



Title	Composite autogenous bone and demineralized bone matrix: an effective graft material
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3129 Bone Regeneration Induced by Autogenic Demineralized Dentin Matrix in Dog Cranial Defects

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Large bony defects in the skull heal by fibrous connective tissue and do not form bone unless osteoinductive materials are placed in the defects. In view of its osteoinductive capacity and lack of immunogenicity, autogenous demineralized dentin matrix is considered a good implant material for bone defect restoration. Eight adult dogs were anesthetized and a midline longitudinal incision along the frontal suture was made over the skull reflecting the skin flap laterally. Periosteal tissue was also removed from the defect site. Two skull defects, 10mm x 5mm were trephined on both sides of the frontal suture. Care was taken not to damage the underlying dura. Slices of demineralized dentin matrix were placed into the right side defect, while the left was filled with blood and served as control. Periosteal and skin flaps were sutured both in place. Harvest periods of 3, 6, 12 and 17 weeks were chosen. Two dogs were euthanized in each period. The calvaria were resected, fixed in 10% neutral buffered formalin, and then demineralized in solutions of formic acid and sodium citrate. For histologic examination tissues were stained with hematoxylin-eosin and Masson's trichrome. The results showed good bone regeneration in all of the grafted defects, while the controls healed only partially by new bone formation from the margins of the defect. Hence we conclude that slices of autogenic demineralized dentin matrix can be used to heal cranial defects, representing an efficacious treatment modality in osseous reconstruction.

3130 Automated Measuring of Mineralized Bone Fraction Around Endosseous Implants.

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Previous studies of osseointegration have employed manual interpretation of photomicrographs. This approach is time consuming and subject to considerable operator bias. In this study, we evaluated the use of image analysis software on directly digitized SEM images to quantify the percentage of mineralized bone as a function of distance from a titanium implant. Geographic Information System (GIS) software is used to characterize land cover, measure distances and calculate areas in satellite images. Percentage implant osseointegration may be measured utilizing backscattered electron images in an analogous approach, albeit at a much smaller scale. A GIS is a collection of relatively simple software tools for manipulating digital images. The GIS contains individual programs to view an image, classify it based on digital number, extract polygons, measure the distance between polygons and pixels, and summarize information in the form of a histogram. These individual programs may be grouped in sequences to automatically perform more complicated analyses. In this investigation, pixels are classified into soft tissue, hypomineralized bone, mineralized bone and implant. The implant polygon is then extracted into a separate file and the distance to all non-implant pixels determined. These distance values are recoded into discrete intervals corresponding to actual distances on the sample. Finally, the two files are recombined so that each digital number corresponds to a unique combination of sample material and distance. The histogram of this last file is processed to provide plots of percentage mineralized bone against distance. The GIS software approach yields results that are comparable to manual interpretation (tracing) followed by careful measurement of area on a digitizing tablet. Because it is implemented entirely in computer software using directly digitized images, this new method has four distinct advantages. The GIS software approach to measuring mineralized bone fraction is reproducible, eliminates investigator bias, is highly automated, and is less costly per image.

3131 Composite Autogenous Bone and Demineralized Bone Matrix: An Effective Graft Material.

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Previous reports have qualitatively evaluated the improved integration of composite endochondral bone grafts (EC) and cortical demineralized bone matrix (DBM). The purpose of the present study was to quantitatively assess the amount of new bone formed in response to composite EC-DBM and to compare it to that produced by EC bone grafts alone. Twelve defects were created in the skulls of adult rabbits, half were grafted with EC bone alone, and the other half with composite EC-DBM. Healing was evaluated, 2 weeks later, by image analysis of PAS stained histologic sections. A total of 108 sections were digitized. On average 47% more new bone was measured in the composite group when compared to that of the EC group. The statistical difference between the bone formed in defects grafted with EC bone was significant while it was insignificant in the composite group. To illustrate the therapeutic potential of composite grafting, patients with varying degrees of nonregenerative defects were treated. In 3 of the patients, defects were repaired with IM + DBM. Within 4 months implants were placed. Bone continuity and function were restored using EC + DBM in mandibular and maxillary reconstruction. In conclusion: DBM augments the bone induction capacity of the host bone as well as the bone graft. Composite autogenous graft and DBM is an effective graft material and merit further clinical investigation. The study was supported by RCG Grant #372/251/6435 University of Hong Kong.

