J. Cell. Mol. Med. Vol 13, No 9B, 2009 pp. 2953-2972

Biomimetism, biomimetic matrices and the induction of bone formation

Ugo Ripamonti *

Bone Research Unit, Medical Research Council/University of the Witwatersrand, Johannesburg, South Africa

Received: August 7, 2008; Accepted: October 16, 2008

Abstract

Bone formation by induction initiates by invocation of osteogenic soluble molecular signals of the transforming growth factor-β (TGF-β) superfamily; when combined with insoluble signals or substrata, the osteogenic soluble signals trigger the ripple-like cascade of cell differentiation into osteoblastic cell lines secreting bone matrix at site of surgical implantation. A most exciting and novel strategy to initiate bone formation by induction is to carve smart self-inducing geometric concavities assembled within biomimetic constructs. The assembly of a series of repetitive concavities within the biomimetic constructs is endowed with the striking prerogative of differentiating osteoblast-like cells attached to the biomimetic matrices initiating the induction of bone formation as a secondary response. Importantly, the induction of bone formation is initiated without the exogenous application of the osteogenic soluble molecular signals of the TGF-β superfamily. This manuscript reviews the available data on this fascinating phenomenon, i.e. biomimetic matrices that arouse and set into motion the mammalian natural ability to heal thus constructing biomimetic matrices that in their own right set into motion inductive regenerative phenomena initiating the cascade of bone differentiation by induction biomimetizing the remodelling cycle of the primate cortico-cancellous bone.

Keywords: bone induction • calcium phosphate bioceramics • biomimetism • smart biomimetic matrices

Introduction: the induction of bone formation, the emergence of the skeleton, of the vertebrates and of Homo species

In a number of reviews on the induction of bone formation, we have often asked how bone differentiation by induction is initiated? Or how the soluble molecular signals of the transforming growth factor-β (TGF-β) supergene family are deployed so as to initiate *de novo* bone formation by induction [1–3]? Somehow more simply but very confusing in a scenario of redundancy of multiple soluble molecular signals initiating bone differentiation by induction in the primate, which are the molecular signals that initiate *de novo* bone formation by induction [1–10]?

To be truthful, we have always assigned a prominent and pivotal role to the osteogenic proteins (OPs) of the TGF-β supergene family [4, 5]. There is no bone formation by induction without the osteogenic soluble molecular signals of the TGF-β supergene family [1–10]. We have, however, always assigned additional prominent

*Correspondence to: Ugo RIPAMONTI, M.D., Ph.D.,
Bone Research Unit, Medical Research Council/University of the Witwatersrand, Medical School, 7 York Road, Parktown, Johannesburg 2193, South Africa.
Tel.: +27 11 717-2144; Fax: +27 11 717-2300
E-mail: ugo.ripamonti@wits.ac.za

doi:10.1111/j.1582-4934.2008.00562.x

critical roles to biomimetic matrices capable of delivering the biological activity of the osteogenic soluble molecular signals [1–10].

To initiate the induction of bone formation and thus to ultimately erect the skeleton, Nature has had a powerful lesson to teach. A lesson that is continuously taught to biomaterial scientists, molecular biologists and tissue engineers alike, who wish to design, manufacture and sculpt new tissue constructs for replacement of lost parts in human patients [2, 5, 11–17]. The induction of bone formation requires three key components [17]: an osteoinductive soluble signal, an insoluble signal or substratum and responding host's cells. The insoluble signal delivers the osteogenic soluble molecular signal and acts as a scaffold for bone formation to occur after transmembrane serine-threonine kinase receptors' phosphorilation of responding host's cells [1, 12, 14, 16, 17].

The osteogenic soluble molecular signals of the TGF-β supergene family need thus to be reconstituted, more figuratively perhaps, recombined, with an insoluble signal or substratum to initiate the cascade of bone differentiation by induction [9–12, 17–19]. This fundamental rule in molecular and cellular biology has now become the cardinal rule to initiate tissue morphogenesis after the molecular dissection of the fascinating phenomenon of '*bone: formation by autoinduction*' [20], though regretfully not always completely understood. The classic experiments of the dissociative extraction and reconstitution of the bone matrix components showed that partially purified [1, 21–24] or highly purified recombinant human bone morphogenetic proteins (BMPs) [25, 26] need to be reconstituted with an insoluble signal or substratum to trigger the cascade of bone differentiation by induction [21]. The experiments were also critical to learn that putative BMPs within the bone matrix could be solubilized by the dissociative extraction of the bone matrix [17, 21, 22]. This has set into motion a ripple-like cascade of biochemical, chromatographic and molecular events that ultimately has resulted in the isolation, characterization and molecular cloning of an entirely new family of protein initiators collectively called the BMPs/OPs [17–19, 25–27].

When writing about osteoinduction, it is important to properly define the terminology related to '*bone: formation by autoinduction*' [20]. The acid test of the induction of bone formation is the *de novo* generation of endochondral bone after heterotopic extraskeletal implantation of the osteogenic soluble molecular signals of the TGF-β supergene family [5, 17, 18]. The heterotopic implantation site avoids the ambiguities of the orthotopic site where some degree of bone formation by conduction may occur from the viable bone interfaces [20].

Different strategies for the induction of bone formation

The classic studies of Sacerdotti and Frattin [28], Huggins [29], Levander [30, 31], Bridges and Pritchard [32], Moss [33], Trueta [34], Urist [20], Urist *et al.* [35] and Reddi and Huggins [36], have shown that several mineralized and non-mineralized extracellular matrices of mammalian tissues including uroepithelium, bone and dentine contain morphogenetic signals capable of initiating *de novo* bone formation by induction [3, 5]. The '*osteogenic activity*', as defined by different authors [31, 37, 38], resides thus within the extracellular matrices of different tissues and when implanted in heterotopic extraskeletal sites of animal models, this '*osteogenic activity*' diffuses out of the implanted matrices interacting with transforming resident mesenchymal cells capable of differentiation into chondroblastic and osteoblastic cell lines initiating bone differentiation by induction [3, 5, 17, 19].

The dissociative extraction and reconstitution of the bone matrix components [21] and of the homology of BMPs/OPs among mammals [22] have provided the key to implement the phenomenon of the induction of bone formation in pre-clinical and clinical contexts [17–19]. Before implantation, recombinant human BMPs/OPs, either hBMP-2 and/or hOP-1, [39, 40] need to be reconstituted with a variety of biomaterial matrices to form the osteogenic device for orthotopic orthopedic and craniofacial applications in clinical contexts [2–5, 17, 39–41]. Alternative approaches are continuously under laboratory and pre-clinical evaluation, *i.e.* directing the differentiation of stem cells into the osteogenic lineage firstly *in vitro* and then *in vivo* when implanted in skeletal defects of animal models [42, 43]. Additional approaches are based on the manipulation of a deliberately created space within the body, such that it serves as an *in vivo* bioreactor [44]. Osteogenesis forms within the surgically manipulated space of the bone bioreactor, which deploys the essential ingredients to induce bone formation, *i.e.* osteogenic soluble molecular signals, responding cells and the extracellular matrix scaffolds of the operated patients. The induced bone is later transplanted in osseous defects of the same patient [44]. The *in vivo* bone bioreactor has available a mesenchymal layer rich in pluripotent cells endowed with the potential to rapidly differentiate into osteoblastic-like cells secreting bone matrix remodelled into newly formed bone for autogenous transplantation [44].

Custom-made bone grafts grown in extraskeletal sites in human beings by hOP-1 and marrow aspirated from the iliac crest have been implanted in the *latissimus dorsi* muscle [45]. Heliotis *et al.* [46] went further by demonstrating that with a single recombinant OP combined with a porous hydroxyapatite (HA) carrier, a prefabricated heterotopic bone graft could be constructed in a human outside the skeleton without the addition of cortical bone, bone marrow aspirates or any other bone precursors to engineer a custom-made prefabricated bone flap for human mandibular reconstruction (Fig. 1) [46].

Biological significance of redundancy and synergistic induction of bone formation

Since the purification and cloning of multiple molecularly different BMPs/OPs, confirmation of osteoinductive activity has been

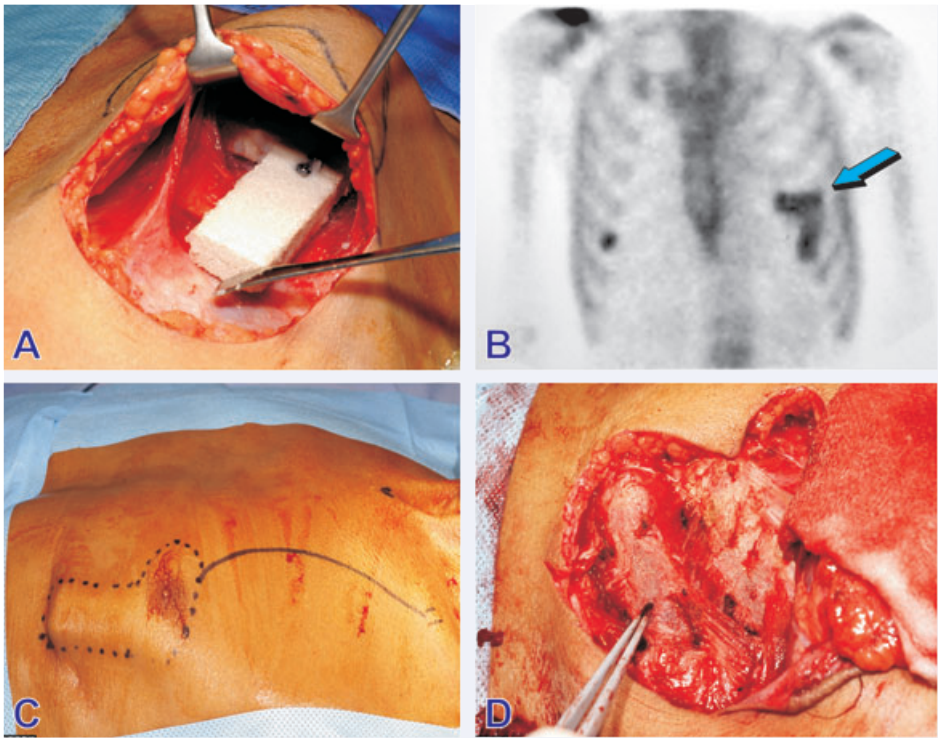

Fig. 1 Preparation and surgical harvest of a prefabricated mandibular graft in the chest of a human patient after combining hOP-1 with Interpore blocks of porous hydroxyapatite. (**A**) L-shaped blocks of coral-derived porous hydroxyapatite inserted into the pectoralis major muscle. (**B**) Skeletal scintigraphy demonstrating osteoinduction in the L-shaped prefabricated graft (arrow). (**C** and **D**) Flap design and mobilization of the prefabricated flap raised as a pectoralis major myo-osseohydroxyapatite flap pedicled on the thoracoacromial artery before transplantation into the recipient left mandibular region [46].

characterized for naturally derived and several human recombinant BMPs/OPs in different animal models [3, 5, 17, 19, 25, 26]. Importantly and conclusively, in the bioassay for bone induction in rodents, the TGF-β isoforms do not initiate the induction of bone formation [47]. In marked contrast, however, the mammalian TGF-β isoforms do induce rapid and substantial endochondral bone formation when implanted in heterotopic extraskeletal sites of *Papio ursinus* [3, 5, 18, 48]. In the primate, the TGF-β isoforms may act upstream to the BMPs/OPs and may induce the induction of heterotopic bone by expressing BMPs/OPs-related gene products resulting in the cascade of bone differentiation by induction [49–51]. Heterotopic implantation of the three mammalian TGF-β isoforms results in expression of BMP-3 and OP-1 as evaluated by Northern blot [49, 50] and RT-PCR analyses [51]. The presence of several molecularly related but different proteins with osteogenic activity in the primate [3, 5, 18] poses important questions about the biological significance of this apparent redundancy, additionally indicating multiple interactions during both embryonic development and the induction of bone formation in postnatal life [5, 18].

We have shown a potent synergistic induction of endochondral bone formation after binary applications of hOP-1 and TGF-β_1 in both heterotopic and orthotopic sites of the non-human primate *Papio ursinus* [48, 49]. The level of tissue induction induced by hOP-1 was raised several fold by the binary application of comparatively low doses of the TGF-β_1 isoform implanted in the *rectus abdominis* muscle of adult baboons [48]. The rapid induction of large corticalized ossicles in the *rectus abdominis* muscle of non-human primate species using binary applications of osteogenic molecular signals has important clinical implications. In the human, the primate *Homo sapiens*, regenerative phenomena are slower than in several other animals including non-human primate species. In human patients, regenerative medicine is often ineffective with delayed healing, contamination and scarring. The rapidity of tissue morphogenesis complete with mineralization of the outer cortex and bone marrow formation by binary application of recombinant hOP-1 and relatively low doses of hTGF-β_1 indicates that osteogenesis and bone induction in *Homo sapiens* should rather be engineered by the synergistic induction of bone formation [3, 52, 53]. The latter overcomes the temporally delayed repair phenomena in human patients where healing progresses slower than in experimental animals including non-human primate species [3, 48, 52, 53].

Biomimetism and biomimetic matrices self-assembling the induction of bone formation

A most fascinating and novel strategy to initiate the induction of bone formation is to construct biomimetic bioactive biomaterial matrices that *per se* initiate the morphogenesis of bone. This is initiated within the porous spaces of *smart* self-inducing biomaterial matrices even when implanted in heterotopic extraskeletal sites and without the addition of osteogenic soluble molecular signals of the TGF-β superfamily (Fig. 2) [2, 3, 5, 7, 27, 54, 55].

