Enhanced activity of demineralised bone matrix augmented with xenogeneic bone morphogenetic protein complex in rats

SADJ August 2012, Vol 67 no 7 p354 - p358

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ABSTRACT

Introduction: Demineralised bone matrix (DBM) is an allograft material widely used as a bone filler and bone graft substitute. DBM contains bone morphogenetic proteins (BMPs), which induce and regulate bone formation during embryogenesis and in postnatal life.

Aims and objectives: To investigate the osteoinductivity of DBM augmented with xenogeneic BMP-complex at different doses.

Materials and methods: Rat DBM was augmented with BMP-complex purified from porcine diaphyseal bone.

Results: Dorsal subcutaneous implantation of 25 mg rat allogeneic DBM augmented with 0, 3, 6 and 12 mg BMP-complex per gram of DBM resulted in dose dependant up-regulation of bone formation on day 21, as scored histologically and biochemically.

Conclusions: Allogeneic DBM can be augmented with xenogeneically sourced BMP-complex to improve DBM performance *in vivo*. This work demonstrates the potential of BMP-complex augmented DBM to induce new bone formation with improved parameters of bone formation.

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ACRONYMS

BMP: Bone morphogenetic protein
DBM: Demineralised bone matrix
ECM: Extracellular matrix
ELISA: Enzyme linked immunosorbent assay
TGF: Transforming growth factor
rh: recombinant human

Key words: bone regeneration; demineralised bone matrix; bone morphogenetic proteins; bone graft substitute; osteoinduction

INTRODUCTION

The discovery[1] and molecular cloning[2,3] of the bone morphogenetic proteins (BMPs), has revolutionised tissue engineering for the regeneration of alveolar bone in periodontal and oral-maxillofacial defects.[4,5] Bone tissue engineering is particularly relevant to periodontal defects and the augmentation of alveolar bone for implant dentistry.[6,7] The BMPs are important molecular signalling factors that cooperate with responding cells and the extracellular matrix to induce and maintain bone.[6,8]

The commercial availability of recombinant human (rh) BMPs in the past decade continues to advance treatment methods used by clinicians for the restoration of periodontal tissues, including the cementum, periodontal ligament and alveolar bone.[6] The high cost of rhBMPs places them largely outside the reach of emerging economies such as South Africa. Cheaper sources of BMPs are potentially available from human cadaveric bone, which have demonstrated a therapeutic potential in the regeneration of bone fractures and defects.[9] The paucity of human bone tissue from which

to purify BMPs limits their commercial availability. Recently, xenogeneically sourced BMPs (OsteaPLEX™, Altis Biologics (Pty) Ltd, Pretoria, South Africa), prepared from porcine bone, have been commercialised to meet this need. OsteaPLEX™ is a protein cocktail derived from the soluble fraction of porcine demineralised bone matrix (DBM) and is standardised against its content of BMP-2, one of many members belonging to the TGF-ß1 superfamily.[8]

The three critical ingredients for tissue engineering of bone are the signalling BMPs, responding cells and appropriate scaffolding structures.[6,10,11] Since the discovery that DBM can induce ectopic bone formation,[1] human DBM has become arguably the most frequently grafted tissue in humans for the repair of skeletal defects caused by trauma, neoplasia, infection and periodontal disease.[12] Urist originally defined osteoinduction through his classic study, demonstrating the 'autoinductive' effect of demineralised bone matrix by inducing differentiation of cartilage and bone when implanted into extraskeletal locations in animals.[1] Human DBM is both osteoinductive and osteoconductive by virtue of the presence of endogenous BMPs bound to the osteoconductive collagenous bone matrix. However, the variation in the quality of human donor material and processing protocols makes the osteoinductive potential of tissue banked DBM somewhat inconsistent.[13] Commercial DBM from different tissue banks differs in the ability to induce new bone formation.[14] The variability is related to the age of the donor, as well as to the content of bone inductive factors in the donor bone.[15]