3132 Pluronic Polyols' Effect on Bone Healing

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Pluronic polyols (Pp) have previously been shown to benefit soft tissue during early wound healing. This study was conducted to evaluate the effects of Pp on graft placement and bone regeneration in 182 Sprague-Dawley rats with 8mm calvaria defects. Animals received various combinations of Pp (F-127 and F-68) either topically or systemically, with or without graft materials (demineralized bone powder, DBP; or tricalcium phosphate, TCP) to result in 15 separate treatment groups of 10 animals per group. Calvaria were harvested at 12 weeks post surgery and evaluated histomorphometrically, by contact radiography with subsequent densitometric analysis, energy spectrometry by scanning electron microscope, and by fluorescent microscopy. Pp did not hinder osseous healing and when used topically greatly facilitated placement of graft materials during surgical procedures. DBP was the graft material of choice and gave greater bone fill compared to TCP on non-grafted controls ($p < 0.05$). Based upon these results the Pluronic polyols should be considered as carriers during placement of graft materials in osseous defects.

3133 Pattern of Angiogenesis in the healing of Composite Intramembranous Bone and Demineralized Bone Matrix.

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The purpose of this work was to gain further insight into the angiogenic pattern in the early stages of healing of composite intramembranous (IM) and demineralized endochondral bone matrix (DBM). Fourteen critical-size 10 x 5 mm, full thickness bony defects were created in the parietal bones of mature rabbits. Defects were filled with DBM alone or with combined IM-DBM. Tissues were retrieved in 1, 2, 3, 4, 5, 6, and 7 days post grafting. Neovascularization was assessed using antibodies to factor VIII antigen (marker for vascular endothelium) and pan-endothelial antibody (CD-31). In the composite IM-DBM grafted group: Two days after grafting, positive staining for endothelial cells were first observed near the periphery of the host bone rim. Small blood vessels were first seen budding from host bed towards the graft by day 3. Differentiating chondroblasts were observed by day 4. With the advent of capillary invasion on day 6, initial signs of osteogenesis was observed and new bone was formed on the surface of cartilage matrix and the implanted matrix by day 7. Positive staining for endothelial cells in the DBM group was not observed until day three and the healing progressed through endochondral ossification as expected. These results lend support to the earlier work by Kusiak et al., where he concluded that IM bone vascularizes faster than EC bone. In this study signs of angiogenesis were observed sooner in the IM-DBM than in the DBM group suggesting a role for the IM in the neovascularization. In conclusions IM bone enhances the neovascularization and subsequently the healing of composite autogenous bone and DBM grafts. This study was supported by the CRCG 372/251/6435, The University of Hong Kong.

3134 Lamellar Pattern of Alveolar Bone Observed in Ovarian Dermoid Cysts.

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The lamellar appearance of bone structure is still the subject of much controversy. Recently, MAROTTI had proposed "A new theory of bone lamellation" (Calcif Tissue Int (1993) 53(Suppl 1):S47-S56). The goal of the present paper was to assess this hypothesis in pathological conditions. It was carried out on alveolar bone (AB) found in ovarian dermoid cysts (ODC). 8 bony structures, arranged all around teeth, one of them resembling a small part of a mandible, were collected from 306 ODC; 21.9% containing teeth. The AB samples fixed in 4% paraformaldehyde, were prepared for Polarized Light Microscopy (PLM), Scanning Electron Microscopy (SEM), Backscattered Electron (BSE) imaging and Energy Dispersive X-ray (EDX) analysis. SEM analysis of cross-sectioned osseons showed lamellae, with high and low density interwoven collagen fibers, alternate, giving them respectively anisotropic (dense fibers) and isotropic (loose fibers) appearance under PLM. The former were differentiated under osteoblast control and the latter during transformation of osteoblasts into osteocytes. BSE of polished carbon coated methyl methacrylate embedded AB showed variations in mineral density. Ca and P concentrations in the lamellae, measured by EDX analysis, were higher than in dense lamellae, respectively 14.17% ($p < 0.0052$) and 10.75% ($p < 0.0038$). These results are in agreement with those of MAROTTI, 1993. They suggest AB architecture and mineralization in ODC are highly dependent on matrix proteins composition. The lamellation seems the result of differences in the proportion of specific protein-protein complexes within bone matrix between adjacent lamellae. The presence of a bone protein with the same epitope as phosphophoryn, involved in mineralization, with a monoclonal anti-phosphophoryn antibody MAb 7G4, (LABOUX et al., Hybndoma (1994) 13:143-146), is under investigation.

