rondebosch, Cape Town, South Africa

1. Introduction

Advances in surface biomaterial science and cell biology are intimately intertwined, with new functionalized visualization targets that will lead to quantum leaps in our understanding of cell induction and differentiation [1–7]. Tissue induction will be determined ab initio by biological invocation of nanopatterned surface topographies of differentiating surfaces of newly developed self-inductive substrata [2,3,8–20]. Such differentiating nanotopographical patterning scenarios would have applications for the construction of geometric surfaces designed for titanium prostheses including titanium dental implants [18–20]. Indeed, new definitions and classifications of biomaterials are now necessary for the rapidly changing field of biomaterial sciences; primarily, this has occurred because of tissue engineering and regenerative medicine, as well as biomimetic biomaterials interacting with the “stem cell niche” [21].
Several papers have shown how surface nanotopography provides a useful tool for guiding cell differentiation and tissue induction \([3,13,15–18]\). Biomimetic topographies present overriding geometric cues to responding cells that sense microscale groove contact guidance for cellular differentiation \([17]\). Induction of mesenchymal stem cells is controlled by nanoscale symmetry and disorder of patterned surfaces designed for bone tissue engineering \([3]\). Designing titanium implants with functionalized nanotopographic surfaces that could stimulate, and above all, induce osteogenesis when implanted in mandibular and/or

<table>
<thead>
<tr>
<th>Duration</th>
<th>Site</th>
<th>n – experimental</th>
<th>n – control</th>
</tr>
</thead>
<tbody>
<tr>
<td>90d</td>
<td>Mandible</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Tibia</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>30d</td>
<td>Mandible</td>
<td>7(^a)</td>
<td>7(^a)</td>
</tr>
<tr>
<td></td>
<td>Tibia</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>31 mo</td>
<td>Mandible</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Tibia</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^a\) 1 sample excluded from histomorphometric analysis.

**Table 1**
Numbers of samples analyzed for histometric analyses.

**Fig. 1.** Scanning electron macro/micrographs (SEM) defining the geometric landscape of the experimental geometric constructs with repetitive concavities coated by highly crystalline hydroxyapatite. (A, B, C) Concavities (blue arrows) machined on the titanium construct prior to hydroxyapatite-coating. (D, E, F, G) Nanotopographic images of the highly crystalline hydroxyapatite coating with pits and concavities (blue arrows) after plasma spraying; surfaces are highly suitable for stem cells attachment and differentiation. Original magnifications: A \( \times 32\), B \( \times 60\), C \( \times 160\), D \( \times 350\), E \( \times 1500\), F \( \times 1000\), G \( \times 2500\). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
heterotopic intramuscular sites, would have significant therapeutic potential [19,20].

The innovation of re-programming somatic stem cells to induce osteogenic differentiation and the induction of bone formation [2,19,20,22] now provides a new approach to control and modify the induction of bone formation in titanium orthopedic, oral and maxillofacial implants to obtain and maintain superior osteointegration [3,15–18]. We have previously shown the remarkable induction of bone formation by calcium phosphate-based macroporous constructs when implanted in heterotopic sites of the rectus abdominis muscle of the non-human primate Chacma baboon Papio ursinus [2,22–25]. We have further shown that the geometric patterning of the calcium phosphate-based macroporous constructs is critical for the induction of bone formation to occur; a sequence of repetitive concavities of specific dimensions and radii of curvature must be cut within the macroporous constructs to set into motion the ripple-like cascade of tissue induction and morphogenesis without the exogenous application of the osteogenic soluble molecular signals of the transforming growth factor-β (TGF-β) supergene family [19,20,25–30]. These studies have shown the importance of surface topography and geometry of the substrata in controlling and inducing the osteogenic phenotype in responding mesenchymal stem cells.

We wanted to develop implants germane for clinical contexts that are themselves osteoinductive due to properties of their surface geometry, and thus do not require any application of soluble osteogenic molecular signals. Here we describe the osteointegration and osteoinduction of planar vs. geometrically-designed titanium dental implants plasma-coated with highly crystalline hydroxyapatite implanted in orthotopic mandibular and tibial sites and in heterotopic sites of the rectus abdominis muscle of P. ursinus. Induction of bone formation was assessed by histological and histomorphometric evaluation.

2. Materials and methods

2.1. Implants

Cylindrical implants of grade Ti-60A1-4V of 15 mm in length and 3.85 mm in diameter were prepared with controlled geometric configurations. For the test implant, the body was machined with a series of repetitive concavities of 1600 µm in diameter and 800 µm in depth. The concavities along the implant body were spaced 1000 µm apart without sharp edges [19,20].