A possible strategy to overcome the drawbacks associated with variable DBM is to augment the biomaterial with exogenously-sourced BMP and provide a naturally-derived bone graft substitute with enhanced and consistent osteoinductive activity for the predictable tissue engineering of bone. Examples of such augmentation strategies have been reported using bone derived BMP,[16] as well as rhBMP.[17] Aspenberg *et al*. showed in the squirrel monkey heterotopic bioassay that allogeneic DBM augmented with recombinant BMP-2 resulted in dose dependent increases in calcium content and frequency of bone formation at six weeks.[17] They concluded that BMP-2 augmented DBM might be a clinically useful bone inducing biomaterial for man. Similarly, enrichment of allogeneic DBM with cadaveric BMP-complex significantly enhanced periodontal regeneration in humans.[16] The objectives of this research were to conduct a three-week study, in order to evaluate the bone induction potential of DBM augmented with commercial xenogeneic BMP-complex (OsteaPLEX™).

MATERIALS AND METHODS

The protocol for this study was approved by the Research Committee, School of Dentistry, Faculty of Health Sciences, University of Pretoria, and by the Animal Ethics Committee (AEC/2006/06/001), Tshwane University of Technology, Pretoria, South Africa (Study Number A55/05/06). The implants were formulated at Altis Biologics (Pty) Ltd, Pretoria, South Africa as described below. Implantation was performed by LaBio Research Laboratories at the Tshwane University of Technology, Pretoria, South Africa. OsteaPLEX™ BMP-complex was purified according to the disclosures of a USA patent 7,728,116 B2.[18]

Morphogens, implants and animals

OsteaPLEX™ BMP-complex in 20mM acetic acid was donated by Altis Biologics (Pty) Ltd, South Africa. OsteaPLEX™ is a porcine bone matrix isolate, containing a number of proteins that include members of the transforming growth factor-ß (TGF-ß) super-family, namely BMP-2, BMP-4, BMP-7 and TGF-ß1. OsteaPLEX™ is standardised to 0.01% m/m with respect to its content of porcine (p) BMP-2 content, using a commercial enzyme linked immunosorbent assay (ELISA) kit (Quantikine®, R&D Systems, USA). OsteaPLEX™ was reconstituted with rat DBM in doses that included negative controls (0mg/g), and doses of 3mg/g, 6mg/g, and 12mg/g of OsteaPLEX™ per gram of DBM. The lyophilised composite was further combined with 10% m/m soluble rat-tail type I collagen, frozen and precipitated with 70% ethanol to sterilise the biomaterial. The implant was lyophilised to dryness in a lyophiliser (VirTis, USA). The BMP-complex augmented DBM implant was transferred into a 1ml capacity sterile syringe, and reconstituted on the day of the implantation with two volumes of sterile, pyrogen free water. The rehydrated implant was injectable through a 15-gauge needle with a short bevel. Four male Wistar rats, between 28 to 32 days old, weighing between 100g and 200g each, were obtained from National Health Laboratory Services, Cape Town. The rats were housed, two per cage, and food and water were provided *ad libitum*. The ambient temperature was controlled between 18 to 23°C at a humidity of between 45 to 60%.

Surgical procedures

The dorsal area was shaved and swabbed with antiseptic solution before the implantation procedure was done under sterile conditions. The animals were sedated using ether and the implants injected into the subcutaneous space overlying the fascia. Each rat received four implantations subcutaneously in the dorsal region. The sites were selected in a predetermined sequence following a Latin square block design that allowed for the rotational allocation of the four doses within a total of 16 implantations.[19] Two bilateral implants were positioned cranially, overlying the fascia of the underlying *latissimi dorsi* muscles. The remaining two implants were positioned caudally, bilaterally overlying the fascia of the *glutei maximi* muscles.

