A comparative pilot case study between irradiated human cancellous bone and a synthetic analogue

# Bone Regeneration in a Bilateral Sinus Lift

Anthony McGee, BDS, Staines, and Dr J. A. Hunt, Liverpool/England

**The procedure of sinus grafting is an accepted technique for the generation of viable bone in the sinus cavity for successful implant placement. Autologous bone is considered the benchmark material for this grafting procedure due to ideal tissue compatibility and bone regeneration potential. Autogenous graft can be harvested from a number of donor sites and used as both bone blocks and in granular form.**

However, autograft is not without disadvantages. Donor site morbidity is a possibility when opting for autologous bone, as post-operative morbidity can be observed in up to 33 per cent of patients in the common practice of harvesting bone from the chin [1], and evidence indicates morbidity rates vary according to the donor site [2]. There is also the risk of infection at the donor site.

In addition to these possible adverse events, a diminished ability of autograft to maintain the volume of the area augmented by autogenous bone graft can result in a volume reduction of approximately 40 per cent in 180 days [3].

As a result of these factors, alternative graft materials have been widely researched, with a view to develop safe, resorbable materials with effective bone regenerative ability.

A commonly used alternative is xenograft, available as granular bovine hydroxyapatite. Although clearly an osteoconductive material, questions remain over the long term resorption profile, with no graft resorption observed at twelve months post implantation [4], and residual graft remaining as far as nine years post implantation [5].

The use of allograft materials for sinus augmentation is also common. These are available in a variety of forms, ranging from bone blocks to irradiated cancellous bone chips.

An increasingly popular form of allograft tissue is Demineralised Bone Matrix (DBM). Extensive processing removes the blood, cells and mineral component of the bone, leaving a collagen matrix. It is available in a wide range of forms and is often applied as a bone graft extender. Results of positive osteoinductivity assays in the rodent model with DBM are encouraging [6] with evidence for the presence of bone morphogenetic proteins (BMP) in the demineralised matrix [7]. However, similar activity in humans is yet to be established and there are reported variations in osteoinductivity [8 to 11] and the levels of BMP [12] in the products available.

Synthetic graft materials have grown in popularity in recent years due to the twofold advantage of eliminating both donor site morbidity and the risks of disease transmission present with human [13] and animal tissue [14].

A number of synthetic graft materials are available for sinus augmentation, some number of which are based on beta-tricalcium phosphate (ß-TCP). ß-TCP offers the significant benefit of total resorption and associated replacement by newly forming bone tissue through the mechanism of osteoconduction [15]. This material has an established research pedigree [16], and has been used with some success in a granular form [17 and 18].

Granular ß-TCP does have some inherent disadvantages as a graft material for sinus elevation. As a result of pressure from the sinus cavity, some movement of the graft material can occur. In addition, a membrane is required over the window into the sinus cavity to prevent soft tissue infiltration, which can be problematic to place and present an additional step to the surgical procedure.


*Fig. 1 Synthetic composite of ß-TCP and calcium sulphate (Fortoss Vital, Biocomposites, UK).*

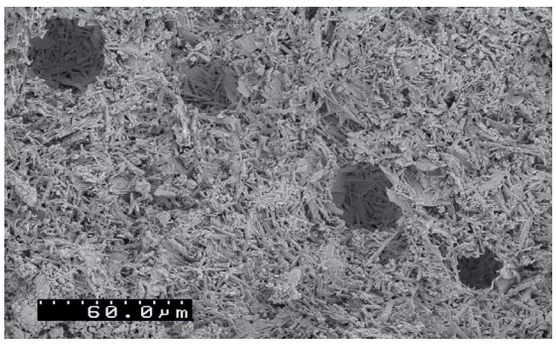


*Fig. 3 Scanning Electron Micrograph (Scale x 500) of the surface of the graft material when set, forming a cell occlusive membrane preventing epithelial ingrowth.*

*Fig. 2 The synthetic graft is prepared as a paste that can be implanted at the surgical site.*

An alternative synthetic graft material is available which possesses qualities that offer advantages in a sinus lift procedure. The material is a synthetic composite of ß-TCP and calcium sulphate (Fortoss Vital, Biocomposites, UK) (Fig. 1).

This combination could offer improved resorption and integration profile of the material, as mixing grafting materials with calcium sulfate can facilitate the process of osteogenesis and increased new bone bonding to remnants of the grafting material with which it is combined [19]. It is prepared as a setting paste, which sets hard at the surgical site (Fig. 2). As a result, the graft material is not moved by pressure from the sinus cavity and forms a cell occlusive membrane at the window in the sinus, preventing epithelial ingrowth (Fig. 3). The material is designed to be fully resorbable allowing implant placement at six months, and can also be used for indirect sinus lifts (Summer's technique [20 and 21]) and simultaneous implant and sinus graft placement.

