

SCULPTURING THE ARCHITECTURE OF MINERALIZED TISSUES: SOLUBLE, INSOLUBLE AND GEOMETRIC SIGNALS

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INTRODUCTION: Nature relies on common and limited molecular mechanisms tailored to provide the emergence of specialized tissues and organs. The bone morphogenetic and osteogenic proteins (BMPs/OPs) family is an elegant example of Nature's parsimony in programming multiple specialized functions deploying molecular isoforms with minor variation in amino acid motifs within highly conserved carboxy-terminal regions. [1-4] BMPs/OPs, pleiotropic members of the transforming growth factor- β (TGF- β) superfamily, are soluble mediators of tissue morphogenesis and powerful regulators of cartilage and bone differentiation in embryonic development and regeneration in postnatal life. A striking and discriminatory feature of BMPs/OPs is their ability to singly induce *de novo* endochondral bone formation in extraskeletal heterotopic sites as a recapitulation of embryonic development. [1-4] Strikingly this prerogative, originally solely assigned to BMPs/OPs, has been extended to the TGF- β isoforms themselves with a marked site and tissue specificity of endochondral bone induction yet remarkably and strictly in primates only. [3,4] The presence of several molecular forms endowed with osteogenic activity poses important questions about the biological significance of this apparent redundancy, additionally indicating multiple interactions during bone tissue formation and tissue morphogenesis of disparate organs and tissues. The menu for enunciating the rules of architecture that sculpt cortico-cancellous structures of the bone and regulate bone regeneration and bone tissue engineering in clinical contexts lists additional complex interactions but requires three key components: an osteoinductive soluble signal; an insoluble substratum which delivers the signal and acts as a scaffold for new bone formation; and host cells capable of differentiation into bone cells in response to the osteoinductive soluble signal. [1,4] The signals responsible for osteoinduction are conferred by the osteogenic members of the TGF- β superfamily. This communication describes site-specific modifications of bioactive biomimetic matrices endowed with the striking prerogative of initiating bone formation by induction even in absence of exogenously applied osteogenic members of the TGF- β superfamily. [4-6]

METHODS: The heterotopic and orthotopic models of tissue morphogenesis by osteoinductive and osteoconductive biomaterials have been described in detail [4,5] and height clinically healthy Chacma baboons (*Papio ursinus*) were implanted heterotopically in the *rectus abdominis* muscle with four different types of resorbable and non-resorbable sintered porous hydroxyapatite (SPHA) discs, 20 mm in diameter with a series of concavities prepared on both planar surfaces. Discs 25 mm in diameter of porous hydroxyapatites were also implanted orthotopically in non-healing calvarial defects. Tissue specimens were harvested on day 30, 90 and 180 and processed for histological, biochemical and molecular analyses. Additional sections were used for immunohistochemical staining of OP-1 and BMP-3, markers of bone formation. Total RNA was extracted and probed for γ -actin, OP-1, BMP-3, TGF- β_1 and collagen type IV cDNAs.

RESULTS: Morphological analyses on day 30 showed mesenchymal collagenic condensations within concavities of the substratum with vascular invasion and capillary sprouting within the invading tissues with capillary elongation in close contact with the implanted hydroxyapatite biomatrix and bone formation by induction (Fig. 1). There was attachment and differentiation of mesenchymal cells at the hydroxyapatite/soft tissue interface of the concavities and expression of BMP-3 and OP-1 mRNA in differentiating osteoblast-like cells resident within the concavities of the *smart* biomimetic matrices which was followed by immunolocalization of the secreted proteins BMP-3 and OP-1 at the interface of the sintered hydroxyapatite biomatrix with the invading mesenchymal tissue. Signal intensity of mRNA expression differed between the different types of SPHAs with marked variation of mRNA expression of BMP-3, OP-1 and type IV collagen. Non-resorbable SPHAs orthotopically implanted induced 32.9 ± 6.5 and 36.8 ± 8.8 bone volume on day 90 and 180 respectively. Resorbable SPHAs 27.1 ± 5.2 and 27.5 ± 11.3 , respectively.

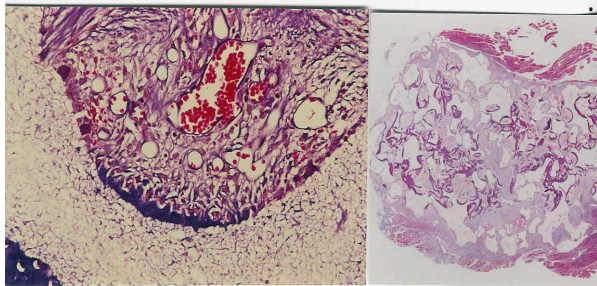


Fig1: Vascular invasion and bone differentiation by induction within concavities of highly crystalline sintered hydroxyapatite biomatrices.

DISCUSSION & CONCLUSIONS:

Morphological, biochemical and molecular evidence has been harnessed in our laboratories to guide the incorporation of specific angiogenic and osteogenic activities into biomimetic matrices of sintered highly crystalline hydroxyapatites. Sintered hydroxyapatites implanted heterotopically in *Papio ursinus* induce the reproducible spontaneous differentiation of bone. The geometry of the insoluble signal is a critical parameter for bone induction to occur: concavities of a specific dimension bind OP-1 and BMP-3 and then initiate a sequential cascade of events driving the emergence of the osteogenic phenotype and the morphogenesis of bone as a secondary response. We have now shown that the spontaneous induction of bone differentiation initiates even in concavities of resorbable biomimetic matrices. Additional and complementary data were deduced by Northern blot analyses to study the mRNA expression of specific gene products induced by responding cells within the concavities of the sintered biomimetic matrices and showing critical differences in expression of mRNA markers of bone formation according to the type of implanted biomatrices. Results indicate that the geometry of the substratum is not the only driving force since the structure of the insoluble signal dramatically influences and regulates gene expression and the induction of bone as a secondary response. We now propose the following cascade of molecular and morphological events culminating in the induction of bone initiating within concavities of the *smart* biomimetic matrices: 1) vascular invasion and capillary sprouting within the invading tissue with capillary elongation in close contact with the hydroxyapatite biomatrix; 2) attachment and differentiation of mesenchymal cells at the interface of the *smart* concavities; 3) expression of TGF- β and BMPs/OPs gene products in osteoblast-like cells resident and differentiating within the concavities; 4) expression and synthesis of specific BMP/OP proteins from transformed and

differentiated osteoblasts onto the sintered crystalline hydroxyapatite; 5) intrinsic osteoinduction depending on a critical threshold of endogenously produced BMPs/OPs initiating bone formation. Soluble signals induce morphogenesis, physical forces imparted by the geometric topography of the insoluble signal dictate biological patterns, constructing the induction of bone and regulating the expression of selective mRNA of gene products as a function of the structure. In the primate only, heterotopic bone induction is initiated by naturally-derived BMPs/OPs and TGF- β 1, recombinant hBMPs/OPs and TGF- β s and sintered hydroxyapatite biomimetic matrices with a specific geometric configuration. This indicates that in primates bone tissue develops as a mosaic structure in which members of the TGF- β superfamily singly, synergistically and synchronously initiate and maintain the developing morphological structures and play different roles at different time points of the morphogenetic cascade. In the primate only TGF- β s and BMPs/OPs are sculpturing tissue constructs that help to engineer skeletal tissue regeneration in molecular terms: morphogens exploited in embryonic development are re-exploited and re-deployed in postnatal tissue regeneration.

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