The rats were euthanised by carbon dioxide asphyxiation at 21 days post-implantation. The skin was separated from the underlying tissue, the implants were removed with minimal surrounding tissue and divided. One part was snap frozen to -80 °C in liquid nitrogen and submitted for alkaline phosphatase activity determination as described elsewhere.[20] Briefly, the tissue was homogenised in phosphate buffer and centrifuged to obtain a clarified supernatant, which was used to determine the amount of enzyme substrate, para-nitrophenyl phosphate (Merck GmbH, Germany) converted to product over time, as more fully explained previously.[20]

The other part was fixed in 10% buffered formalin, wax embedded, not decalcified, and processed for light microscopic histological examination at Vetpath Laboratories, Onderstepoort, Pretoria.

Histology, microscopy and imaging

The sections of tissue were cut at a thickness of 5 µm, mounted on microscope slides and stained using haematoxylin and eosin, and Goldner's trichrome. These were microscopically evaluated using a light microscope (Leica, Germany). Images were captured using a digital camera (Canon, S70, Japan).

The extent of the histological parameters of osteogenesis, osteoblasts, mineralization, inflammation and vascularisation were scored according to a semiquantitative grading scale ranging from zero to three (Table 1) by a single-blinded, independent veterinary histopathologist (IDEXX Laboratories, Onderstepoort, South Africa), following a similar protocol previously published.[21] The histopathologist first identified as a control the section with the lowest score, and then compared the remaining sections, scoring them against this control. Data were captured in Microsoft Office Excel (2003), running on a computer with a Pentium 4 processor.

Statistical analysis

A non-parametric method, Kruskal-Wallis analysis which is a nonparametric equivalent of analysis of variance for comparing doses was used for the categorical results while one-way analysis of variance (ANOVA) was used for the ratio data (continuous measure). The statistical software package used was STATA 12.1 (StataCorp LP).

RESULTS

The augmentation of DBM resulted in increased osteogenesis and osteoblast scores at all BMP-complex doses when compared to non-augmented native DBM, but the sample size (n=4) was too small to observe statistical significance using the non-parametric methods (Table 2). The highest increase was for the 3mg/g and 6mg/g dose, with a reduction in osteogenesis observed at 12mg/g doses of BMP-complex pointing to a biphasic response. Implant mineralisation showed a downward trend throughout the dose series, contrasting with the osteogenesis scores. Inflammation was low at all doses, with a small drop noted at the 6mg/g dose but this was not significant. The parameters of vascularisation and inflammation appeared independent of dose of BMP-complex.

The temporal study of alkaline phosphatase activity shows a strong dose-dependent effect at 12 days (data courtesy Altis Biologics (Pty) Ltd), peaking at the highest dose of 12mg/g (Figure 2). At 21 days, the picture is different, with a subsidence of the alkaline phosphate activity to low levels and a biphasic dose effect, which was not statistically significant (Table 3).

Table 1: Grading scale for the scoring of the histological parameters

Classification	Scores	Extend of parameter (versus control)
None	0	No evidence
Mild	1	Minimal evidence
Moderate	2	Up to 50% of field
Severe	3	Greater than 50% of field

Table 3: Comparison matrix of P-values from one way ANOVA for the continuous data. No significant differences (P>0.05) were found for alkaline phosphatase activity between dose groups at 21 days.

dose mg/g	0	3	6
3	0.938		
6	0.488	0.813	
12	0.843	0.994	0.918

DISCUSSION

Heterotopic ossification brought about by the implantation of DBM alone and DBM augmented with BMP-complex was observed histologically (Figure 1). The histological specimen shows bone formation induced by heterotopic implantation of DBM (Figure 1A). Newly formed islands of cartilage are being replaced by mineralising bone, embedded with newly forming osteocytes. These observations agree with Urist's classic definition of osteoinduction, exemplified in the present case as new bone formation evidenced by DBM implanted at extraskeletal sites.[1] The implanted devitalised DBM is clearly evidenced as a poorly-staining material bearing empty lacunae (Figure 1A and 1B). In contrast, intramuscular implantation of recombinant BMP-2 augmented DBM in nude rat intramuscular sites, induced ossicles with a bone shell, no surrounding implant particles and a marrow cavity as reported by the work of Aspenberg *et al*.[17] It has been proposed that the intramuscular site provides higher osteoinductivity scores for BMP implants, and therefore direct comparison of these studies with the present study must take implantation sites and differences in BMP potencies into consideration.[22]