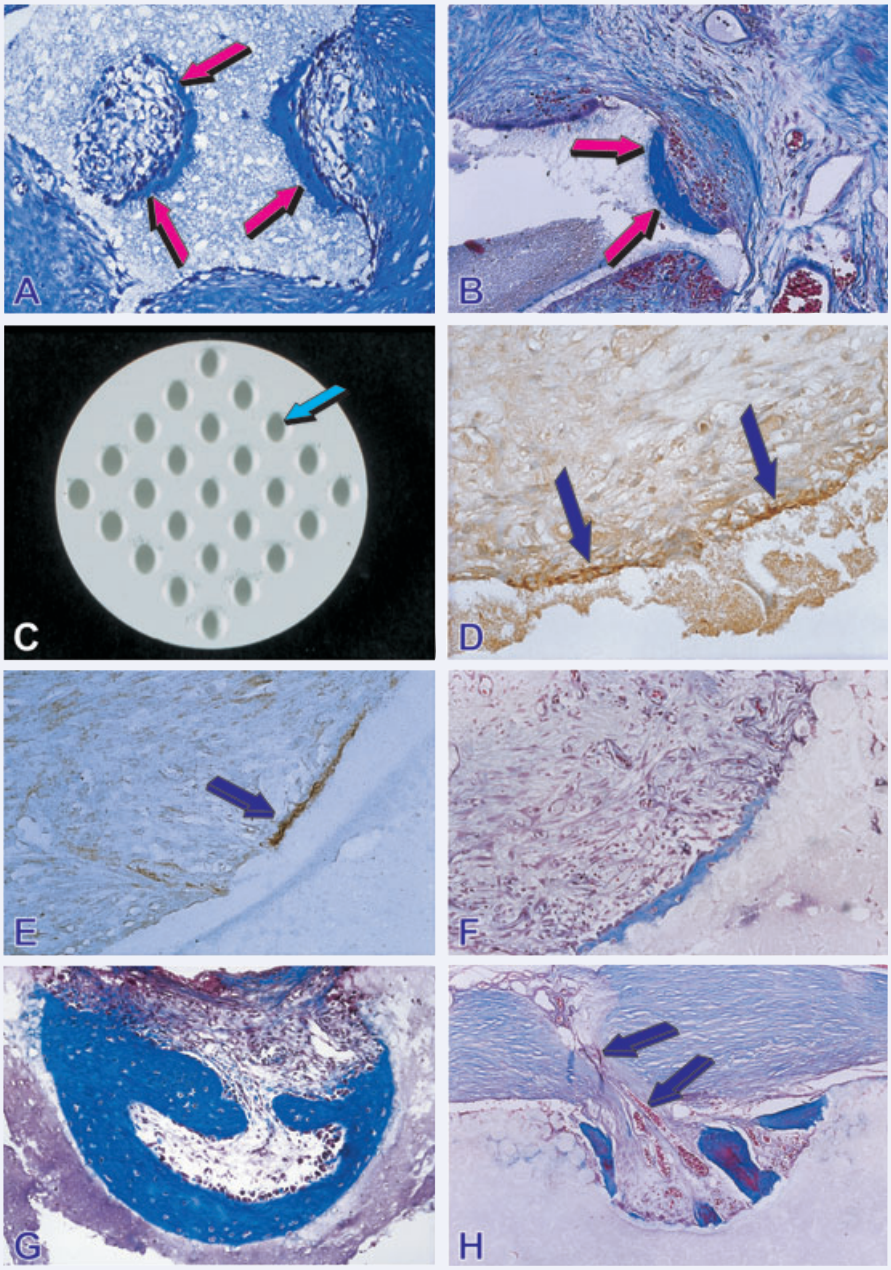

Fig. 2 Morphology of tissue induction and regeneration by geometric *cues* of smart biomimetic matrices implanted heterotopically in the *rectus abdominis* muscle of adult baboons *Papio ursinus* without the addition of exogenously applied osteogenic proteins. (**A** and **B**) Induction of bone formation (magenta arrows) in concavities of highly crystalline porous hydroxyapatite implanted in the *rectus abdominis* muscle of adult baboons and harvested on day 30. Bone (arrows) forms by induction within the concavities of the substratum. (**C**) To determine thus the critical role of specific geometries of the substratum, slurry preparation of hydroxyapatite powders were sintered to form solid monolithic discs (20 mm in diameter) of highly crystalline hydroxyapatite with concavities of 1600 μm in diameter and 800 μm in depth (blue arrow) [55]. (**D** and **E**) Tissue sections of harvested specimens on day 30 from the *rectus abdominis* were used to immunolocalize BMP-3 (**D**) and OP-1 (**E**) embedded into the biomimetic matrices (blue arrows). Bone formation by induction thus forms by day 30 (**F**) facing a highly vascular tissue within the concavity; bone formation increases by day 90 (**G**) with prominent vascular invasion (**H**) perforating the fibrous tissue enveloping the implanted disc (blue arrows) and targeting the newly formed bone within the concavity of the biomimetic matrix. Original magnification: (**A**) ×125; (**B**) ×125; (**C**) ×175; (**D**) ×175; (**E**) ×275; (**F**) ×275.

After millions of years of evolution, Nature has had the capacity to nucleate and evolve highly sophisticated tissues and organs. A classical example is the evolution of the skeleton, thus providing for the emergence of the vertebrates, deambulation and body erection freeing the upper limbs for superior foraging and more industrious Homo like activities including the use of tools for hunting, foraging above all, however, for maternal care contributing thus to the speciation of the genus Homo ultimately directing the emergence of *Homo sapiens*. The emergence of osteogenesis and later in evolution of the vertebrates, of the skeleton and *Homo sapiens* after a billion years from *Drosophila melanogaster* has permitted the precision self-assembly of the bone unit or osteosome [56] and the remodelling processes of the skeleton [57, 58]. The concavities as sculpted in the bio-inspired biomimetic bioceramics biomimetize the remodelling cycle of the osteonic corticocancellous bone (Fig. 3) [5, 8, 9, 59].

Nature has relied on common yet limited molecular mechanisms to direct morphogenesis and the emergence of specialized tissues and organs [3, 5, 17–19]. The BMPs/OPs reflect Nature's parsimony in controlling multiple specialized functions or pleiotropy, deploying several osteogenic molecular signals with minor variation in amino acid motifs within highly conserved carboxy terminal regions [2, 5]. The pleiotropic activity of the BMPs/OPs gene products is vast and spans from neurothropism to nephrogenesis, from cementogenesis to chondrogenesis, from air follicle induction to tooth morphogenesis, from angiogenesis to neurogenesis and from osteogenesis to cardiogenesis. The pleiotropic activity of the secreted proteins indicates that they are critical in development and are involved in several unrelated events that control pattern formation in embryonic development, morphogenesis and regeneration in postnatal life [3, 5, 17–19].

The evolutionary conservation of the OPs of the TGF-β superfamily is superbly demonstrated by the remarkable observation that recombinant decapentaplegic and 60A proteins, gene products of the fruit fly *Drosophila melanogaster*, induce bone formation in mammals [60].

Decapentaplegic and 60A in *D. melanogaster* are highly homologous to BMP-2, BMP-4 and BMP-5 and BMP-7, respectively, indicating the primordial role of BMPs/OPs sequences for the emergence of the vertebrates [5, 60]. Nature has thus usurped phylogenetically ancient amino acid sequences deployed for dorsal-ventral patterning in *D. melanogaster* to set the unique vertebrate trait of the induction of bone and of the skeleton rather than evolving novel gene products initiating the induction of bone formation [5, 60]. The skeleton with its supramolecular assembly of structural proteins, collagens and vascular structures permeating the osteonic walls bathed by the bone marrow organ is a superior example of design architecture and engineering [56]. The skeleton has evolved through millions of years of evolution and the extant skeleton has appeared as a result of expression and secretion of complex soluble molecular signals. Molecular signals interacting with insoluble signals or substrata of the extracellular matrix of bone populated by several different responding cells within the bone bone/marrow organ. The remodelling of the skeleton, the formation of bone by osteoblasts and the resorption of bone by osteoclasts, is a closely integrated homeostatic system [56]. When thinking about the molecular cell biology of bone remodelling and maintenance, it is important to visualize the geometric pattern of the remodelling cycle as initiated in any given time on each trabecula of the cortico-cancellous bone during the remodelling cycle (Fig. 3). The sequential phases of bone remodelling in the primate cortico-cancellous bone are quiescence, remodelling activation, resorption, reversal, formation/induction and quiescence again [56–58].

Remodelling of the cortico-cancellous bones entails, at any given time along the trabeculae of bone, three fundamental molecular, cellular and morphological processes that characterize the remodelling cycle of the primate cortico-cancellous bone: (*i*) resting, *i.e.* surfaces lined by resting lining cells as yet to be differentiated; (*ii*) resorption, *i.e.* areas of trabeculae actively resorbed by activated osteoclasts. Of critical importance for tissue engineering of bone, the bone resorption lacunae as formed by osteoclastogenesis are in the form of concavities (Fig. 3) [8, 9, 57–59]. The concavities are thus regulators of bone initiation and deposition during the remodelling processes of the skeleton.

Biomimetic matrices of highly crystalline HAs [27, 55] or biphasic HA/β-tricalcium phosphate (TCP) bioceramics [61] constructed with a series of repetitive concavities offer a geometric configuration which vividly reproduces and biomimetizes the bone remodelling processes of primate osteonic bone (Fig. 3) [5, 8, 9, 27, 55, 59, 61]. In several experiments in the *rectus abdominis* of the non-human primate *Papio ursinus,* we have bioassayed discs of highly crystalline HA and biphasic HA/β-TCP with concavities of specific dimensions on both planar surfaces (Fig. 2) [27, 55, 61]. Concavities were thus prepared to mimic the supramolecular assembly of the rigid mineralized extracellular matrix of primate osteonic bone biomimetizing self-assembling geometric cues *de novo* initiating bone formation by induction [5, 8–10, 61].

Independently, other research groups using different bioceramics and animal models reported the *intrinsic* induction of bone formation [62–69]. de Groot [70] hypothesized that after heterotopic implantation of calcium phosphate ceramics *in vivo*, there is '*de- and re-mineralization processes that may concentrate and immobilize the experimental animal's own naturally derived BMP/OP complex*' [70]. Whether BMPs/OPs attached to biomimetic matrices implanted heterotopically in animal models are originating from systemic circulation and/or extracellular matrices invading the porous spaces and concavities or *de novo* expressed in cellular elements resting on the biomimetic matrices, has been elucidated by Northern blot analyses showing the local expression of mRNA of osteogenic soluble molecular signals in tissues formed within the concavities of the implanted substrata [61]. Concavities are endowed with the striking prerogative of *de novo* initiating bone differentiation by induction in heterotopic extraskeletal sites of adult non-human primates *Papio ursinus* (Figs. 2, 3 and 7) [5, 27, 55, 61]. Our systematic studies have shown that the driving force of the intrinsic induction of bone formation by bioactive biomimetic matrices is the shape of the implanted substratum; the language of shape is the language of geometry; the language of geometry is the language of a sequence of repetitive concavities which biomimetizes the

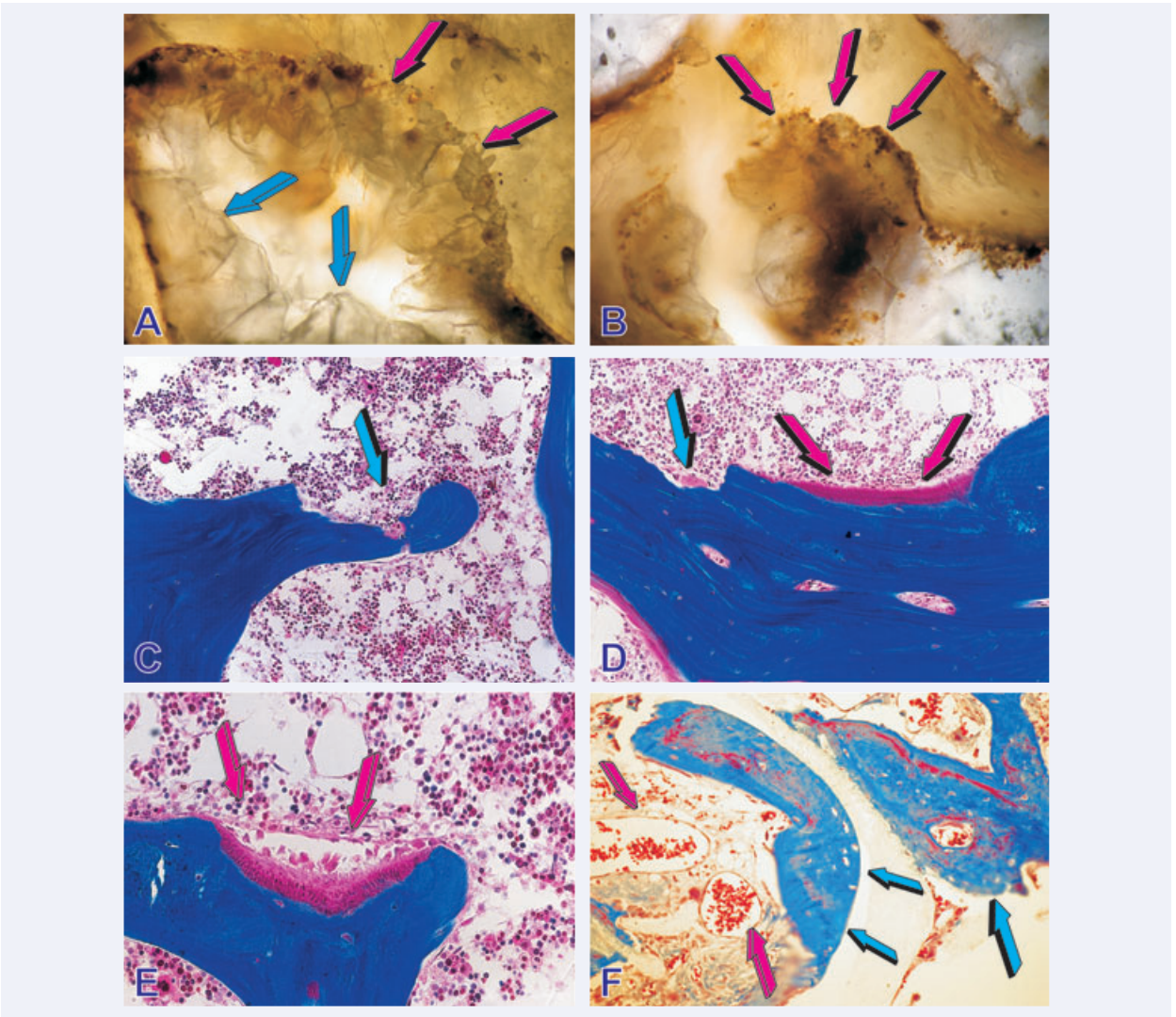

Fig. 3 Biomimetism of the concavity induced by osteoclastogenesis during the remodelling cycle of the osteonic bone with the induction of bone in concavities of the porous biomimetic substratum even if implanted in heterotopic extraskeletal sites and without the addition of exogenously applied osteogenic proteins. (**A** and **B**) Sculpted concavities by remodelling cycles in ancient hominid skeletal fossilized remains of cortico-cancellous bone of the *Australopithecus africanus*, the man ape of Southern Africa, temporally confined on faunal and paleomagnetic grounds to 2.5 to 3 million years before the present. Arrows (magenta) indicate the extent of osteoclastic activity within the trabecular bone of the extinct hominid; blue arrows point to calcite crystals occupying the cancellous spaces of the fossilized cortico-cancellous remains of *A. africanus*. (**C, D** and **E**) Cortico-cancellous bone remodelling and osteoclastogenesis (blue arrows) in extant non-human primate *Papio ursinus*. Concavities induced by osteoclastogenesis along trabeculae of bone biomimetize the concavities prepared in biomimetic bioceramics setting formation after resorption (magenta arrows in **D** and **E**), (**F**) self-inducing the spontaneous induction of bone formation (blue arrows) in biomimetic matrices when implanted in heterotopic extraskeletal sites of an adult baboon *Papio ursinus*. (**F**) Arrows indicate the rich vascular network and capillary sprouting that always correlate with the induction of bone formation (magenta arrows) within the porous biomimetic matrices. Original magnification: (**A**) ×125; (**B**) ×125; (**C**) ×175; (**D**) ×175; (**E**) ×275; (**F**) ×275.

remodelling cycle of the primate osteonic bone (Figs. 2, 3 and 7) [5, 8, 9, 55, 61, 71].