Concavities cut within the titanium profile created a space between the rotary instrumented bone or the enveloping muscular tissues of the rectus abdominis muscle such that it served as an “in vivo bioreactor” [31] by invocation of soluble and insoluble signals to induce formation of new bone within the bioreactor spaces of the macroporous constructs [2,25,29]. The coating of hydroxyapatite was applied to the implant body by air–plasma spraying of highly crystalline, low porosity hydroxyapatite using conventional deposition techniques resulting in a uniform layer of crystalline hydroxyapatite, 30 µm in thickness [19,20]. A Metco 9 MB plasma spray gun operating with an Ar/H2 sub 2 plasma at 35 kW was used to deposit the coating (Metco–Plasma Technik, product AMDRY 6020). Prior to spraying, the fixtures were prepared for coating, i.e. roughened by grit blasting with alumina grit. The typical spray parameter was: plasma gas primary Argon (5 bar); secondary Hydrogen (5 bar); gun power 25–35 kW with a power fed rate of 10–30 g/min at a stand-off distance of 60–100 mm. The gun velocity was 100 mm/min.

2.2. In vitro bioreactor for cellular attachment and differentiation

To further test the concept of the inductive microenvironment as generated by concavities created in macroporous calcium phosphate-based constructs [19,20,27–30], macroporous coral-derived calcium carbonate/calcium phosphate constructs (Pro-Osteon Interpore International, Irvine CA, USA) [23] were used to study the critical role of surface topography to align and differentiate osteoblast-like cells in vitro. The in vitro study was set to provide an ex-vivo bioreactor for heterotopic implantation in rodents, non-human [22] and human primates [32]. Mouse-derived fibroblasts (NIH3T3) and pre-osteoblast (MC 3T3-E1) cells, 2–3 × 10^5 in 50 µl of Medium 199 with 10% fetal calf serum and 1% ITS Premix (Insulin, Transferrin and Selenium; Collaborative Research Systems Inc., Bedford, MA) were applied to coral-derived hydroxyapatite constructs 7 mm in diameter, 3 mm in height in Costar tissue culture clusters (Costar, Cambridge, MA). After 90 min, the wells were flooded with medium. Tissue culture wells were coated with albumin to minimize cell attachment to the plastic. The cells were grown in Medium 199 with 10% fetal calf serum at 37 °C with 5% CO2 with daily medium changes. Cellular constructs were fixed at 3 and 7 days in Bouin’s fluid, dehydrated through a graded series of ethanol, infiltrated and embedded in JB4 resin. Undecalcified sections, cut at 2 µm, were stained with toluidine blue.

2.3. Primate model for tissue induction and osteointegration

Eight clinically healthy Chacma baboons P. ursinus with a mean weight of 18.2 ± 2.02 kg were selected from the primate colony of the University of the Witwatersrand, Johannesburg. Research protocols were approved by the Animal Ethics Screening Committee (AESC no. 93/79) and conducted according to the Guidelines for the Care and Use of Experimental Animals prepared by the University and in compliance with the national code for Animal use in Research, Education and Diagnosis in South Africa [33]. Criteria for selection, housing conditions and diets were as described [23]. P. ursinus species share similar bone and osteogenic bone remodeling with man [34], together with post-menopausal and post-menotropic trabecular micro-architectural deterioration with associated altered biochemical markers [35].

On the day of surgery, the baboons were immobilized with an intramuscular injection of ketamine hydrochloride (10 mg/kg), and anesthesia was induced with intravenous sodium thiopentone (15 mg/kg). The baboons were maintained on 100% oxygen after orotracheal intubation [23,24]. Mandibular molar and premolar were extracted to prepare edentulous hemi-mandibles for later implantation. After 7–8 months, edentulous hemi-mandibles were implanted with 3 geometrically designed and 3 planar plasma-sprayed titanium constructs. Three geometrically-modified fixtures were also implanted along the anterior-medial aspect of the right (test implants) and left (control planar implants) tibia after periosteal exposure. Similarly, 3 geometrically-modified fixtures and 3 planar constructs were implanted in heterotopic sites of the right and left rectus abdominis muscle, respectively (Table 1). Postoperative pain was controlled with intramuscular

![Fig. 2. Concavities of the in vitro bioreactor orient and polarize MC 3T3-E1 cells along concavities of the coral-derived macroporous construct. (A) Alignment and orientation of MC 3T3-E1 cells along a concavity of the substrate; the concavity forces the polarization of MC 3T3-E1 cells in vitro to fit within the geometric cues of the concavities. (B) Optimal polarization and alignment of MC 3T3-E1 cells within a concavity of the bioreactor grown in vitro.](image-url)
injections of buprenorphine hydrochloride (0.3 mg). Individually housed animals were fed and monitored as described [22,23].