Tissue alkaline phosphatase (ALP) activity is an indicator of osteogenesis. This osteoinduction enzyme marker has previously been shown to peak on day 10 and to decline thereafter.[20] In the present work, ALP activity was still detectable on day 21, and at much lower levels than was observed at 12 days (Figure 2), corroborating the results of others,[20] and eliminating the possibility of the present findings being attributable to an inactive BMP-complex. The biphasic curve generated by reduced ALP activity at the highest dose may point to the earlier temporal ossification followed by earlier resorption at increasing doses in this model. Previously, a biphasic response to implanted rhBMP-7 has been observed *in vivo* in the rat ectopic bioassay, corroborating the present results.[23]

It has previously been proposed that the time period allowed for the formation of ectopic *de novo* bone is of critical importance.[24] If harvested too early, evidence for bone transformation may be lost, whereas if harvested too late, resorption may have taken place.[24] In the present study, the 21-day rodent assay period provides new insights into the fate of BMP-complex augmented DBM at the three-week period.

Table 2: Median histological scores for DBM with different doses of OsteaPLEX™, implanted in rats (n=4), and probability values from Fisher's exact probability test, and Kruskal-Wallis procedures.

Parameter	OsteaPLEX™ dose mg per gram DBM				Calculated Fisher's (chi-squared) exact probability	Kruskal-Wallis procedure (chi-squared statistic (df=3))
	0	3	6	12		
Osteogenesis	1.0	2.5	2.0	2.0	0.707	0.388 (3.023)
Osteoblasts	1.5	2.0	2.5	1.5	0.439	0.235 (3.579)
Mineralisation	2.0	1.5	1.5	1.0	0.942	0.584 (1.947)
Inflammation	1.0	1.0	0.5	1.0	1.000	0.730 (1.295)
Vascularisation	2.0	2.5	2.0	2.0	0.49	0.299 (3.668)