## Case study

In this pilot study we report the comparative bone regeneration capability, using histological techniques, of this synthetic composite of tricalcium phosphate and calcium sulphate versus an irradiated cancellous human bone (Rocky Mountain Tissue Bank, US), when used in a bilateral sinus graft procedure.

A male smoker, aged 53, presented with pneumatised sinuses (Fig. 4). A bilateral sinus graft was performed using the modified Caldwell-Luc technique. Irradiated cancellous human bone from a commercial bone bank was placed in the right hand sinus, and the synthetic composite was placed in the left hand sinus. Healing was uneventful in both sides.

The patient was assessed at six months post surgery (Fig. 5). X-ray results indicated suitable bone augmentation for the proposed implantation of

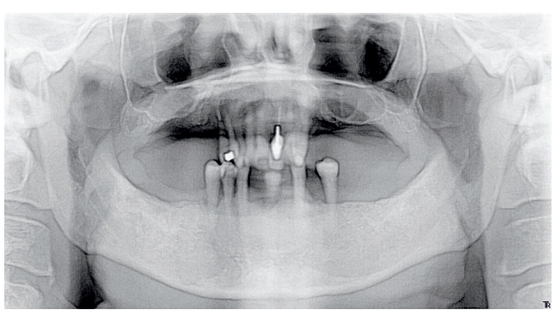

*Fig. 4 X-ray prior to graft placement, with pneumatised sinuses evident.*

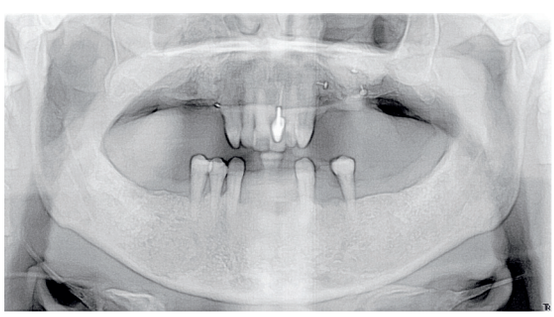

*Fig. 5 X-ray at six months post graft placement.*

## Synthetic Graft Site

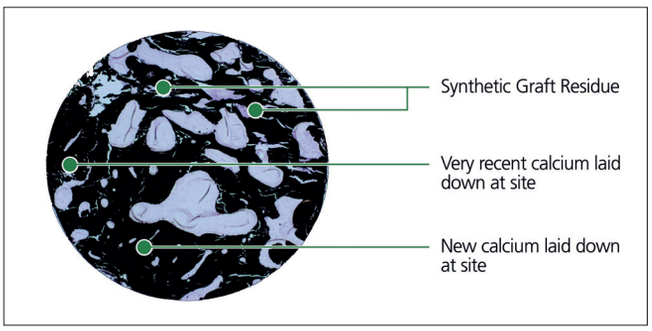


*Fig. 6 Synthetic composite site. Core section. Von Kossa staining (Calcium staining).*

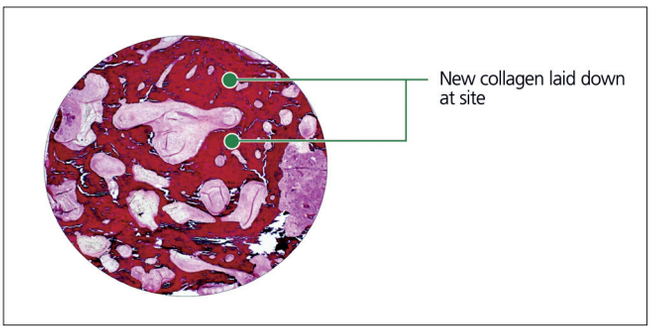


*Fig. 7 Synthetic composite site. Core section. Van Gieson staining (Collagen staining).*

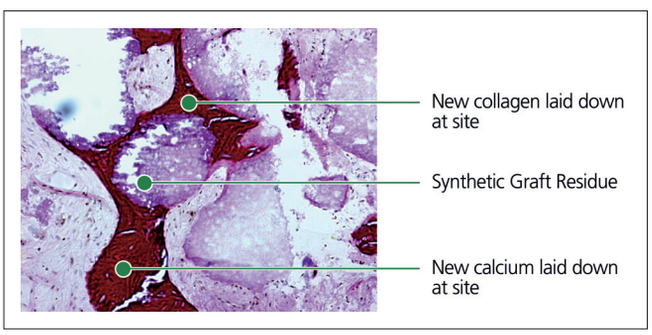


*Fig. 8 Synthetic composite site. Core section. x 10 magnification. Van Gieson staining (Collagen staining).*