The term biomimetism has been recently introduced in the lexicon of regenerative medicine and tissue engineering with the intended meaning of the creative imitation of various specific biological systems [72–74]. Biomimetism is particularly appealing in the creation and assembly of biomaterials matrices which are endowed with specific *smart* functionalities as imparted by the

biomimetic organization coupled to the bioactive matrices. Biomaterial biomimetic matrices biomimetize specific biological systems with multifunctional shape memories with self-assembly capacities initiating and promoting angiogenesis and osteogenesis. The induction of angio-osteogenesis exploits and recapitulates events that initiate the induction of bone formation. Bone forms in the concavities as initiated by osteoclastogenesis during the remodelling cycle of the osteonic cortico-cancellous bone of non-human and human primates (Figs. 2 and 3) [5, 9, 17, 61, 71].

The concavity: the shape of life and the induction of bone formation

In collaboration with the Council for Scientific and Industrial Research division of Materials Science and Technology we have thus encapsulated into solid matrices repetitive sequences of concavities as biologically programmed morphogenetic cues that result in the differentiation of resident mesenchymal cells into osteoblastic-like cells expressing, secreting and embedding soluble osteogenic molecular signals into the concavities initiating bone formation by induction as a secondary response (Fig. 2) [7–10, 55, 61, 71].

We have proposed that there is a direct spatial and temporal relationship of molecular and morphological events that emphasize the pronounced biomimetism of the geometric induction of bone formation, *i.e.* the induction of bone in *smart* concavities assembled in biomimetic matrices with the remodelling cycles of cancellous bone (Fig. 3) [8–10, 59, 61, 71]. The basic multicellular unit of the cortico-cancellous bone excavates a trench across the surface rather than a tunnel leaving in its wake (with some degree of geometrical latitude) a hemi-osteon rather than an osteon [58], *i.e.* a trench with a cross-sectional geometric cue of a concavity at different stages of osteoclastogenesis, *i.e.* concavities with different radii of curvatures and depths as induced by osteoclastic activity.

Predating formation during the remodelling cycle of primate cortico-cancellous bone, osteoblasts eventually appear at the resorption site, *i.e.* lacunae with a geometric configuration of concavities (Fig. 3). Which are the molecular signals and/or physical forces imparted by the geometric topography of the substratum that terminate osteoclastogenesis and recruit osteoblastic-like cells initiating bone formation by induction within the concavities (Fig. 3)?

The available molecular and morphological data on the subject from several research groups have permitted to formulate the following conceptualization of the spontaneous induction of bone formation within porous biomimetic matrices: the net result of the induction of bone formation in concavities of the substratum is nothing but the language of geometry set by the concavities assembled within the biomimetic biomaterials.

In the adult skeleton, the demand of osteoblasts is created by bone resorption, *i.e.* the concavities induced by osteoclastogenesis, whereas the demand of osteoclasts is governed by the purpose of bone remodelling [58]. The concavities assembled in the biomimetic matrices are endowed with multifunctional pleiotropic self-assembly capacities initiating and promoting angiogenesis and differentiating resident mesenchymal cells into osteoblastic cell lines expressing, secreting and embedding osteogenic molecular signals within the concavities of the biomimetic matrices [27, 61, 71]. The molecular and morphogenetic mechanisms initiating the spontaneous induction of bone formation within concavities of the *smart* biomimetic matrices originate and progress with blood vessels and capillary sprouting developing within the mesenchymal tissue invading the concavities [7–10]. The extracellular matrix components of type IV collagen and laminin around the invading capillaries bind morphogenetic proteins involved both in angiogenesis and osteogenesis [59, 71, 75–79]. Bound morphogenetic proteins are then presented locally in an immobilized form to responding mesenchymal cells and osteoprogenitors alike to initiate osteogenesis in angiogenesis [5, 9, 59, 71].

Morphogenetic progression is sustained by continuous recruitment of mesenchymal cells and capillary invasion within the concavities. Resting mesenchymal cells attach to the matrix and differentiate into osteoblastic-like cells within the concavities of the biomimetic matrices. Differentiating osteoblastic-like cells express OPs of the TGF-β superfamily; mRNA expression, as evaluated by Northern blot analyses, is then followed by secretion and embedding of the expressed gene products within the *smart* concavities of the biomimetic matrices (Fig. 2D and E) [61, 71]. The induction of bone formation then follows as a secondary response [2, 5, 27, 55, 71].

Synthetic biomimetic matrices mimic the super-*smart* functionality of living tissue and allow the engineering of *smart* self-inducing biomimetic matrices for tissue engineering of bone [9]. The assembly of a series of repetitive concavities within porous biomimetic matrices adds selected functionalities or super-*smart* functionalities to the biomimetic matrices that intrinsically *per se* induce the spontaneous induction of bone formation without the exogenous application of osteogenic soluble molecular signals of the TGF-β superfamily (Figs 2 and 7) [9]. A critical step is the embedding of *smart* biological functions within intelligent scaffolds for tissue engineering of bone, *i.e.* embedding biological signals into biomaterials designed with super-*smart* biomimetic functionalities, ultimately resulting in the intrinsic induction of bone formation [61, 71].

Secreted BMPs/OPs within the concavities of the biomimetic matrices may be the result of osteoblastic-like cell differentiation or macrophages/osteoclasts expressing selected BMPs/OPs during the remodelling cycle of the primate cortico-cancellous bone. Macrophages/osteoclastic cells are known to express BMPs/OPs during the remodelling cycle of the cortico-cancellous bone [80]. Macrophages and osteoclastic cells are continuously involved during the osteointegration processes of implanted biomaterials [81] and are essential for effective tissue regeneration as they regulate the recruitment, proliferation and attachment of fibroblasts, osteoblasts and endothelial cells, significantly contributing to

construct osteogenesis in angiogenesis [81–85]. Whether BMPs/OPs are secreted and embedded into the *smart* concavities by differentiating osteoblast-like cells or macrophages/osteoclastic cells still deserves experimental investigation. The embedded soluble molecular signals will then differentiate osteoblastic-like cells, further secreting bone matrix and the induction of bone formation as a secondary response. Besides BMPs/OPs [82, 83], macrophages are also known to secrete TGF-β_1 [86]. While the evidence is still lacking, it is tempting to suggest that the induction of bone within concavities of *smart* biomimetic matrices recapitulates embryonic development by expressing and secreting BMPs/OPs synchronously and synergistically with TGF-β proteins, initiating the synergistic cascade of bone differentiation [18, 48].

Influence of geometry on the expression of the osteogenic phenotype

Experiments by Reddi and Huggins [87] have shown that the temporal sequence of fibroblast–chondroblast–osteoblast transformation is profoundly influenced by the geometry of the transformant, *i.e.* demineralized rodent incisors [87]. The specific geometric configuration of the inductor ultimately results in the induction of endochondral bone either with an anlage of cartilage or bone with bone marrow without the induction of chondrogenesis [87]. Subcutaneous implantation of coarse demineralized bone matrix powders (particle size 420–850 μm) resulted in the local differentiation of endochondral bone. On the other hand, implantation of fine matrix with particle size of 44–74 μm did not induce the bone [87].

Since implantation of fine demineralized bone matrix (particle size 44–74 μm) did not induce endochondral bone differentiation [87], it was interesting to determine whether extracts of fine bone matrix contain endochondral bone differentiation activity or, if the geometry of the implanted matrix particles solely drives the induction of bone [88]. When protein extracts of fine matrix were combined with coarse inactive collagenous matrix residues, there was restoration of the induction of endochondral bone formation [88]. Fine particles thus contain inductive proteins and the geometry of the matrix carrier is critical for the initiation of the bone induction cascade [88].

These powerful experiments demonstrated that although the fine matrix with particle size 74–420 μm contains osteoinductive proteins, the geometry of the inductor, *i.e.* the implanted matrix, is a critical factor to drive the biochemical cascade of bone formation by induction [87, 88]. Several studies have clearly highlighted that tissue induction and morphogenesis can be greatly altered by the geometry of the substratum [27, 54, 55, 61, 87–94].

In previous experiments, we have shown that the biological activity of BMPs/OPs could be expressed and delivered by a substratum other than the insoluble collagenous bone matrix as carrier [90]. The dramatic differences observed between substrata of granular and disc configuration underscored the importance of geometry in bone formation by induction [90]. Substrata of coral-derived HAs in disc configuration reconstituted with bovine osteogenic fractions purified greater than 50,000-fold with respect to crude guanidinium extract induced heterotopic endochondral bone formation when implanted subcutaneously in rodents [90]. Identical coral-derived HAs but in granular configuration did not induce bone differentiation even if pre-treated with identical doses of highly purified naturally derived BMPs/OPs [90]. Remarkably thus, the geometric configuration of the substratum can inhibit and overrule the osteogenic activity of BMPs/OPs both in rodents and non-human primates *Papio ursinus* [90, 94].

Identical coral-derived porous HAs with distinct geometric configurations were implanted in the *rectus abdominis* muscle of adult baboons. Bone differentiation occurred only in blocks of HA in rod configuration [94]. As in the rodent bioassay, though without the addition of highly purified osteogenic fractions, implants of particulate granular HA implanted in the *rectus abdominis* muscle of adult baboons, failed to induce bone differentiation within the porous spaces [94]. Instrumental for our understanding of the critical role of the concavity in driving the cascade of bone differentiation, minimal yet some bone formation was only found in a concavity of a particulate granular coral-derived HA specimen harvested on day 90 from the *rectus abdominis* muscle (Fig. 4H) [94]. The lack of bone differentiation in implants of granular HA implies a critical role of implant geometry on bone differentiation by induction [90, 94]. This has important implications for the construction of appropriate porous bone substitutes for reconstructive bone tissue engineering in clinical contexts [90, 94].

Predating the induction of bone formation, and in close proximity to developing bone, there is always a rich capillary network invading the porous spaces of the biomimetic matrix, particularly in concavities of the substratum [27, 54, 55]. Angiogenesis and vascular invasion are prerequisites for osteogenesis [34, 95]. Trueta has stressed the importance of the blood vessels in osteogenesis, and defined the vascular invasion during bone formation as *osteogenetic vessels*, suggesting that the endothelium may be capable of osteoblastic differentiation [34]. While circumstantial evidence is lacking, it is tempting to suggest that *osteogenetic vessels*, penetrating the porous spaces of the substratum, might have provided a temporally regulated flow of cell populations capable of expression of the osteogenic phenotype (Figs 2H and 4C, D) [34, 54, 95].

The implantation of calcium carbonate coral-derived HAs in the *rectus abdominis* of adult baboons showed that osteocyte-like cells are embedded within a tissue that had intermediate features between fibrous tissue and bone (Fig. 4) [54, 96, 97]. The development of mesenchymal condensation at the HA interface is a critical developmental event predating the initiation of spontaneous bone differentiation in porous bioceramics when implanted in the *rectus abdominis* muscle of *Papio ursinus* [54, 87, 88]. Indeed, mesenchymal condensations are critical for the initiation of skeletal development [98].