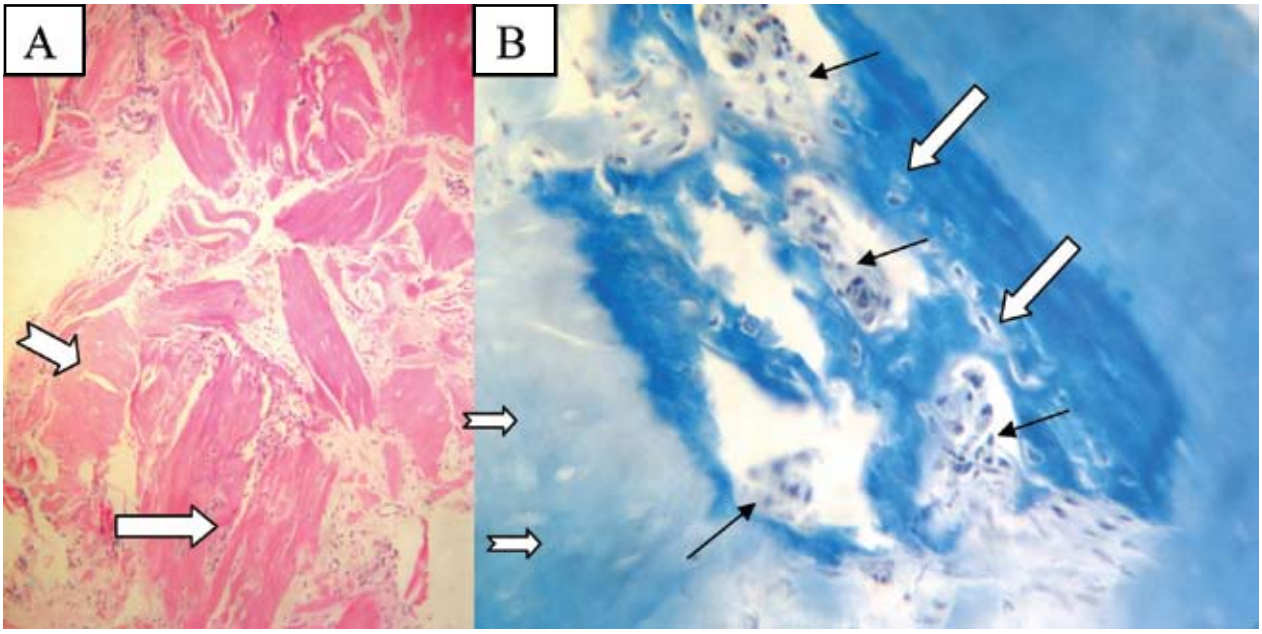

Figure 1: Histological sections of 21 day old implants; **(A)** Haematoxylin and eosin staining of allogeneic DBM augmented with 0 mg/g BMP-complex (magnification 100x). **(B)** Goldner's trichrome staining of allogeneic DBM augmented with 3 mg/g BMP-complex (magnification 400 x). Solid arrows point to islands of cartilage, open arrows to areas of newly formed bone, and open fluted arrows to implanted, devitalised DBM bearing empty lacunae.

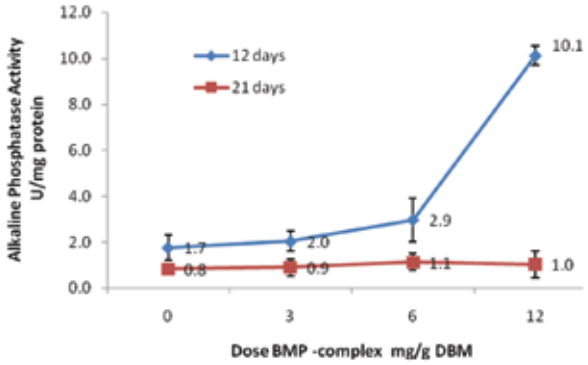

Figure 2: Biphasic dose curve of alkaline phosphatase activity at 21 days of rat DBM augmented with different doses of xenogeneic BMP-complex. The 12-day data of the same implantation protocol from a different study verify the biologic activity for BMP-complex used in the study (courtesy Altis Biologics (Pty) Ltd).

Future studies should be designed to determine the fate of BMP-complex augmented DBM at earlier and extended time periods, with larger sample sizes to improve the statistical strength of the experiments.

Extracellular matrix (ECM) has been shown to be very important in adhesion, growth and differentiation of cells during embryogenesis, as well as post-natal repair and regeneration of bone trauma. Subcutaneous implantation of allogeneic rat DBM in the rat has been associated with local *de novo* endochondral bone differentiation.[20] Dissociatively extracted and partially fractionated matrix protein fractions from human, monkey and bovine bone matrix, reconstituted with biologically inactive collagenous rat bone matrix, induces new bone in the rat, indicating homology of bone-inductive activity from different species' bone matrices.[25] These findings demonstrate the potential utilisation of xenogeneic porcine BMPs, combined with allogeneic DBM, to induce clinically relevant *de novo* bone formation in man.

Previous work has demonstrated that members of the TGF-ß superfamily found in the ECM of bone, cooperate synergistically to induce exuberant bone formation.[26,27] It has been shown that 5µg of TGF-ß interacts synergistically with 125µg BMP-7 during ectopic bone induction in the rectus abdominis of the baboon heterotopic bone induction assay.[26] Combined delivery of TGF-ß1 and BMP-2 similarly, with osteoprogenitor cell transplantation, allowed physiologic doses of these growth factors to enhance bone formation *in vivo*.[28] Thus a cocktail or complex of multiple factors is expected to have higher biologic activity than singly applied factors. Cocktails of rhBMP-2 and rhBMP-7 have been used to regenerate periodontal defects in baboons (*Papio ursinus*).[29] Recombinant BMP-7 alone, failed to achieve the same results in *Papio ursinus*, generating mainly cementogenesis without restoration of the other components of the periodontium.[30] In contrast, co-application of BMP-2 with BMP-7 resulted in periodontal tissue regeneration in *Papio ursinus*.[29] These studies support the premise that multiple growth factors working in concert significantly enhance the formation of functional bone.[31]