## Human Bone Graft Site

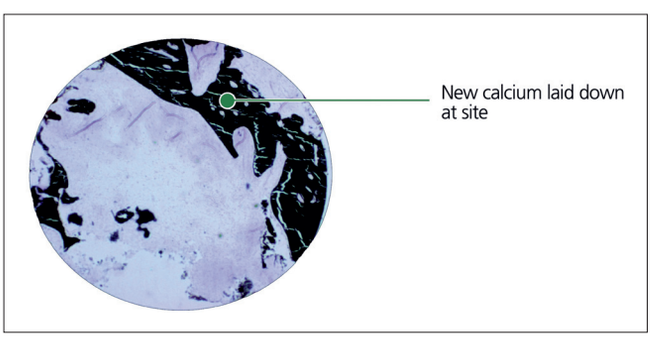


*Fig. 9 Cancellous graft site. Core section. Von Kossa staining (Calcium staining).*

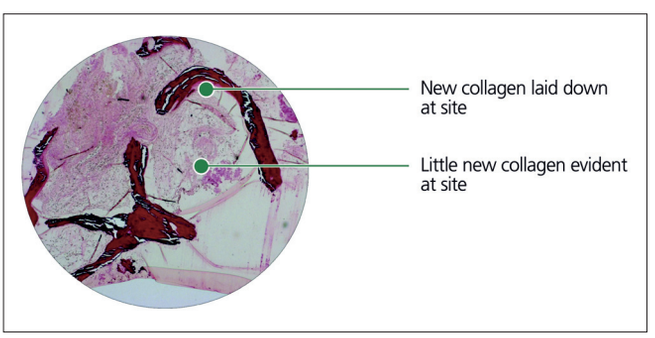


*Fig. 10 Cancellous graft site. Core section. Van Gieson staining (Collagen staining).*

three implants placed on each side. At the time of implant placement, core biopsy samples were taken, one into each site of augmentation material implantation. The diameter of core samples taken was 3 mm.

On removal of the core biopsies, it was noted that the core from the right hand sinus (human bone graft site) was soft and broke into two fragments on removal from the core drill, whilst the left hand sinus core (synthetic graft site) exhibited firm integrity. This initial observation suggested a difference between the quality of bone generated in the bilateral sites.

The core biopsy samples were immediately fixed in formalin, and then processed for histological evaluation by embedding in glycol methacrylate with subsequent 4 µm thick sectioning. Tissue sections were tincturally stained using Von Kossa and Van Gieson.

Von Kossa staining is routinely used to characterize biological mineralisation, by staining the calcium mineral component dark brown. For this histological examination, the biopsy sample was also treated using Van Gieson staining which stains the collagen component of bone with a red colouration. When used in combination, these two techniques indicate the quantity of bone regenerated at the sinus graft sites.

The biopsy from the site implanted with synthetic composite material demonstrated the presence of bone in close apposition, or completely surrounding the residues of the graft material (Von Kossa-stained dark brown). The new bone present at the site was mineralised (Von Kossa-stained brown) and contained collagen (Van Gieson-stained red), which histopathologically appeared to be normal healthy bone; equivalent to bone in the upper sinus (Figs. 6 to 8).

The biopsy from the site implanted with irradiated bone demonstrated little new bone generation, indicated by small amounts of mineralisation and collagen tissue (Figs. 9 and 10).

In this challenging case, the synthetic graft material proved more effective for bone generation in the sinus graft procedure than the irradiated human cancellous bone. The use of the synthetic composite material as an effective alternative to allograft tissue warrants further application specific study. ■

## CONTACT ADDRESSES:

Anthony McGee BDS
Fairfield Dental Surgery,
10 Station Path, Staines, Middlesex TW18 4LW
ENGLAND
Phone: +44 1784 458425
Fax: +44 1784 881860
fatdent@btopenworld.com

Dr J. A. Hunt
UK Centre for Tissue Engineering,
UKBioTEC Laboratories, Department of Clinical Engineering, University of Liverpool,
Duncan Building, Daulby Street, Liverpool L69 3GA
ENGLAND
Phone: +44 151 706 5264
Fax: +44 151 706 5803
huntja@liv.ac.uk

## REFERENCES

[1] Joshi, A. :An investigation of post-operative morbidity following chin graft surgery. Br Dent J. 2004 Feb 28; 196(4): 215-8.

[2] Silva, F.M., Cortez, A.L., Moreira, R.W., Mazzonetto, R. :Complications of intraoral donor sites for bone grafting prior to implant placement. Implant Dent. 2006 Dec; 15(4): 420-6.