By including the analysis of early periods of observation (*i.e.* days 30 and 60), morphological and histochemical data have

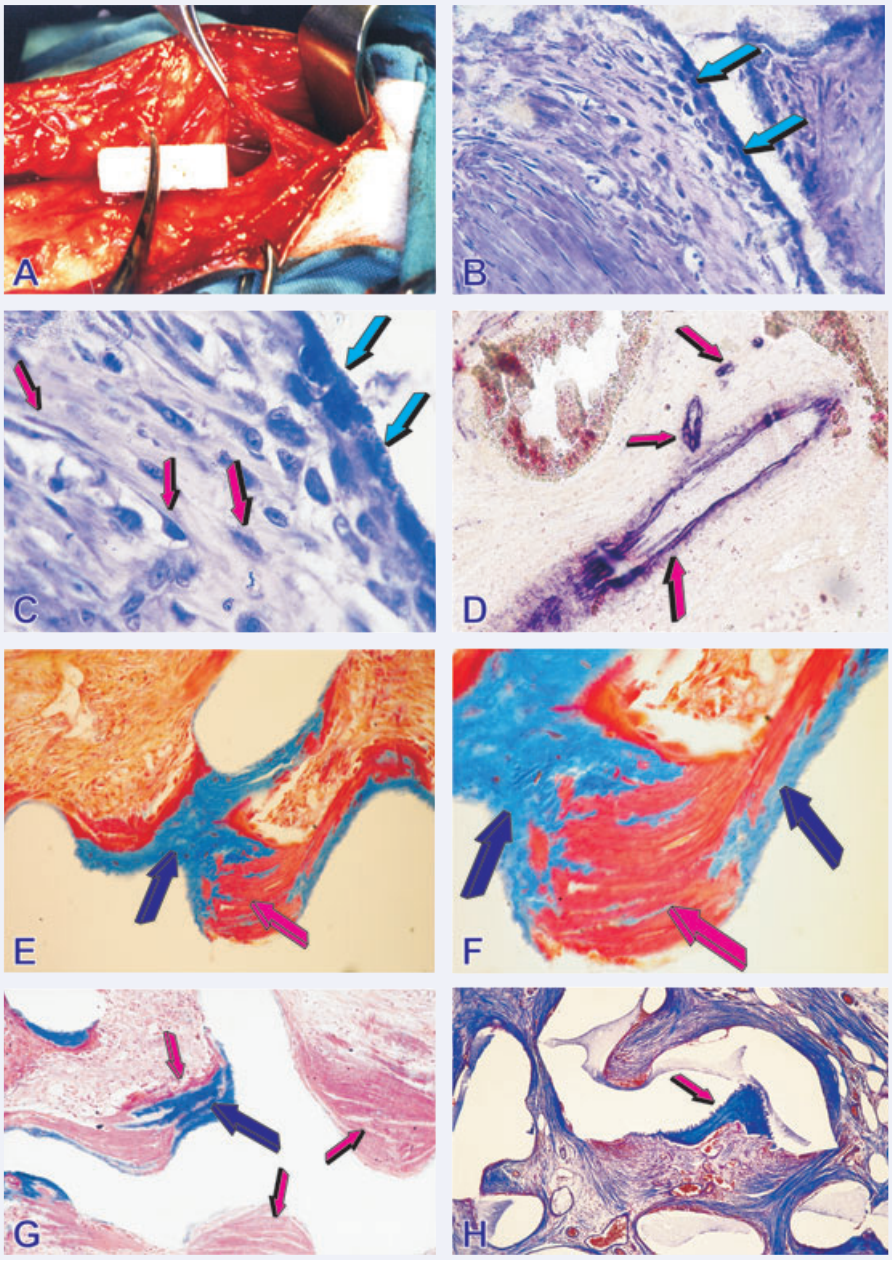

Fig. 4 Effects of the substratum of coral-derived hydroxyapatite biomatrices on tissue induction and morphogenesis within the porous spaces of the biomimetic matrix implanted heterotopically in the *rectus abdominis* muscle of *Papio ursinus*. (**A**) Heterotopic implantation of a rod of coral-derived porous hydroxyapatite (Interpore, USA). (**B** and **C**) Differentiation of osteoblastic-like cells at the hydroxyapatite interface highlighted in **C**. '*Osteogenetic vessels*' as defined by Trueta [34] penetrate the mesenchymal condensation seemingly providing migrating cellular progenitors (magenta arrows) capable of osteoblastic cell differentiation when in contact with the hydroxyapatite substratum. (**D**) Alkaline phosphatase staining of invading capillaries, the '*osteogenetic vessels*' within the porous spaces of the coral-derived porous hydroxyapatite (blue arrows). (**E, F** and **G**) Undecalcified sections stained freefloating with a modified Goldner' trichrome showing mineralization (blue arrows) of collagenic condensations (magenta arrows) surfacing the hydroxyapatite substratum. Mineralized bone (blue arrows) is surfaced by osteoid seams populated by osteoblastic cells. (**H**) Tissue morphogenesis in an implant of particulate granular coral-derived hydroxyapatite harvested on day 90 from the *rectus abdominis* muscle of an adult baboon. Bone formation (arrow) only within a concavity of the implanted matrix. Digital images G and H were instrumental for the understanding of the role of specific geometric configurations in the induction of bone and thus for the preparation and testing of solid discs of highly crystalline hydroxyapatite with concavities on both planar surfaces as shown in Fig. 2C. Original magnification: (**A**) ×125; (**B**) ×125; (**C**) ×175; (**D**) ×175; (**E**) ×275; (**F**) ×275.

shown that the differentiation of large, hyper-chromatic and intensely alkaline phosphatase positive cells at the HA interface is a critical morphogenetic event preceding the differentiation of bone (Fig. 4B and C) [54]. A critical step during the developmental cascade of the spontaneous and/or intrinsic bone induction in porous bioceramics without the addition of exogenously applied BMPs/OPs is the differentiation of resident mesenchymal cells in contact with the biomimetic substrata into osteoblastic cell lines resting upon the surface of the implanted matrices (Fig. 4B and C) [54, 61]. We now propose the following cascade of molecular, cellular and morphological events culminating in the induction of bone formation in heterotopic sites of non-human primates *Papio ursinus* initiating within concavities of '*smart*' biomimetic matrices:

1. Vascular invasion and capillary sprouting within mesenchymal fibrovascular condensations invading specific geometries of the substratum with capillary elongation in close contact with the implanted biomimetic matrix.
2. Invading mesenchymal cells attach and differentiate at the HA/soft tissue interface of the concavities. There is no differentiation of osteoblastic cell lines without the driving force of the concavities. This crucial step of differentiation is shown by Northern blot and PCR analyses of tissue harvested from the concavities of the biomimetic matrices and by the immunolocalization of BMP-3 and OP-1 within differentiating osteoblastic cell lines.
3. Synthesis and secretion of osteogenic soluble molecular signals of the TGF-β superfamily from resident resting and transforming osteoblastic-like cells and/or macrophages/osteoclastic cell lines attached to the concavities of the substratum as shown by intercellular immunolocalization and finally embedding of the secreted gene products into the concavities of the HA biomimetic matrices.
4. *Intrinsic* osteoinduction with further differentiation of osteoblastic cell lines; bone formation by induction within the concavities of the biomimetic matrices is dependent on a critical threshold of endogenously produced BMPs/OPs initiating formation by induction as a secondary response.

The intrinsic or *spontaneous* induction of bone formation in a variety of porous biomaterials is a very interesting phenomenon which originally has been shown by implanting porous polyhydroxyethyl-methacrylate in the sub-cutis of white pigs [99]. In the late 1980s, systematic studies were started in non-human primates *Papio ursinus* after the remarkable finding of *intrinsic* osteoinductivity in the porous spaces of coral-derived HAs (Figs. 4–6) [5, 54, 96, 97, 100, 101]. Coral-derived HAs induced substantial amounts of membranous bone when implanted heterotopically in the *rectus abdominis* muscle of adult *Papio ursinus* (Figs. 5 and 6) [54, 96, 97, 100, 101]. In the same animals, coral-derived (Figs 8 and 9) and sintered porous HAs (Fig. 10) implanted in calvarial defects also induced extensive bone deposition culminating in complete calvarial regeneration [5, 7, 55, 101].

Importantly for application in clinical contexts, the extent of the *spontaneous* induction of bone formation in calcium phosphate ceramics varies significantly in different animal models. Using coral-derived HA substrata, minimal, if any, bone formation was shown in dogs and rabbits as compared to adult baboons [100]. In contrast to other studies, calcium phosphate ceramics showed bone formation within the porous spaces of the implanted matrices in canine models [70, 102]. The bone also formed in direct contact of calcium phosphate ceramic particles implanted heterotopically in sheep muscles [66, 103, 104] and in goats [62]. Further studies on the cross-species comparison of heterotopic bone induction in biphasic calcium phosphate HA scaffolds showed that the extent of heterotopic bone formation is controlled by the animal species as well as the nature of the implanted scaffolds [67]. Implantation of calcium phosphate bioceramics in the rat heterotopic bioassay results in lack of bone differentiation although specimens harvested from heterotopic sites of murine models showed the differentiation of bone to a varying degree [67].

Several papers have stressed the importance of biomimetic matrices capable of concentrating several proteins including BMPs/OPs from the body fluids and/or the extracellular matrix [70]. It has been proposed that ceramics sintered at a lower temperature would have the ability to concentrate more circulating and/or locally produced BMPs/OPs [62, 67, 70]. The capacity to concentrate BMPs/OPs as found in the circulation or in the microenvironment of the extracellular matrix suggests that the incidence of the spontaneous induction of bone formation varies with animal species as well as the implanted biomimetic matrix. Additional critical parameters for the spontaneous induction of bone formation in a variety of porous bioceramics are the porosity, pore size and distribution, interconnectivity, fenestration, distribution as well as orientation of pores [62, 67]. Constructs of porous β-TCP also showed the induction of heterotopic bone in dogs [85]. Kondo *et al.* have suggested that the microporous surfaces of the β-TCP constructs enhanced protein adsorption and cell attachment contributing to the osteoinductivity of the tested β-TCP biomatrices also showing *in vitro* that microstructure is a key factor for osteoblastic differentiation and proliferation [85].

Data from several investigations *in vivo* in canine and ovine models have suggested that the microporosity of the implanted bioceramics play important roles as the storage microenvironment for several extracellular matrix components, including BMPs, and are ultimately responsible for the induction of bone formation. The reason why, however, the microstructure and interconnected pores are so critical for osteoblastic-like cells differentiation, expression of OPs, and thus the induction of bone formation as a secondary response still remain unclear. Similarly, the reason for the differences in biomaterials-induced bone formation in the tested animal species is as yet to be defined [66, 67, 69, 100]. More importantly perhaps, morphological and molecular experiments are now mandatory to assign to specific cellular elements the expression and secretion of the OPs of the TGF-β superfamily.

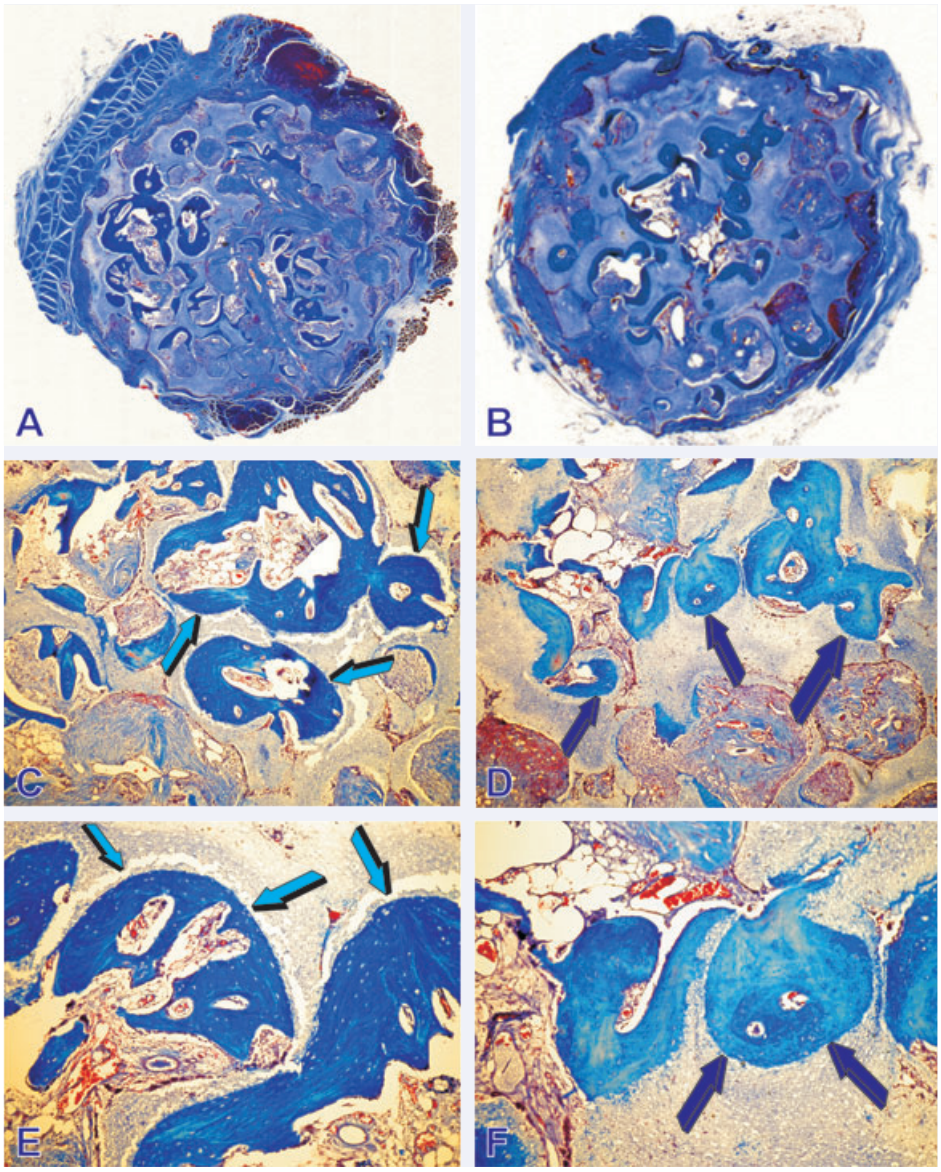

Fig. 5 The morphogenesis of bone in porous coral-derived hydroxyapatites harvested on day 90 from the *rectus abdominis* muscle of adult baboons *Papio ursinus*. (**A** and **B**) Low-power photomicrographs showing substantial bone differentiation within the porous spaces of the biomimetic matrix. (**C** and **D**) High-power views of the distribution of the newly formed bone (arrows) within the porous spaces in tight contact with the biomimetic substratum. (**D** and **E**) Details of previous images showing the biomimetism of the concavity inducing bone formation within specific geometries of the biomimetic matrix (blue arrows). Original magnification: (**A**) ×125; (**B**) ×125; (**C**) ×175; (**D**) ×175; (**E**) ×275; (**F**) ×275.