3135 Lectin Histochemistry of Rat Submandibular and Sublingual Glands.

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Previous studies from our laboratory indicated that repeated doses of β -adrenergic agonist isoproterenol produced differential expression of several salivary proteins in rat submandibular and parotid glands (Bedi, G.S., Crit. Rev. Oral Biol. Med., 4, 565, 1993). In the present study we used lectin histochemistry to study the effects of isoproterenol on the glycosylation patterns of rat submandibular and sublingual gland glycoproteins. Female Wistar rats (175-200 g) were treated with daily intraperitoneal injections of isoproterenol (25 mg/kg body wt.) for 7 consecutive days. After autopsy, the salivary glands were removed and fixed in 10% formalin. An avidine-biotin technique was used to study the binding of lectins from *Ulex europaeus* (UEA-I), *Dolichos biflorus* (DBA), *Glycine max* (SBA), *Erythrina cristagalli* (ECL), *Phaseolus vulgaris* (PRA); *Ricinus communis* (RCA) and *Triticum vulgaris* (WGA) to specific sugars on paraffin sections. Histochemical staining of sublingual glands revealed that about 20-30% of the mucous acinar cells from untreated animals stained positively with UEA-I (fucose-directed) and ECL (Gal β -1,4 GlcNAc-directed), suggesting the presence of terminal fucose and/or Gal-GlcNAc structure in sublingual mucous glycoproteins. Isoproterenol treatment of rats for 7 consecutive days resulted in positive staining in 50-60% of the sublingual mucous acinar cells by these two lectins. Submandibular glands of both untreated and treated animals stained poorly with UEA-I, but strong staining of ductal cells was observed with ECL and SBA, showing that α -fucose and α - and/or β -GalNAc form the major carbohydrate moieties of the secretory glycoproteins from granular tubules. Such lectin histochemical studies will be useful in understanding the synthesis, processing and packaging of salivary gland glycoproteins. This study was supported by NIH Grant DE-09690.

3136 Immunohistochemistry of Carbonic Anhydrase in Developing Rat Submandibular Gland.

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Carbonic anhydrase (CA) has been localized to many structures involved in bicarbonate transport, including the granular, striated and excretory ducts, (GD, SD, ED) of the rat submandibular gland by both enzyme (Carpentier et al., *Biol. Cell* 54:241, 1985) and immunohistochemistry (Honnigar et al., *Anat. Rec.* 207:605, 1983). The purpose of this study was to immunohistochemically assess developmental changes in the CA isozymes I, II and VI in the rat submandibular gland. Glands were excised from one or more rats of each sex from each of 4 litters at ages 1, 7, 14, 28, 35, 42 and 80 (adult standard) days, fixed in Helly's fluid for 3 hr, then 2% $K_2Cr_2O_7$ for 2 hr and embedded in 56° C m.p. paraffin. Sections were cut at 6 μ m and incubated in normal sheep serum, then in either polyclonal (pc) sheep anti-human CA I or II Ab-HRP (Biossigen Int'l), or in pc rabbit anti-human CA VI Ab (purified by Protein A-Sepharose CL-4B) followed by pc goat anti-rabbit Ab-HRP. The chromogen was DAB. CA II reactions were +++ (scale of 0 to +++) in ED and SD and ++ in the ID (intercalated ducts) and the transient type I and III cells of acini (AC) at 1 and 7d, and changed gradually to +++ in SD and ED, ++ in GD, + in ID and 0 in CA by 42 d. Muscle controls were +++. Parenchymal cells were 0 with DAB alone or the goat anti-rabbit Ab-HRP followed by DAB. CA I and VI followed the same pattern but generally were + and ++ lighter, respectively. Myoepithelium was 0 at all ages. The order of usefulness of these CA isozymes as immunohistochemical markers for the functional differentiation of the type I and III cells and the SD and ED of the developing rat submandibular gland is II > I > VI. Supported by Dept. of Veterans Affairs and University of Umeå.