[3] Schlegel, K.A., Fichtner, G., Schultze-Mosgau, S., Wiltfang, J. :Histologic Findings in Sinus Augmentation with Autogenous Bone Chips Versus a Bovine Bone Substitute. Int J Oral Maxillofac Implants, 2003;18:53–58.

[4] Lee, Y.M., Shin, S.Y., Kim, J.Y., Kye, S.B., Ku, Y., Rhyu, I.C. :Bone reaction to bovine hydroxyapatite for maxillary sinus floor augmentation: histologic results in humans. Int J Periodontics Restorative Dent. 2006 Oct; 26(5): 471-81.

[5] Traini, T., Valentini, P., Lezzi, G., Piattelli, A. :A Histologic and Histomorphometric Evaluation of Anorganic Bovine Bone Retrieved 9 Years After a Sinus Augmentation Procedure. J Periodont. 2007 May; 78 (5): 955-961.

[6] Edwards, J.T., Diegmann, M.H., Scarborough, N.L. :Osteoinduction of human demineralised bone: characterization in a rat model. Clin Orthop Relat Res. 1998 Dec; (357):219-28.

[7] Pietrzak, W.S., Woodell-May, J., McDonald, N. :Assay of bone morphogenetic protein-2, -4, and -7 in human demineralized bone matrix. J Craniofac Surg. 2006 Jan;17(1):84-90.

[8] Takiwawa, S., Bauer, T.W., Kambic, H., Togawa, D. :Comparative evaluation of the osteoinductivity of two formulations of human demineralised bone matrix. J Biomed Mater Res A. 2003 Apr 1;65(1):37-42.

[9] Peterson, B., Whang, P.G., Iglesias, R., Wang, J.C., Lieberman, J.R. : Osteoinductivity of commercially available demineralised bone matrix. Preparations in a spine fusion model. J Bone Joint Surg Am. 2004 Oct;86-A (10): 2243-50.

[10] Lee, Y.P., Jo, M., Luna, M., Chien, B., Lieberman, J.R., Wang, J.C. :The efficacy of different commercially available demineralised bone matrix substances in an athymic rat model. J Spinal Discord Tech. 2005 Oct;18(5):439-44.

[11] Wang, J.C., Alanay, A., Mark, D., Kanim, L.E., Campbell, P.A., Dawson, E.G., Lieberman, J.R. :A comparison of commercially available demineralized bone matrix for spinal fusion. Eur Spine J. 2007 Jan 5; [Epub ahead of print] .

[12] Bae, H.W., Zhao, L., Kanim, L.E., Wong, P., Delamarter, R.B., Dawson, E.G. :Intervariability and intravariability of bone morphogenetic proteins in commercially available demineralized bone matrix products. Spine. 2006 May 20;31(12):1299-306; discussion 1307-8.

[13] Chapman, L.E. :Xenotransplantation: public health risks - patient vs. society in an emerging field. Curr Top Microbiol Immunol. 2003;278:23-45.

[14] Wang, S., Zinderman, C., Wise, R., Braun, M. :Infections and human tissue transplants: review of FDA MedWatch reports 2001-2004. Cell Tissue Bank. 2007 Feb 3; [Epub ahead of print].

[15] Frayssinet, P., Trouillet, J.L., Rouquet, N., Azimus, E., Autefage, A. : Osseointegration of macroporous calcium phosphate ceramics having a different chemical composition. Biomaterials 1993;14(6): 423-9.

[16] Rejda, B., Peelen, J., De Groot, K. :Tricalcium phosphate as a bone substitute. J.Bioeng 1997 1: 93-101.

[17] Horch, H.H., Sader, R., Pautke, C., Neff, A., Deppe, H., Kolk, A. :Synthetic, pure-phase beta-tricalcium phosphate ceramic granules (Cerasorb) for bone regeneration in the reconstructive surgery of the jaws. Int J Oral Maxillofac Surg. 2006 Aug;35(8):708-13. Epub 2006 May 9.

[18] Zerbo, I.R., Zijderveld, S.A., de Boer, A., Bronckers, A.L., de Lange, G., ten Bruggenkate, C.M., Burger, E.H. :Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: a prospective study. Clin Oral Implants Res. 2004 Dec;15(6):724-32.

[19] Al Ruhaimi, K.A. :Effect of Adding Resorbable Calcium Sulfate to Grafting Materials on Early Bone Regeneration in Osseous Defects in Rabbits. Int J Oral Maxillofac Implants 2000;15:859-864.

[20] Summers, R.B. :A new concept in maxillary implant surgery: the osteotome technique. Compendium. 1994 Feb;15(2):152, 154-6, 158 passim.

[21] Summers, R.B. :The osteotome technique: Part 4--Future site development. Compend Contin Educ Dent. 1995 Nov;16(11):1080, 1092 passim.