Porous biomaterials with a series of repetitive concavities with optimal surface topography and microstructure provide porous spaces that are architecturally conducive to differentiate resident mesenchymal cells into osteoblastic cell lines. Expression, secretion and embedding of osteogenic molecular signals are followed by rapid vessel ingrowths, capillary sprouting and the induction of bone formation as a secondary response [71]. The surface topography and the geometry of the concavity affect cellular morphology, and cellular shape will influence function [105–107] during the differentiation of osteoblastic cell lines expressing and secreting osteogenic molecular signals. The differentiation of osteoblastic cell lines expressing and secreting osteogenic soluble molecular signals of the TGF-β superfamily is at the crux of the intrinsic osteoinductivity of a variety of porous calcium phosphate and biphasic TCP HA bioceramics in a variety of animal models including man.

Morphological analyses of coral-derived HAs implanted in the *rectus abdominis* muscle of *Papio ursinus* showed the differentiation of large hyper-chromatic cells arranged in one or two layers directly opposed to the HA substratum [54]. Such cells, interpreted as differentiating osteoblasts, were close to a rich capillary network lined by large and hyper-chromatic endothelial cells [54].

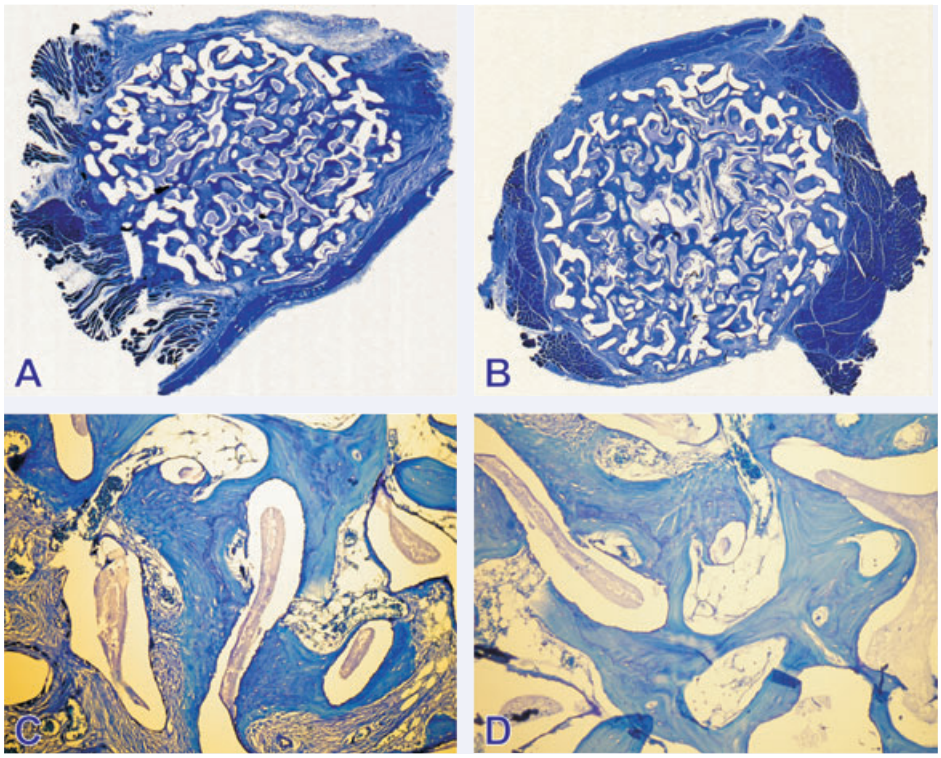

Fig. 6 Substantial bone morphogenesis by induction in porous coral-derived hydroxyapatites long-term implanted in the *rectus abdominis* muscle of adult baboons *Papio ursinus*. (**A** and **C**) Low- and high-power views of bone induction within the implanted biomimetic matrix harvested 6 month after implantation in the *rectus abdominis* muscle of adult baboon. (**B** and **D**) Morphogenesis of bone in coral derived porous hydroxyapatites harvested 9 months after implantation. Lamellar/osteonic bone with bone marrow. Original magnification: (**A**) ×125; (**B**) ×125; (**C**) ×175; (**D**) ×175; (**E**) ×275; (**F**) ×275.

The differentiation of large, hyper-chromatic and intensely alkaline phosphatase positive osteoblastic cells in an intimate relationship with endothelial cells and the implanted biomimetic matrix is a critical morphogenetic event preceding the differentiation of bone [54, 55].

The induction of bone is constructed by regulating the expression of selected mRNA of gene products as a function of the structure [2, 9, 10, 61]. Biomimetic matrices of highly crystalline HAs [9, 55] or biphasic HA/TCP bioceramics [61] constructed with a series of repetitive concavities offer a geometric configuration which vividly reproduces and biomimetizes the remodelling processes of the primate osteonic bone [5, 8, 9, 57, 58].

Ultimately, which are the resident mesenchymal cells capable of transformation/ differentiation into secreting osteoblastic-like cells at the HA interface? It is obvious that the *rectus abdominis* muscle of adult baboons is endowed with a stem cell *niche* [108, 109] that provides a large number of differentiating cells including osteogenic progenitors which attach and differentiate onto the biomimetic matrices. The presence of a stem cell *niche* in *rectus abdominis* muscles where stem cells reside and undergo self-renewal continuously producing large number of progenitor cells [108, 109] is additionally supported by the recent identification of myendothelial cells in human skeletal muscle [110]. Myogenic and endothelial cells may derive from a common somatic precursor, and cells co-expressing myogenic and endothelial cell markers residing in the interstitial spaces of skeletal muscle, *i.e.* myoendothelial cells, may contribute to postnatal tissue morphogenesis [110]. Importantly, in the context of the spontaneous induction of bone formation in porous biomimetic matrices implanted in the *rectus abdominis* muscle, clonally expanded myoendothelial cells differentiate into myogenic, chondrogenic and osteogenic cells under appropriate culture conditions [110]. Multipotent myoendothelial cells residing in stem cell niches within the *rectus abdominis* muscle do respond to endothelial cell mitogens including angiogenic factors [110]; myoendothelial cells may also respond to BMPs previously bound to collagen type IV and other extracellular matrix components further inducing the ripple-like cascade of the induction of bone formation within the porous spaces of the biomimetic matrices biomimetizing the cortico-cancellous remodelling cycle of the primate bone [8, 9, 57, 58].

The final leap into the '*intrinsic*' induction of bone formation will rest upon the mechanistic understanding of how microstructured surface areas of biphasic or not calcium phosphate biomaterial matrices differentiate inducible stem cells into osteoblastic-like cells initiating the induction of bone formation as a secondary response. The effect of calcium phosphate microstructure on the '*intrinsic*' osteoinductivity of biphasic calcium phosphate biomatrices has been elegantly investigated by Li *et al.* [69]. The authors suggested that increased surface areas of calcium phosphate

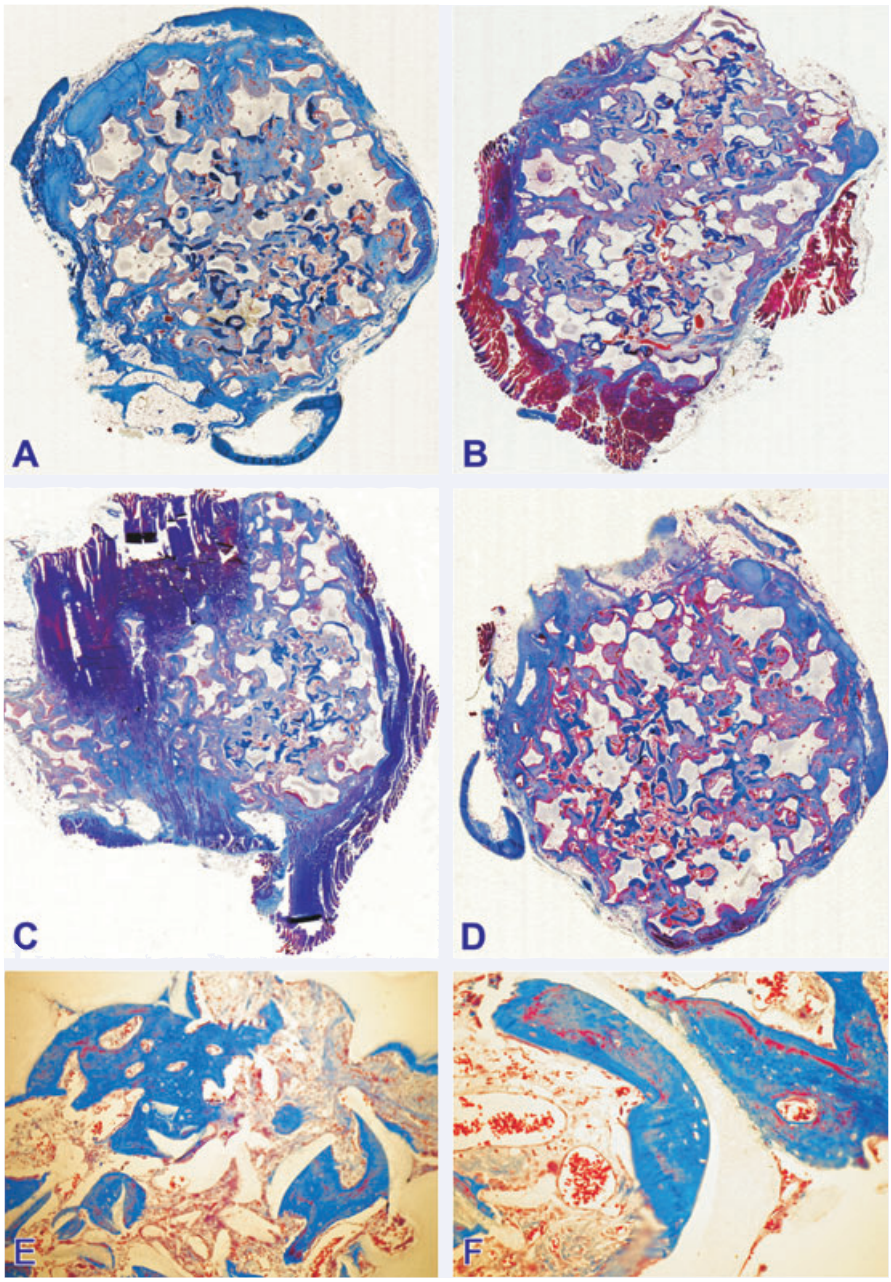

Fig. 7 Biomimetism of the concavity, the shape of life: Construction of biomimetic matrices and the induction of bone formation in highly crystalline sintered porous hydroxyapatite matrices when implanted in the *rectus abdominis* muscle of adult baboons without the exogenous application of osteogenic proteins of the TGF-β superfamily and harvested on day 90 after implantation. (**A, B, C** and **D**) Low-power views of sintered bioceramics with a series of repetitive concavities with substantial bone formation across the porous spaces. (**E** and **F**) Detail of bone formation by induction within the biomimetic concavities of the highly crystalline sintered biomatrix together with prominent angiogenesis and vascular invasion. Original magnification: (**A**) ×125; (**B**) ×125; (**C**) ×175; (**D**) ×175; (**E**) ×275; (**F**) ×275.

biomaterial matrices are endowed with osteoinductivity; microstructured surfaces with increased surface areas might promote superior protein adsorption [69]; selected adsorbed proteins would directly influence cell attachment and proliferation to further promote the induction of the osteogenic phenotype in attached and resident stem cells into osteoblastic-like cells synthesizing, expressing, secreting and embedding osteogenic soluble molecular signals into the biomimetic matrices inducing the ripple-like cascade of bone differentiation by induction [61, 71].

Conclusions and therapeutic perspectives of porous biomimetic matrices with intrinsic osteoinductivity

Tissue engineers, molecular biologists, reconstructive surgeons and developmental biologists alike have learned that tissue engineering in postnatal life recapitulates events that occur in the

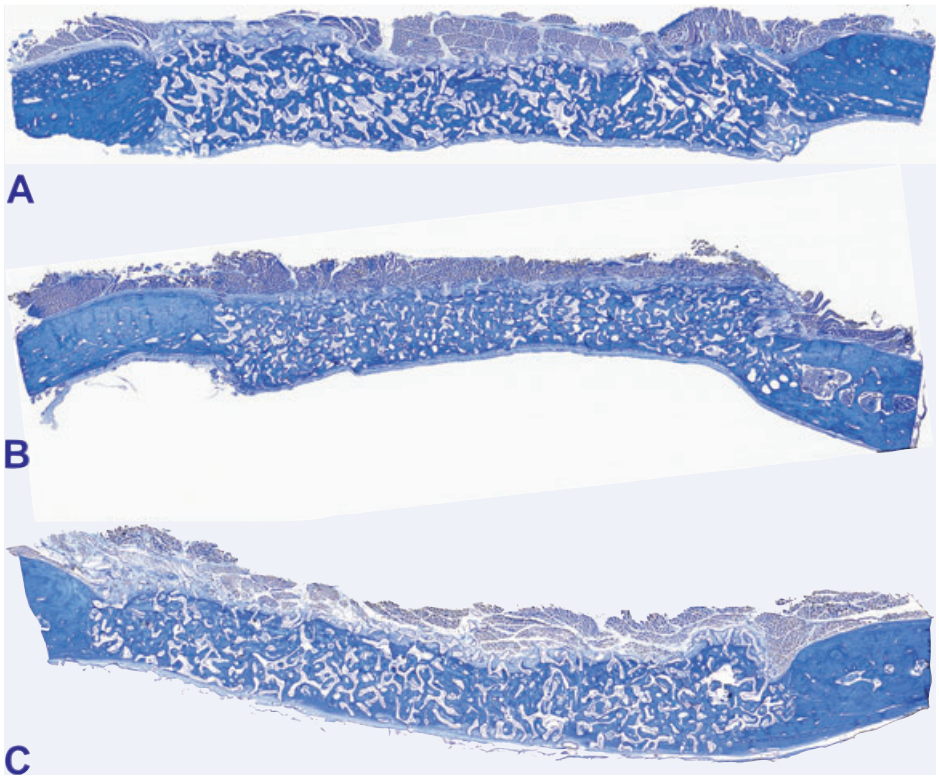

Fig. 8 (**A, B** and **C**) Complete regeneration of bone across the porous spaces of coral-derived hydroxyapatites implanted in non-healing calvarial defects of the adult baboon and harvested 6 months after calvarial implantation. Original magnification: (**A**) ×125; (**B**) ×125; (**C**) ×175; (**D**) ×175; (**E**) ×275; (**F**) ×275.

normal course of embryonic development [2, 5, 17]. More figuratively perhaps, tissue engineering of postnatal tissues recapitulates and exploits the very mechanisms of embryonic development though with different ratios and quantities of synchronously expressed soluble molecular signals and insoluble extracellular matrix substrata that nature has so parsimoniously developed through million years of evolution. The summary of several hundred years of research into the mechanisms of regenerative medicine is surprisingly simple: morphogens exploited in embryonic development are re-exploited and re-deployed for postnatal tissue induction and morphogenesis.