Anaesthetized animals were killed with an intravenous overdose of sodium pentobarbitone on days 30 and 90; one animal had to be euthanized 5 days after surgery and the remaining animal was euthanized 31 months after implantation (2.7 years). Anaesthetized animals were subjected to bilateral carotid perfusion with buffered saline followed by 10% buffered formalin [23–27]. Harvested tissue was further fixed in phosphate-buffered formalin for 48 h, rinsed in tap water and stored in 70% ethanol. Undecalcified tissue blocks were treated according to a previously described method [36] with minor modifications. Briefly, the tissues were processed in ascending concentration of Technovit 7200 VLC (Heraeus Kulzer GmbH, Wehrheim, Germany) and embedded in a fresh solution of the same resin. Undecalcified sections were then ground and polished to 40–60 μm and stained with a modified Goldner’s trichrome [29]. All sample preparation was performed using the EXAKT precision cutting and grinding system (EXAKT Apparatebau, Nordstedt, Hamburg, Germany) [36].

2.4. Histology and histomorphometry

Sections were examined with a Provis AX70 research microscope (Olympus Optical Co., Japan); scans of digital images were analyzed by the

![Fig. 3](image)

**Fig. 3.** Morphological conceptualization of the critical role of the concavity prepared along the surfaces of titanium constructs later coated with plasma sprayed highly crystalline hydroxyapatite and implanted in orthotopic bony sites. (A) Induction of bone formation (magenta arrow) within a concavity of a granular/particulate coral-derived hydroxyapatite construct 90 days after implantation in the rectus abdominis muscle of *Papio Ursinus* [40]. (B, C, D) Repetitive concavities (magenta arrows) induce the induction of bone formation in highly sintered crystalline hydroxyapatite constructs [B] [30] or in coral-derived macroporous specimens (C, D) [23,24]. Digital microphotography A, B, C and D together with Fig. 2B were instrumental for the conceptualization and fabrication of self-inducing geometrically-designed titanium implants as represented in (E) [19,20]. (E) The concavity motif is reassembled in a titanium construct coated with highly crystalline hydroxyapatite; the concavities (blue arrows) preferentially adsorb plasma and plasma products during the implantation procedure. There is no binding of plasma products and/or blood along the inter-concavity spaces even though identically coated by highly crystalline hydroxyapatite (Fig. 1A, B). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Olympus AnalySIS system mounted on the microscope. Digital images of the sections at both low and intermediate magnification were captured on the Olympus AnalySIS system and linear measurements obtained using the system software [29]. Measurements of bone in contact (BIC) on both the planar and geometric surfaces were made and the bone/implant ratio (%) determined.

2.5. Scanning electron microscopy analyses

Scanning electron microscopic (SEM) images were used to obtain the exact image of the concavities under high magnification as well as the nanopatterned surface after plasma spraying of the treated titanium surfaces. Carbon-coated samples (coating 5 nm thick) were examined on a FEI Nova Nanolab SEM (FEI Company, Oregon, USA), at 30 kV.

3. Results

3.1. Generation and surface morphology of geometrically-designed titanium implants

To engineer bone, we created an in vivo bioreactor with a series of repetitive concavities within the titanium constructs to generate an inductive microenvironment for the induction of bone formation. The anatomical shape of the sequence of repetitive concavities together with surface nanotopographies generated by the hydroxyapatite coating were defined from digital images as presented in Fig. 1. Depressions and indentations of the plasma-coated

![Morphology of osteointegration and bone in contact with the hydroxyapatite-coated titanium constructs harvested from the tibia on day 30. (A, B) Digital microphotographs of planar control implants showing a thin layer of osteointegrated bone along the hydroxyapatite-coated surfaces (blue arrows). (C, D) Pronounced induction of bone formation within concavities of the geometric implants as well as along the newly formed bone surrounding the implants; newly formed bone both within the bioreactor of the concavities and surrounding the implanted constructs is characterized by prominent osteoid seams (blue arrows) surfacing newly formed mineralized bone. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image-url)
hydroxyapatite layer are visible at higher magnifications (Fig. 1D, E). The observed topographical landscape mimics pits, lacunae and concavities of the trabecular cortico-cancellous bone as patterned by sequential episodes of osteoclastogenesis [26–29,37–39].