In what is probably the first human clinical study involving DBM augmentation using naturally sourced BMPs, Bowers *et al*. showed that human osteogenin combined with human DBM enhances periodontal regeneration when compared with DBM alone.[16] Similarly, the co-application of allogeneic collagenous bone matrix with naturally derived bovine BMP-complex in surgically created furcation defects in *Papio ursinus* led to connective tissue attachment and bone regeneration.[32]

CONCLUSION

Previous research has reported the quantitative assessment of the osteoinductivity of bone matrix from different donors and after different processing methods in order to assess the potential clinical effectiveness of these grafts.[12] Access to commercially available xenogeneically derived BMP-complex may overcome limitations associated with the scarcity of human tissue, and offer tissue engineers a way of enriching human DBM with xenogeneically sourced signalling factors, to enhance the performance of implanted DBM for the tissue engineering of bone. Future research employing

BMP-complex augmented DBM may elucidate the utility of this biomaterial for periodontal regeneration.

Declaration: Nicolaas Duneas owns shares in Altis Biologics (Pty) Ltd.

Acknowledgements

The authors wish to thank Altis Biologics for the donation of BMP-complex, and Dr S Olorunju, Biostatistics Unit, Medical Research Council of South Africa, for statistical analyses.

G U Mohangi acknowledges Prof F D Verwayen for assistance in the initial stages of this study.