Perhaps indeed the different ratios and quantities of synchronously expressed soluble molecular signals interacting with insoluble signals or substrata pinpoints the very different capabilities to regenerate between embryos and adult animals, particularly the regenerative potential of *Homo sapiens*. Embryonic development and postnatal tissue regeneration are equally regulated by selected few and highly conserved families of secreted proteins, members of the TGF-β superfamily [2, 17, 18]. We have learned that in primates and in primates only, there is an apparent redundancy of soluble molecular signals initiating the induction of bone formation in heterotopic extraskeletal sites. We have learned though that we still have to grapple with the reality of our discoveries, that when implanted in heterotopic sites of the *rectus abdominis* muscle of non-human primates *Papio ursinus*, the mammalian TGF-β isoforms do induce substantial endochondral bone formation by induction [5, 48–51].

We have further learned, and again biomimetizing nature that combines signals to induce tissue morphogenesis, that combining osteogenic soluble molecular signals with relatively low doses of the TGF-β_1 isoform, the rapidly generated tissue constructs are the result of a synergistic interaction which we have labelled '*synergistic induction of bone formation*' [2, 5, 18, 48]. The '*synergistic induction of bone formation'* has remarkably showed to us that bone tissue develops as a mosaic structure in which members of the TGF-β superfamily singly, synergistically and synchronously initiate and maintain tissue induction and morphogenesis [2, 5].

The morphogenesis of structurally organized chondrogenic zones, highly reminiscent of rudimentary embryonic growth plates [48], is a finding that vividly illustrates the concept that regeneration of cartilage and bone in postnatal life shares common cellular and molecular mechanisms with embryonic bone development, and that the '*memory*' of developmental events in embryo can be redeployed postnatally by the application of morphogen combinations [48]. The '*synergistic induction of bone formation*' reflects nature's parsimony in deploying low doses of molecularly different molecular signals yet resulting in rapid and complete tissue induction and regeneration [48, 49]. Nature's key is to synchronously and synergistically deploy low doses of molecularly different single gene products during both embryogenesis and tissue

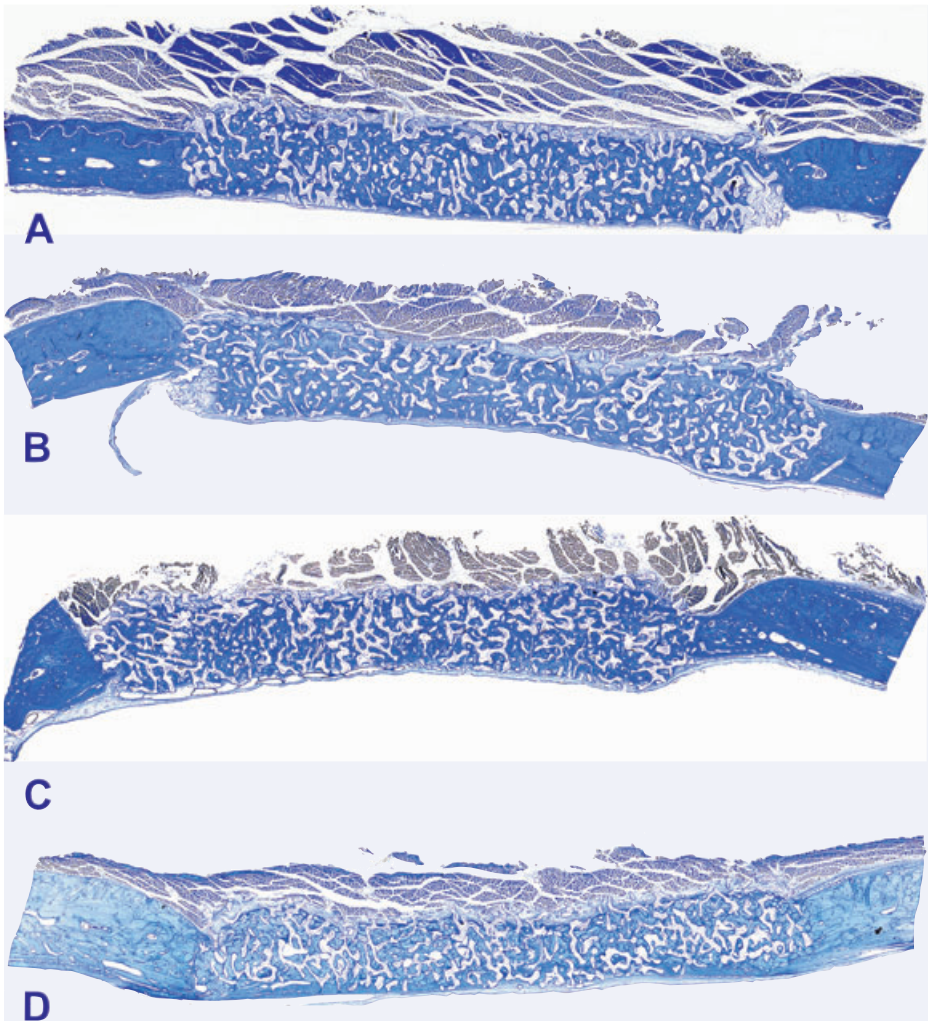

Fig. 9 (**A**, **B**, **C** and **D**) Remodelling and maintenance of the induced bone across the coral-derived porous hydroxyapatites 9 months after calvarial implantation in the adult baboon. Original magnification: (**A**) ×125; (**B**) ×125; (**C**) ×175; (**D**) ×175; (**E**) ×275; (**F**) ×275.

regeneration in postnatal life, parsimoniously achieving complete tissue regeneration [18, 111]. Supra-physiological doses of single recombinant human BMPs/OPs [111, 112] contravene nature's elegant deployment of synchronously active molecular signals; this often generates incomplete and partial tissue induction in clinical contexts [112].

Biominetic matrices of HA/β-TCP bioceramics have shown remarkable intrinsic oteoinduction in a variety of animal models [113–116]. Long-term experiments in the non-human primate *Papio ursinus* were set to investigate the induction of bone formation by biphasic biomimetic matrices (HA/β-TCP) 40 to 60 and 20 to 80, respectively [117, 118]. One year after implantation in orthotopic calvarial sites there was prominent osteogenesis coupled with resorption/dissolution of the implanted biomimetic matrices (Fig. 11) [118]. Solid discs of biphasic HA/β-TCP, with concavities prepared on one planar surface only, were implanted heterotopically in the *rectus abdominis* muscle of adult baboons [117, 118]. Histological analyses of heterotopic specimens harvested on days 90 and 365 showed the induction of bone formation also on the planar surfaces of the implanted biphasic HA/β-TCP discs. Osteoclasts/macrophages excavate resorption lacunae and pits upon which osteoblastic cell lines secrete bone matrix within the microconcavities cut by osteoclastogenesis [117, 118]. Morphological analyses 1 year after heterotopic implantation showed the induction of substantial bone with marrow on both planar surfaces with further dissolution of the implanted HA/β-TCP biomimetic matrix with bone formation within the excavated resorption lacunae [117, 118]. Orthotopic calvarial specimens showed complete induction of bone with resorption and dissolution of the implanted biphasic biomimetic matrices (Fig. 11) [117, 118].

Resorption thus initiates the induction of bone formation in a '*continuum*' of molecular and morphological processes that ultimately results in significant amounts of bone formation by induction

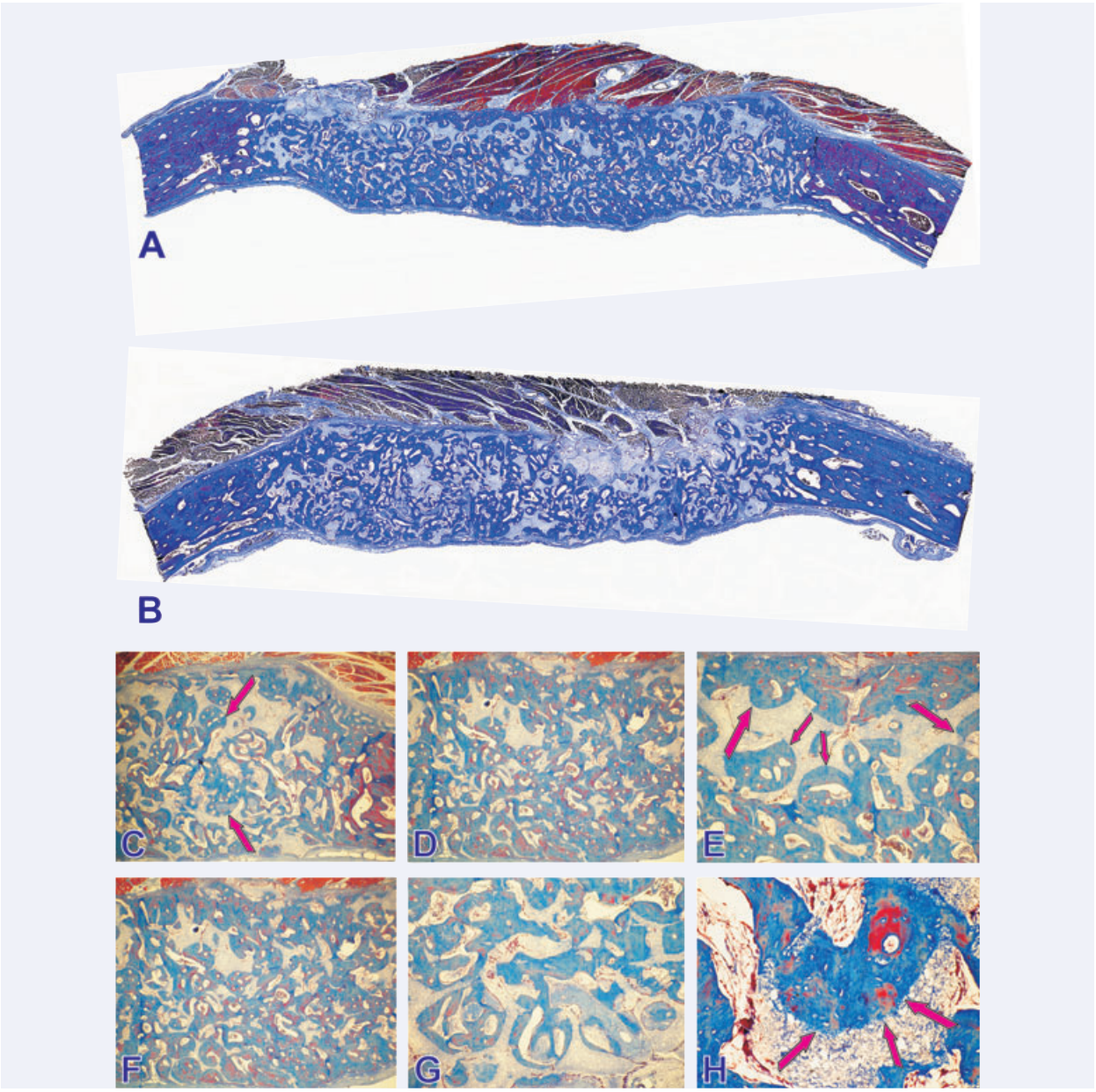

Fig. 10 Morphogenesis of bone across the porous spaces of highly crystalline sintered hydroxyapatites implanted in calvarial defects of adult baboons and harvested on day 90. (**A** and **B**) Low-power view of two specimens of sintered porous hydroxyapatites showing the induction of bone across the porous spaces of the sintered ceramics. (**C, D, E, F, G** and **H**) Details of previous sections showing the morphogenesis of bone in direct contact with the sintered biomatrix biomimetizing the geometric concavities of the bone remodelling cycle and the induction of bone formation. Arrows (**E, G** and **H**) indicate the induction of bone formation as driven by the concavities of the biomimetic highly crystalline substratum. Original magnification: (**A**) ×125; (**B**) ×125; (**C**) ×175; (**D**) ×175; (**E**) ×275; (**F**) ×275.

'*in toto*' replacing the implanted '*smart*' biomimetic matrices [117, 118]. Above all, the overall geometric configuration of novel biomimetic matrices will provide biomimetic constructs to optimally deliver low doses of OPs of the TGF-β superfamily. The incorporation of specific biological activities into biomimetic biomaterial matrices by manipulating the geometry of the substratum, defined as '*geometric induction of bone formation*' [5, 8, 9, 55] is now helping to engineer therapeutic osteogenesis in clinical contexts.