3.2. In vitro studies: topographic and geometric cues

Histological evaluation of the pre-seeded macroporous calcium phosphate/calcium carbonate constructs with NIH3T3 and MC3T3-E1 cell lines showed the critical role of nanotopographic and geometric cues for cell differentiation, attachment and alignment along the available concavities (Fig. 2). Concavities of the stratum effectively induced cell orientation and alignment of MC3T3-E1 cells along the geometric cues of the macroporous constructs (Fig. 2B).

3.3. Morphology of tissue induction and osteointegration

Fig. 3 further illustrates the rationale of the concavity, as designed for the induction of bone formation, for the generation of solid hydroxyapatite-coated titanium surfaces with repetitive concavities (Fig. 3E). Fig. 3A demonstrates the induction of bone formation within the concavity (magenta arrow) of a particulate/granular coral-derived calcium carbonate/calcium phosphate construct implanted in the rectus abdominis muscle of a non-human primate P. ursinus and harvested on day 90 [40]. Arrows in Fig. 3B, C, D indicate the spontaneous and intrinsic induction of bone formation within a series of repetitive concavities constructed by the coral-derived macroporous biomatrix [23–25,27–29].

The concavity rationale has been translated to solid titanium surfaces coated with highly crystalline hydroxyapatite (Fig. 3E), in which blue arrows point to the concavity microenvironment that specifically adsorbs plasma and plasma products. Of note, there was no adsorption along the inter-concavity spaces though sprayed by an identical hydroxyapatite-coating (Fig. 3E).

3.4. Tissue development and morphogenesis: osteointegration

We followed the evolution of the bioreactor/concavity space over time; on day 30 both titanium surfaces whether implanted in the tibiae or in edentulous hemi-mandibles showed a different morphology of osteointegration. Planar surfaces showed osteointegration but limited trabecular and osteoid activities along the integrated surfaces (Fig. 4A, B). Geometrically-designed constructs showed prominent induction of bone formation, trabeculation and osteoid synthesis within and outside the bioreactor of the exposed concavities (Fig. 4C, D). Histomorphometric analyses on day 30 showed no statistically significant differences in the amount of bone in contact (BIC) between experimental and control planar surfaces (Fig. 5).

Tissue development and morphogenesis on day 90 engineered prominent osteointegration by geometrically-constructed titanium specimens (Fig. 6) with greater BIC than control planar surfaces (Fig. 5). Fig. 6B shows how the concavities designed along the implant’s surface specifically bind plasma and plasma products during the implantation procedure (Fig. 6B). Compact bone had formed and remodeled along the treated surfaces with repetitive concavities (Fig. 6C); newly formed and mineralized bone had integrated with the hydroxyapatite-coating often generating marrow spaces within the bioreactor of the concavities (Fig. 6D arrow); mineralized remodeled bone has tightly integrated with the hydroxyapatite coating (Fig. 6E–G), with remodeling and blending into the hydroxyapatite coating (Fig. 6H arrow).

![Bone in contact - Planar vs Concavity](image)

**Fig. 5.** Osteointegration, expressed as bone in contact (BIC) of control planar and geometric concavities by hydroxyapatite-coated titanium implants harvested on day 30 and 90 from the edentulous hemi-mandibles and the anterior-medial aspect of the tibia. No major differences were found on day 30 between controls planar vs. concavity geometric surfaces; on day 90, there was a statistically significant difference between the control and geometric implants (*p < 0.05 on day 90). There were no major differences between the sites of implantation, intra-tibial vs. intra-mandibular.

On day 90, geometrically-designed implants showed greater BIC and osteointegration when implanted in the anterior-medial aspect of the tibiae or the hemi-mandibles (Fig. 5).

3.5. Self-inducing geometric titanium implants

Important results were gained in the animals designed for the long-term study. Notably, one animal had to be euthanized 5 days after surgical implantation and thus provided an interesting insight into early events when experimental and control planar titanium specimens were harvested from the rectus abdominis muscle and processed for SEM analyses. SEM evaluation (Fig. 7) showed a prominent multicellular driven cellular attachment and differentiation with the morphogenesis of cellular condensations and alignment along the exposed surfaces of the concavities (Fig. 7C, D); the multifaceted cellular activities along geometrically-designed substrata markedly contrasted with the limited cellular activity as seen along control planar surfaces (Fig. 7A).