References

1. Urist MR. Bone: Formation by auto induction. Science 1965; 150: 893-9.
2. Wozney JM, Rosen V, Celeste AJ, *et al*. Novel regulators of bone formation: molecular clones and activities. Science 1988;242:1528-34.
3. Ozkaynak E, Schnegelsberg PN, Oppermann H. Murine osteogenic protein (OP-1): high levels of mRNA in kidney. Biochem Biophys Res Commun 1991;179:116-23.
4. Ripamonti U, Reddi AH. Growth and morphogenetic factors in bone induction: role of osteogenin and related bone morphogenetic proteins in craniofacial and periodontal bone repair. Crit Rev Oral Biol Med 1992;3:1-14.
5. Ripamonti U, Reddi, AH. Tissue engineering, morphogenesis and regeneration of the periodontal tissues by bone morphogenetic proteins. Crit Rev Oral Biol Med 1997;8:154-63.
6. Chen F-M, Jin Y. Periodontal Tissue Engineering and Regeneration: Current Approaches and Expanding Opportunities. Tissue Eng 2010;16:219-55.
7. Sykaras N, Opperman LA. Bone morphogenetic proteins (BMPs): how do they function and what do they offer the clinician? J Oral Sci 2003;45:57-73.
8. Reddi AH. Role of morphogenetic proteins in skeletal tissue engineering and regeneration. Nat Biotechnol 1998;16:247-52.
9. Johnson EE, Urist MR, Finerman GA. Resistant non-unions and partial or complete segmental defects of long bones: Treatment with implants of a composite of human bone morphogenetic protein (BMP) and autolyzed, antigen-extracted, allogeneic (AAA) bone. Clin Orthop Relat Res 1992;277:229-37.
10. Reddi AH. Morphogenesis and tissue engineering of bone and cartilage: inductive signals, stem cells, and biomimetic biomaterials. Tissue Eng 2000;6:351-9.
11. Ramoshebi LN, Matsaba TN, Teare J, Renton L, Patton J, Ripamonti U. Tissue engineering: TGF-[beta] superfamily members and delivery systems in bone regeneration. Expert Rev Mol Med 2002;4:1-11.
12. Zhang M, Powers RM Jr, Wolfinbarger L Jr. A quantitative assessment of osteoinductivity of human demineralized bone matrix. J Periodontol 1997;68:1076-84.
13. Peterson B, Whang PG, Iglesias R, Wang JC, Lieberman JR. Osteoinductivity of commercially available demineralized bone matrix. Preparations in a spine fusion model. J Bone Joint Surg Am. 2004;86-A(10):2243-50.
14. Schwartz Z, Mellonig JT, Carnes DL Jr, de la Fontaine J, Cochran DL, Dean DD, Boyan BD. Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation. J Periodontol 1996;67:918-26.
15. Tissue Banking of Bone Allografts Used in Periodontal Regeneration. The Committee on Research, Science and Therapy of the American Academy of Periodontology Position Paper; J Periodontol 2001;72 : 834-8.
16. Bowers G, Felton F, Middleton C, Glynn D, Sharp S, Mellonig J, Corio R, Emerson J, Park S, Suzuki J, *et al*. Histologic comparison of regeneration in human intrabony defects when osteogenin is combined with demineralized freeze-dried bone allograft and with purified bovine collagen. J Periodontol 1991;62:690-702.
17. Aspenberg P, Wang E, Thorngren K-G. Bone morphogenetic protein induces bone in the squirrel monkey, but bone matrix does not. Acta Orthop Scand 1992;63:619-22.
18. Duneas N (2010) Method of preparing an osteoinductive protein fraction USA 7,728,116 B2.
19. Ripamonti U, Ma SS, Cunningham NS, Yeates L, Reddi AH. Reconstruction of the bone-bone marrow organ by osteogenin, a bone morphogenetic protein, and demineralized bone matrix in calvarial defects of adult primates. Plast Reconstr Surg 1993;91:27-36.
20. Reddi AH, Huggins C. Biochemical sequences in the transformation of normal fibroblasts in adolescent rats. Proc Natl Acad Sci USA 1972;69:1601-5.
21. Mankani MH, Kuznetsov SA, Avila NA, Kingman A, Robey PG. Bone formation in transplants of human bone marrow stromal cells and hydroxyapatite–tricalcium phosphate: Prediction with quantitative CT in mice. Radiology 2004;230:369-76.
22. Włodarski KH, Włodarski P. Short letter - Peculiarities of the rodent subcutaneous space for testing of bone inducing agents. Growth Factors 2000;17:269-85.
23. Ripamonti U, van den Heever B, Crooks J, Tucker MM, Sampath T K, Rueger DC, Reddi AH. 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;15:1798-1809.
24. Ripamonti U, Ramoshebi LN, Teare J, Renton L, Ferretti C. The induction of endochondral bone formation by transforming growth factor-beta(3): experimental studies in the non-human primate Papio ursinus. J Cell Mol Med 2008;12:1029-48.
25. 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.
26. Duneas N, Crooks J, Ripamonti U. Transforming growth factor-β1: 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.
27. Ripamonti U, Crooks J, Matsaba T, Tasker J. Induction of endochondral bone formation by recombinant human transforming growth factor- ß2 in the baboon (Papio ursinus). Growth Factors 2000;17:269-85.
28. Simmons CA, Alsberg E, Hsiong S, Kim WJ, Mooney DJ. Dual growth factor delivery and controlled scaffold degradation enhance *in vivo* bone formation by transplanted bone marrow stromal cells. Bone 2004;35:562-9.
29. Ripamonti U, Crooks J, Petit J-C, Reuger DC. Periodontal tissue regeneration by combined applications of recombinant human osteogenic protein-1 and bone morphogenetic protein-2. A pilot study in Chacma baboons (*Papio ursinus*). Eur J Oral Sci 2001;109 :41-8.
30. Ripamonti U, Heliotis, M, Rueger DC, Sampath TK. Induction of cementogenesis by recombinant human osteogenic protein-1 (hOP-1/ BMP-7) in the baboon. Arch Oral Biol 1996;41:121-6.
31. Hsiong SX, Mooney DJ. Regeneration of vascularized bone. Periodontol 2000 2006;41:109-22.
32. Ripamonti U, Reddi AH. Periodontal regeneration: potential role of bone morphogenetic proteins. J Periodont Res 1994;29:225-35.