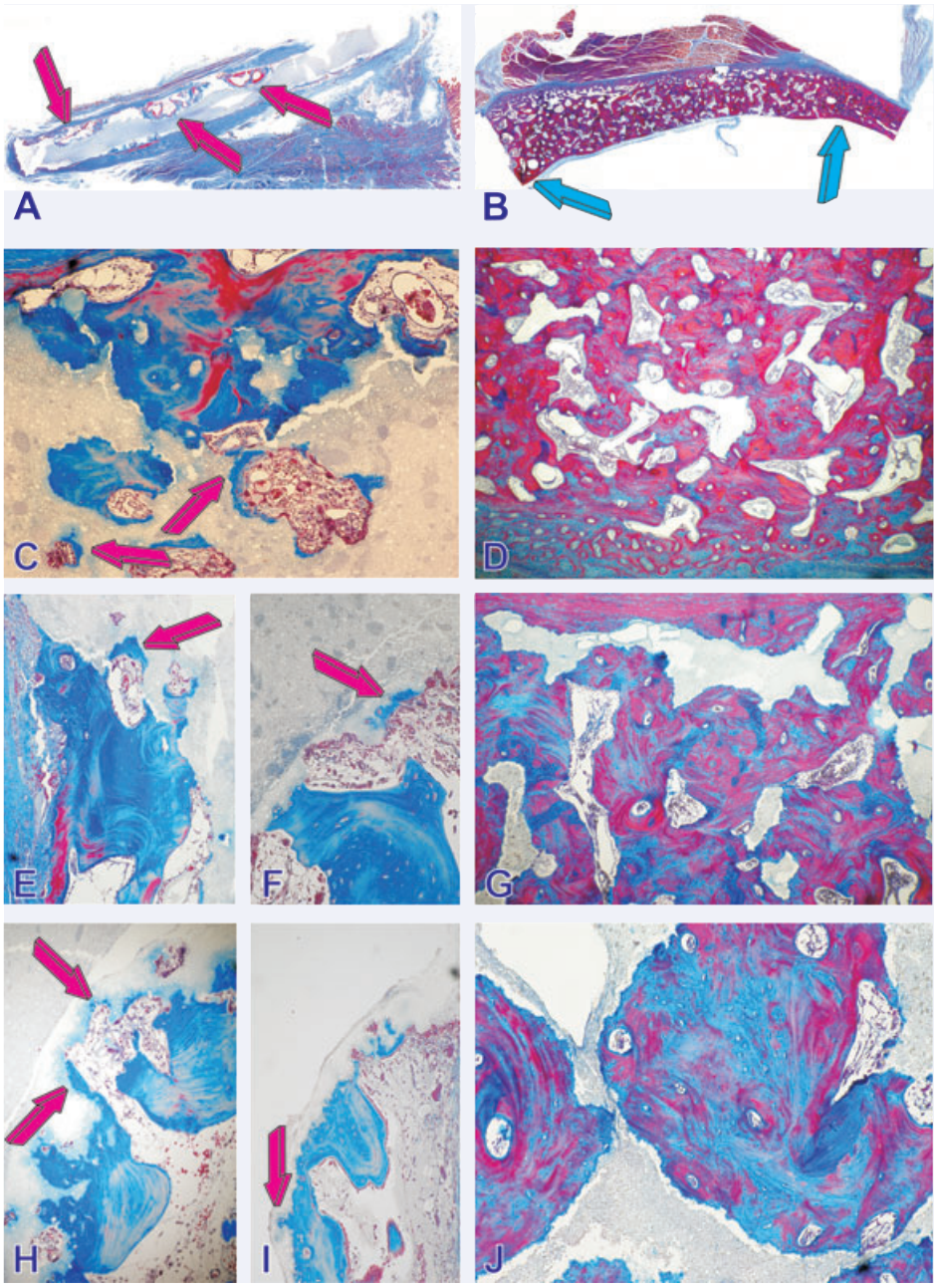

Fig. 11 Induction of bone formation by post-sinter 19/81 hydroxyapatite/β-tricalcium phosphate hydroxyapatite biomimetic matrices harvested from heterotopic *rectus abdominis* (**A, C, E, F, H, I**) and orthotopic calvarial sites (**B, D, G, J**) from adult baboons *Papio ursinus* on day 365 after implantation. (**A**) Magenta arrows point to substantial bone differentiation in pre-carved concavity of the bioactive biomimetic matrix. Opposite facing the muscle, there is bone differentiation on the planar surface without pre-cut concavities. (**C, E, F, H, I**) High-power microphotographs detailing the induction of bone formation (magenta arrows) in a continuum of morphological processes of resorption/dissolution and bone formation. (**B**) Low-power view of substantial induction of bone formation throughout the porous spaces of the implanted biomimetic matrices across the defects (blue arrows). (**D, G, J**) Porous spaces filled by newly formed bone tightly attached to the biomimetic matrices showing resorption/dissolution of the implanted scaffolds. Original magnification: (**A**) ×3.7; (**B**) ×2.7; (**C**) ×65; (**D**) ×35; (**E, F, H, I**)) ×175; (**G**) ×35; (**J**) ×75.

Acknowledgements

This work on the fascinating scenario of bone: formation by autoinduction, could not have been possible without the continuous support of the South African Medical Research Council, the University of the Witwatersrand, Johannesburg, the National Research Foundation and several *ad hoc* grants to the Bone Research Unit. This paper, summarizing our mechanistic knowledge on the intrinsic induction of bone formation, is dedicated to all mentors, scientists, technologists, post-graduate students and scientific friends who have made possible the continuous and constant effort to unravel the fascinating phenomenon of the *geometric induction of bone formation*. I thank the late Michael Thomas and William Richter, Council for Scientific and Industrial Research, Pretoria, for the preparation of the sintered bioceramics for heterotopic and orthotopic implantation. Special thanks to Marshall Urist and Hari A. Reddi for teaching me the understanding of the cascade of 'bone: formation by autoinduction'. And so many thanks to Kathleen for understanding.

References

1. **Ripamonti U, van den Heever B, Crooks J, *et al.*** Long-term evaluation of bone formation by osteogenic protein-1 in the baboon and relative efficacy of bone-derived bone morphogenetic proteins delivered by irradiated xenogeneic collagenous matrices. *J Bone Miner Res.* 2000; 15: 1798– 809.
2. **Ripamonti U, Ramoshebi LN, Patton J, *et al.*** Soluble signals and insoluble substrata: novel molecular cues instructing the induction of bone. In: Massaro EJ, Rogers JM, editors. The skeleton. Totowa: Humana Press; 2004. pp. 217–27.
3. **Ripamonti U, Ferretti C, Heliotis M.** Soluble and insoluble signals and the induction of bone formation: molecular therapeutics recapitulating development. *J Anat.* 2006; 209: 447–68.
4. **Ripamonti U, Duneas N.** Tissue morphogenesis and regeneration by bone morphogenetic proteins. *Plast Reconstr Surg.* 1998; 101: 227–39.
5. **Ripamonti U.** Soluble osteogenic molecular signals and the induction of bone formation. *Biomaterials.* 2006; 27: 807–22.
6. **Ripamonti U.** Recapitulating development: a template for periodontal tissue engineering. *Tissue Eng.* 2007; 13: 51–71.
7. **Ripamonti U, Ramoshebi LN, Matsaba T, *et al.*** Bone induction by BMPs/OPs and related family members in primates. The critical role of delivery systems. *J Bone Joint Surg.* 2001; 83: 116–27.
8. **Ripamonti U.** Molecular signals in geometrical cues sculpt bone morphology. *S Afr J Sci.* 2004; 100: 355–67.
9. **Ripamonti U.** Soluble, insoluble and geometric signals sculpt the architecture of mineralized bone. *J Cell Mol Med.* 2004; 8: 169–80.
10. **Ripamonti U, Ramoshebi LN, Patton J, *et al.*** Sculpting the architecture of mineralized tissue: tissue engineering of bone from soluble signals to smart biomimetic matrices. In: Muller RH, Kayser O, editors. Pharmaceutical biotechnology. Hoboken, NJ: Wiley-VCH; 2004. pp. 281–97.
11. **Khouri RK, Koudsi B, Reddi AH.** Tissue transformation into bone *in vivo.* A potential practical application. *J Am Med Assoc.* 1991; 226: 1953–5
12. **Reddi AH.** Regulation of cartilage and bone differentiation by bone morphogenetic proteins. *Curr Opin Cell Biol.* 1992; 4: 850–5.
13. **Langer R, Vacanti JP.** Tissue engineering. *Science.* 1993; 260: 920–6.
14. **Reddi AH.** Symbiosis of biotechnology and biomaterials: applications in tissue engineering of bone and cartilage. *J Cell Biochem.* 1994; 56: 192–5.
15. **Hubbell JA.** Biomaterials in tissue engineering. *Biotechnology.* 1995; 13: 565–76.
16. **Ripamonti U, Duneas N.** Tissue engineering of bone by osteoinductive biomaterials. *MRS Bulletin.* 1996; 21: 36–9.
17. **Reddi AH.** Morphogenesis and tissue engineering of bone and cartilage: inductive signals, stem cells, and biomimetic biomaterials. *Tissue Eng.* 2000; 6: 351–9.
18. **Ripamonti U.** Osteogenic proteins of the TGF-β superfamily. In: Henry HL, Norman AW, editors. Encyclopedia of hormones. Amsterdam: Austin Academic Press; 2003. pp. 80–6.
19. **Reddi AH.** Role of morphogenetic proteins in skeletal tissue engineering and regeneration. *Nature Biotechnol.* 1988; 16: 247–52.
20. **Urist MR.** Bone: formation by autoinduction. *Science.* 1965; 150: 893–9.
21. **Sampath TK, Reddi AH.** Dissociative extraction and reconstitution of extracellular matrix components involved in local bone differentiation. *Proc Natl Acad Sci USA.* 1981; 78: 7599–603.
22. **Sampath TK, Reddi AH.** Homology of bone-inductive proteins from human, monkey, bovine and rat extracellular matrix. *Proc Natl Acad Sci USA.* 1983; 80: 6591–5.
23. **Luyten FP, Cunningham NS, Ma S, *et al.*** Purification and partial amino acid sequence of osteogenin, a protein initiating bone differentiation. *J Biol Chem.* 1989; 264: 13377–80.
24. **Ripamonti U, Ma S, Cunningham N, *et al.*** Initiation of bone regeneration in adult baboons by osteogenin, a monemorphogenetic protein. *Matrix.* 1992; 12: 369–80.
25. **Wozney JM, Rosen V, Celeste AJ, *et al.*** Novel regulators of bone formation: molecular clones and activities. *Science.* 1988; 242: 1528–34.
26. **Sampath TK, Maliakal JC, Hauschka PV, *et al.*** Recombinant human osteogenic protein-1 (hOP-1) induces new bone formation *in vivo* with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation in vitro. *J Biol Chem.* 1992; 267: 20352–62.
27. **Ripamonti U.** *Smart* biomaterials with intrinsic osteoinductivity; geometric control of bone differentiation. In: Davis JE, editor. Bone engineering. Toronto: EM2 Corporation; 2000. 215 pp.
28. **Sacerdotti C, Frattin G.** Sulla produzione eteroplastica dell'osso. *R Acad Med Torino.* 1901; 27: 825–36.
29. **Huggins CB.** The formation of bone under the influence of epithelium of the urinary tract. *Arch Surg.* 1931; 22: 377–408.
30. **Levander G.** A Study of bone regeneration. *Surg Gynec Obstetr.* 1938; 67: 705–14.
31. **Levander G.** Tissue induction. *Nature.* 1945; 155: 148–9.
32. **Bridges JB, Pritchard JJ.** Bone and cartilage induction in the rabbit. *J Anat.* 1958; 92: 28–38.
33. **Moss ML.** Extraction of an osteogenic inductor factor from bone. *Science.* 1958; 127: 755–6.
34. **Trueta J.** The role of the vessels in osteogenesis. *J Bone Joint Surg.* 1963; 45: 402–18.
35. **Urist MR, Silverman BF, Buring K, *et al.*** The bone induction principle. *Clin Orthop.* 1967; 53: 243–83.
36. **Reddi AH, Hugging CB.** Biochemical sequences in the transformation of normal fibroblasts in adolescent rats. *Proc Natl Acad Sci USA.* 1972; 69: 1601–5.
37. **Levander G, Willestaedt H.** Alcohol-soluble osteogenetic substance from bone marrow. *Nature.* 1946; 3992: 587.
38. **Urist MR, McLean FC.** Osteogenetic potency and new bone formation by induction in transplants to the anterior chamber of the eye. *J Bone Joint Surg Am.* 1952; 34: 443–76.
39. **Friedlaender GE, Perry CR, Cole JD, *et al.*** Osteogenic protin-1 (bone morphogenetic protein-7) in the treatment of tibial nonunions. *J Bone Joint Surg Am.* 2001; 83A; S151–8.
40. **Govender S, Csimma C, Genant HK, *et al.*** Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. *J Bone Joint Surg Am.* 2002; 84A: 2123–34.
41. **Ripamonti U, Reddi AH.** Bone morphogenetic proteins: applications in plastic and reconstructive surgery. *Adv Plast Reconstr Surg.* 1995; 11: 47–73.
42. **Heng BC, Cao T, Stanton LW, *et al.*** Strategies for directing the differentiation of stem cells into the osteogenic lineage *in vitro. J Bone Miner Res.* 2004; 19: 1379–94.