Geometric implants showed dramatically how the geometric design of the concavity is the “shape of life” [25,28] when implanted in the rectus abdominis muscle, known to harbor several mesenchymal paravascular/pericytic and myoblastic stem cell niches [41]. The concavities induced multiple cellular attachments, cell differentiation and cellular trafficking across the modified surfaces and at the margins of the exposed concavities (Fig. 7E, F). This morphological and spatial organization of the invading myoblastic/pericytic stem cells attached to the borders of the concavities are responsible for the induction of bone across concavities as shown in discs of highly crystalline hydroxyapatite when implanted in the rectus abdominis muscle of P. ursinus (Fig. 8).

Morphological analyses of ground sections of hydroxyapatite-coated titanium implants harvested from the rectus abdominis muscle 31 months after implantation showed the induction of bone formation within the “in vivo bioreactor” of the concavities of the implanted constructs (Fig. 9). High power views of ground sections show partial resorption and dissolution of the mineralized coating with induction of mineralized bone (Fig. 9A, B) with osteocytes and...
mineralized collagenic fibers protruding into the osteoid matrix (Fig. 9A–D).

4. Discussion

The basic tissue engineering paradigm has been tissue induction and morphogenesis through combinatorial molecular protocols whereby soluble molecular signals are re-combined with insoluble signals and substrata acting as tridimensional constructs for the initiation of de novo tissue induction and morphogenesis [42–46]. This paradigm for bone tissue engineering has been modified by the language of geometry with a shift to develop biomimetic and bioinspired matrices endowed with shape-memory geometries that in their own right can induce the
ripple-like cascade of bone differentiation by induction, even when implanted in heterotopic extraskeletal sites, without the osteogenic soluble molecular signals of the TGF-β supergene family, the bone morphogenetic/osteogenic proteins (BMPs/OPs) and, in primates only, the three mammalian TGF-β isoforms [25,27,29]. Studies in \textit{P. ursinus} have shown that the driving force of the intrinsic osteoinductivity by bioactive biomimetic matrices is the shape of the implanted scaffold [27–30]. The language of the shape is the language of geometry; the language of geometry is the language of a sequence of repetitive concavities that biomimetize the remodeling cycle of the primate osteonic bone [27,37–39]. To engineer bone and to induce greater osteointegration along hydroxyapatite-plasma coated titanium surfaces, we deployed a series of self-inducing osteoinductive repetitive concavities [22,25–30] that created an \textit{in vivo} bioreactor, as defined by Stevens et al. [31], which controlled tissue induction and morphogenesis within the exposed concavities of the implanted substrata.

Incisive recent experimentation has shown that “stem cells feel the difference” [4–6] of the composition and of the physical pattern of the surfaces of the substratum upon which they attach and spread; the fact that stem cells and differentiating stem cells sense the substratum implies that migrating and attaching cells onto the substratum are able to convert mechanical cues into soluble molecular signals [1,6,7]. It is now clear that the nanotopography and surface geometry of different substrata are the critical cues to set into motion selected gene expression pathways in responding and attaching stem cells. Attaching stem cells by virtue of nano-patterned surface geometric modification [1,3,5,7,47,48] will express osteogenic gene products [15–18] controlling cell proliferation and differentiation [19]. Importantly, recent studies have additionally highlighted that the combination of micron-scale roughness and high surface energy synergistically promotes osteoblast responses [48] and that nanotopographic surface definitions synergistically induce a significant up-regulation of osteogenic markers [14].

Ultimately, the critical role of the concavity as an “\textit{in vivo} bioreactor” [31] initiating the induction of bone formation has been demonstrated by the induction of bone within concavities of hydroxyapatite plasma-coated titanium fixtures long-term implanted in the \textit{rectus abdominis} muscle of \textit{P. ursinus} as shown in Fig. 9. High power morphological analyses of the heterotopic specimens has indicated some degree of resorption/dissolution of the crystalline hydroxyapatite coating; this might have resulted in
Ca\(^{2+}\) release, with induction of angiogenesis, followed by stem cell differentiation into osteoblastic cells [49–54].

We have shown that the importance of geometry for bone tissue engineering is not limited to the internal configuration of a macroporous calcium-phosphate construct, but extends to the external design of solid prostheses to be used for orthopedic and dental applications [19,20]. The described induction of bone formation by the hydroxyapatite-coated titanium concavities is the innovative application of the results of the senior author’s research experimentation on the importance of the geometric configuration of a biomaterial construct for the spontaneous initiation of bone formation even when not in direct contact with viable bone [19,20].