43. **Young HE, Duplaa C, Katz R, *et al.*** Adult-derived stem cells and their potential for use in tissue repair and molecular medicine. *J Cell Mol Med.* 2005; 9: 753–69.
44. **Steven MM, Marini RP, Schaefer D, *et al.*** *In vivo* engineering of organs: the bone bioreactor. *Proc Natl Acad Sci USA.* 2005; 202: 11450–5.
45. **Warnke PH, Springer ING, Wiltfang J, *et al.*** Growth and transplantation of a custom vascularised bone graft in a man. *Lancet.* 2004; 364: 766–70.
46. **Heliotis M, Lavery KM, Ripamonti U, *et al.*** Transformation of a prefabricated hydroxyapatite/osteogenic protein-1 implant into a vascularised pedicled bone flap in the human chest. *Int J Oral Maxillofac Surg.* 2006; 35: 265–9.
47. **Roberts AB, Sporn MB, Assoian RK, *et al.*** Transforming growth factor type β: rapid induction of fibrosis and angiogenesis in vivo and stimulation of collagen formation in vitro. *Proc Natl Acad Sci USA.* 1986; 83: 4167–71.
48. **Ripamonti U, Duneas N, van den Heever B, *et al.*** Recombinant transforming growth factor-β_1 induces endochondral bone in the baboon and synergizes with recombinant osteogenic protein-1 (bone morphogenetic protein-7) to initiate rapid bone formation. *J Bone Miner Res.* 1997; 2: 1584–95.
49. **Duneas N, Crooks J, Ripamonti U.** Transforming growth factor-β_1: induction of bone morphogenetic protein genes expression during endochondral bone formation in the baboon, and synergistic interaction with osteogenic protein-1 (BMP-7). *Growth Factors.* 1998; 15: 259–77.
50. **Ripamonti U, Crooks J, Matsaba T, *et al.*** Induction of endochondral bone formation by recombinant human transforming growth factor-β_2 in the baboon (*Papio ursinus*). *Growth Factors.* 2000; 17: 269–85.
51. **Ripamonti U, Ramoshebi LN, Teare J, *et al.*** The induction of endochondral bone formation by transforming growth factor-β_3: experimental studies in the non-human primate *Papio ursinus. J Cell Mol Med.* 2008; 12: 1029–48.
52. **Ripamonti U, Ferretti C, Heliotis M.** Soluble molecular signals and the induction of bone formation (Chapter 31). In: Guyuron B, Persing JA, Eriksson E, editors. Plastic surgery: indications and practice. Elsevier Global Medicine; 2008; in press.
53. **Ripamonti U, Heliotis M, Ferretti C.** Bone morphogenetic proteins and the induction of one formation: from laboratory to patients. *Oral Maxillofac Surg Clin North Am.* 2007; 19: 575–89.
54. **Ripamonti U, van den Heever B, van Wyk J.** Expression of the osteogenic phenotype in porous hydroxyapatite implanted extra skeletally in baboons. *Matrix.* 1993; 13: 491–502.
55. **Ripamonti U, Crooks J, Kirkbride AN.** Sintered porous hydroxyapatites with intrinsic osteoinductive activity: geometric induction of bone formation. *S Afri J Sci.* 1999; 95: 335–43.
56. **Reddi AH.** Bone morphogenesis and modeling: soluble signals sculpt osteosomes in the sold state. *Cell.* 1997; 89: 159–61.
57. **Manolagas SC, Jilka RL.** Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. *N Eng J Med.* 1995; 332: 305–11.
58. **Parfitt AM, Mundy GR, Roodman GD, *et al.*** A new model for the regulation of bone resorption, with particular reference to the effects of bisphosphonates. *J Bone Miner Res.* 1996; 11: 150–9.
59. **Ripamonti U, Herbst N-N, Ramoshebi LN.** Bone morphogenetic proteins in craniofacial and periodontal tissue engineering: experimental studies in the non-human primate (*Papio ursinus). Cytokine Growth Factor Rev.* 2005; 16: 357–68.
60. **Sampath TK, Rashka KE, Doctor JS, *et al.*** *Drosophila* TGF-β superfamily proteins induce endochondral bone formation in mammals. *Proc Natl Acad Sci USA.* 1993; 90: 6004–8.
61. **Ripamonti U, Richter PW, Thomas ME.** Self-inducing shape memory geometric cues embedded within *smart* hydroxyapatite-based biomimetic matrices. *Plast Reconstr Surg.* 2007; 120: 1797–807.
62. **Habibovic P, Sees TM, van den Doel MA, *et al.*** Osteoinduction by biomaterials – physicochemical and structural influences. *J Biomed Mater Res.* 2006; 77: 747–62.
63. **Habibovic P, de Groot K.** Osteoinductive biomaterials – properties and relevance in bone repair. *J Tissue Eng Regen Med.* 2007; 1: 25–32.
64. **Yuan H, Li Y, de Bruijn JD, *et al.*** Tissue responses of calcium phosphate cement: a study in dogs. *Biomaterials.* 2000; 21: 1283–90.
65. **Yuan H, Yang Z, de Bruijn JD, *et al.*** Material-dependent bone induction by calcium phosphate ceramics: a 2.5-year study in dog. *Biomaterials.* 2001; 22: 2617–23.
66. **Le Nihouannen D, Daculsi G, Saffarzadeh A, *et al.*** Ectopic bone formation by microporous calcium phosphate ceramic particles in sheep muscles. *Bone.* 2005; 36: 1086–93.
67. **Yuan H, van Blitterswijk CA, de Groot K, *et al.*** Cross-species comparison of ectopic bone formation in biphasic calcium phosphate (BCP) and hydroxyapatite (HA) scaffolds. *Tissue Eng.* 2006; 12: 1607–15.
68. **Fellah BH, Gauthier O, Weiss P, *et al.*** Osteogenicity of biphasic calcium phosphate ceramics and bone autograft in a goat model. *Biomaterials.* 2008; 29: 1177–88.
69. **Li X, van Blitterswijk CA, Feng Q, *et al.*** The effect of calcium phosphate microstructure on bone-related cells *in vitro. Biomaterials.* 2008: 29: 3306–16.
70. **de Groot K.** Carriers that concentrate native bone morphogenetic protein in vivo. *Tissue Eng.* 1998; 4: 337–41.
71. **Ripamonti U.** Embedding molecular signals in biomimetic matrices for regenerative medicine. *S Afr J Sci.* 2006; 102: 211–6.
72. **Sanchez C, Arribart H, Guille MMG.** Biomimetism and bioinspiration as tools for the design of innovative materials and systems. *Nature Mat.* 2005; 4: 277–88.
73. **Fratzl P.** Biomimetic materials research: what can we really learn from nature's structural materials? *J R Soc Interface.* 2007; 4: 637–42.
74. **Ingber DE, Mow VC, Butler D, *et al.*** Tissue engineering and developmental biology: going biomimetic. *Tissue Eng.* 2006; 12: 3265–83.
75. **Vlodavsky I, Folkman J, Sullivan R, *et al.*** Endothelial cell-derived basic fibroblast growth factor: synthesis and deposition into subendothelial extracellular matrix. *Proc Natl Acad Sci USA.* 1987; 84: 2292–6.
76. **Folkman J, Klagsbrun M, Sasse J, *et al.*** A heparin-bind angiogenic protein – basic fibroblast growth factor – is stored within basement membranes. *Am J Pathol.* 1988; 130: 393–400.
77. **Ingber DE, Folkman J.** How does extracellular matrix control capillary morphogenesis? *Cell.* 1989; 58: 803–5.
78. **Paralkar VM, Nandekar AKN, Pointer RH, *et al.*** Interaction of osteogenin, a heparin binding bone morphogenetic protein, with type IV collagen. *J Biol Chem.* 1990; 265: 17281–4.
79. **Paralkar VM, Vukicevic S, Reddi AH.** Transforming growth factor β type1 binds to collagen type IV of basement membrane matrix: implications for development. *Dev Biol.* 1991; 143: 303–8.

80. **Anderson HC, Hodges PT, Aguilera M, *et al.*** Bone morphogenetic protein (BMP) localization in developing human and rat growth plate, metaphysis, epiphysis, and articular cartilage. *J Histochem Cytochem.* 2000; 48: 1493–502.
81. **Schilling AF, Filke S, Brink S, *et al.*** Osteoclasts and biomaterials. *Eur J Trauma.* 2006; 32: 107–13.
82. **Champagne CM, Takebe J, Offenbacher S, *et al.*** Macrophage cell lines produce osteoinductive signals that include bone morphogenetic protein-2. *Bone.* 2002; 30: 26–31.
83. **Takebe J, Champagne CM, Offenbacher S, *et al.*** Titanium surface topography alters cell shape and modulates bone morphogenetic protein 2 expression in the J774A.1 macrophage cell line. *J Biomed Mater Res.* 2003; 64: 207–16.
84. **Honda Y, Anada T, Kamakura S, *et al.*** Elevated extracellular calcium stimulates secretion of bone morphogenetic protein 2 by a macrophage cell line. *Biochem Biophys Res Commun.* 2006; 345: 1155–60.
85. **Kondo N, Ogose A, Tokunaga K, *et al.*** Osteoinduction with highly purified β-tricalcium phosphate in dog dorsal muscles and the proliferation of osteoclasts before heterotopic bone formation. *Biomaterials.* 2006; 27: 4419–27.
86. **Hunt TK, Burke J, Barbul A, *et al.*** Wound healing. *Science.* 1999; 284: 1775.
87. **Reddi AH, Huggins CB.** Influence of geometry of transplanted tooth and bone on transformation of fibroblasts. *Proc Soc Exp Biol Med.* 1973; 143: 634–7.
88. **Sampath TK, Reddi AH.** Importance of geometry of the extracellular matrix in endochondral bone differentiation. *J Cell Biol.* 1984; 98: 2192–7.
89. **Reddi AH.** Bone matrix in the solid state: geometric influence on differentiation of fibroblasts. *Adv Biol Med Phys.* 1974; 15: 1–18.
90. **Ripamonti U, Ma S, Reddi AH.** The critical role of geometry of porous hydroxyapatite delivery system in induction of bone by osteogenin, a bone morphogenetic protein. *Matrix.* 1992; 12: 202–12.
91. **Kuboki Y, Takita H, Kobayashi D, *et al.*** BMP-induced osteogenesis on the surface of hydroxyapatite with geometrically feasible and non feasible structures: topology of osteogenesis. *J Biomed Mat Res.* 1998; 39: 190–9.
92. **Kuboki Y, Saito T, Murata M, *et al.*** Two distinctive BMP-carriers induce zonal chondrogenesis and membranous ossification, respectively: geometrical factors of matrices for cell-differentiation. *Connect Tissue Res.* 1995; 32: 219–26.
93. **Kuboki Y, Jin Q, Takita H.** Geometry of carriers controlling phenotypic expression in BMP-induced osteogenesis and chondrogenesis. *J Bone Joint Surg Am.* 2001; 83A: 105–15.
94. **van Eeden SP, Ripamonti U.** Bone differentiation in porous hydroxyapatite in baboons is regulated by the geometry of the substratum: implications for reconstructive craniofacial surgery. *Plast Reconstr Surg.* 1994; 93: 959–66.
95. **Keith A.** Concerning the origin and nature of osteoblasts. *Proc Royal Soc Med.* 1927; 21: 301–7.
96. **Ripamonti U.** Inductive bone matrix and porous hydroxylapatite composites in rodents and nonhuman primates. Handbook of bioactive ceramics. Boca Raton, FL: CRC Press; 1990. Vol II: pp. 245–53.
97. **Ripamonti U.** The morphogenesis of bone in replicas of porous hydroxyapatite obtained from conversion of calcium carbonate exoskeletons of coral. *J Bone Joint Surg Am.* 1991; 73: 692–703.
98. **Hall BK Myake T.** All for one and one for all: condensations and the initiation of skeletal development. *Bioessays.* 2000; 22: 138–47.
99. **Winter GD, Simpson BJ.** Heterotopic bone formed in a synthetic sponge in the skin of young pigs. *Nature.* 1969; 223: 88–90.
100. **Ripamonti U.** Osteoinduction in porous hydroxyapatite implanted in heterotopic sites of different animal models. *Biomaterials.* 1996; 17: 31–5.
101. **Ripamonti U.** Calvarial reconstruction in baboons with porous hydroxypatite. *J Craniofac Surg.* 1992; 3: 149–59.
102. **Yamasaki H, Sakai H.** Osteogenic response to porous hydroxyapatite ceramics under the skin of dogs. *Biomaterials.* 1992; 13; 308–12.
103. **Gosain AK, Song L, Riordan P, *et al.*** A 1-year study of osteoinduction in hydroxyapatite-derived biomaterials in an adult sheep model: part I. *Plast Reconstr Surg.* 2002; 109: 619–30.
104. **Gosain AK, Riordan P, Song L, *et al.*** A 1-year study of osteoinduction in hydroxyapatite-derived biomaterials in an adult sheep model: part II. Bioengineering implants to optimize bone replacement in reconstruction of cranial defects. *Plast Reconstr Surg.* 2004; 114: 1155–63.
105. **Ingber DE, Folkman J.** How does extracellular matrix control capillary morphogenesis? *Cell.* 1989; 58: 803–5.
106. **Folkman J, Greenspan HP.** Influence of geometry on control of cell growth. *Biochim Biophys Acta.* 1975; 417: 211–36.
107. **Bissell MJ, Barcellos-Hoff MH.** The influence of extracellular matrix on gene expression: is structure the message? *J Cell Sci Suppl.* 1987; 8: 327–43.
108. **Li L, Xie T.** Stem cell niche: structure and function. *Ann Rev Cell Dev Biol.* 2005; 21: 605–31.
109. **Lensch MW, Daheron L, Schlager TM.** Pluripotent stem cells and their niches. *Stem Cell Rev.* 2006; 2: 185–202.
110. **Zheng B, Cao B, Crisan M, *et al.*** Prospective identification of myogenic endothelial cells in human skeletal muscle. *Nat Biotechnol.* 2007; 25: 1025–34.
111. **Ripamonti U.** Bone induction by recombinant human osteogenic protein -1 (hOP-1, BMP-7) in the primate *Papio ursinus* with expression of mRNA of gene products of the TGF -β superfamily. *J Cell Mol Med.* 2005; 9: 911–28.
112. **Termaat MF, Den Boer FC, Bakker FC, *et al.*** Bone morphogenetic proteins. Development and clinical efficacy in the treatment of fractures and bone defects. *J Bone Joint Surg Am.* 2005; 87: 1367–78.
113. **Daculsi G, Laboux O, Malard O, *et al.*** Current state of the art of biphasic calcium phosphate bioceramics. *J Mater Sci Mater Med.* 2003; 14: 195–200.
114. **Daculsi G, Layrolle P.** Osteoinductive properties of micro macroporous biphasic calcium phosphate bioceramics. *Key Eng Mater.* 2004; 254–56: 1005–8.
115. **Yang Z, Yuan H, Tong W, *et al.*** Osteogenesis in extraskeletally implanted porous calcium phosphate ceramics: variability among different kinds of animals. *Biomaterials.* 1996; 17: 2131–7.
116. **Li S, de Wijn JR, Li J, *et al.*** Macroporous biphasic calcium phosphate scaffold with high permeability/ porosity ratio. *Tissue Eng.* 2003; 9: 535–48.
117. **Ripamonti U, Richter PW, Nilen RWN, *et al.*** Biomimetic *smart* hydroxyapatite/biphasic tricalcium phosphate biomatrices induce bone formation. *Key Eng Mater.* 2008; 361–363: 981–84.
118. **Ripamonti U, Richter PW, Nilen RWN, *et al.*** The induction of bone formation by *smart* biphasic hydroxyapatite tricalcium phosphate biomimetic matrices in the non-human primate *Papio ursinus*. *J Cell Mol Med.* 2008; 12: 1029–48.