The induction of bone formation within the hydroxyapatite-coated titanium surfaces is the first reported instance of osteoinductive hydroxyapatite-coated titanium implants constructed for implantation in clinical contexts. Can bone be formed by uncoated titanium substrata? The only available experiment reporting the induction of bone formation by titanium constructs when implanted intramuscularly in a canine model has been reported by Fujibayashi et al. [55]. Importantly, however, the titanium constructs were not prepared as titanium dental implants for insertion in edentulous ridges but rather as macroporous titanium blocks [55]. The implanted bioactive titanium also showed superior in vitro apatite-forming ability bonding directly to living bone in vivo [55]. Additionally, studies have shown that chemically and thermally-treated titanium macroporous blocks possessed an in vitro apatite-forming ability with an in vivo osteoinductive capacity [56]. SEM analyses of titanium macroporous blocks after chemical and thermal treatment show a high degree of nanotopographical complexity [55] which is functionalized as initiating cellular induction and transformation of trafficking and attaching stem cells, coupled with in vivo apatite-forming ability. This might have initiated the induction of bone formation. It is noteworthy that bone formation by macroporous titanium blocks was only found 12 months after implantation in the dorsal musculature of adult beagle dogs [55]. Similarly, the induction of bone formation within the concavities of the hydroxyapatite-coated titanium constructs was seen at 31 months and not 30 and 90 days after implantation in heterotopic sites of *P. ursinus*.

5. Conclusion

The current requirement of biomaterials for bone tissue engineering and regenerative medicine at large is to implant functionalized surfaces with bioactive nanotopographic geometric cues priming the available stem cell niches. These will set into motion gene expression, secretion, synthesis and embedding of the osteogenic gene products within the functionalized surfaces, initiating the spontaneous induction of bone formation as a secondary response. The concavity, as cut within titanium surfaces, generates an in vivo bioreactor and biomimetizes the ancestral repetitive and multi-million-year old tested designs and topographies of Nature. The concavities are endowed with functional shape-memory geometric cues in which soluble signals induce morphogenesis, and physical forces, imparted by the geometric topography of the substratum, dictate biological patterns, causing the induction of bone formation and regulating the expression of gene products as a function of the structure.

Acknowledgments

The studies on the “geometric induction of bone formation” have been constantly supported by the Medical Research Council of South Africa, the University of the Witwatersrand, Johannesburg, and the National Research Foundation of South Africa since the first histological sections cut by Barbara van den Heever in 1988 demonstrating the morphogenesis of bone in coral-derived macroporous constructs. The senior author and the staff of the Bone Research Laboratory are indebted to the creative work of Doctors Thomas, Richter and Kotze of the Council for Scientific and Industrial Research — Materials Science and Bioceramics Group — with whom the Unit has collaborated for many years in joint experimentation resulting in several publications including patents. A special word of thanks to Mrs. Bhavanbhai for the expert bibliographical assistance and for the continuous support for the research activities of the Unit and to Dr Carlo Brambilla for making available wireless Internet to the senior author when working in Italy on the several drafts of the manuscript. The senior author is indebted to Hari A Reddi, distinguished Professor and Ellison Chair, Musculoskeletal Molecular Biology, Center for Tissue Regeneration and Repair, the University of California, Davis, for a special flair of understanding and for teaching the senior author the critical role of geometry on the induction of bone formation. The senior author is also indebted to Jean-Claude Petit for all the help in experimentation and surgical implantation. The reported work was done by the senior author and Laura Roden (née Yeates) when working at the
Bone Cell Biology Section of the NIH and at the Dental Research Institute, MRC/University of the Witwatersrand, Johannesburg, in the late eighties-early nineties.

References


Fig. 9. Induction of bone formation within the bioreactor spaces of the concavities of the geometric implant coated with highly crystalline hydroxyapatite and long-term implanted in the rectus abdominis muscle of Papio ursinus. Low (A) and high (B) power views of the newly formed mineralized bone onto the plasma sprayed layer of crystalline hydroxyapatite (blue arrow); note mineralized collagenic fibers inserting into as yet to be mineralized osteoid (red arrow). (C, D) Induction of bone formation within concavities of the plasma-coated titanium constructs with cellular elements within the non-mineralized secreted matrix. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)


