

Evaluation of demineralised, freeze-dried, irradiated bone allografts in the treatment of osseous defects in the oral cavity

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Abstract Demineralised, freeze-dried bone allografts (DFDBA) have been used extensively by dentists in the treatment of periodontal and periapical osseous defects resulting from inflammatory diseases. Their use in India however, is limited by the availability of quality allografts and the high cost of imported alternatives. A study was conducted to assess the osteogenic potential of DFDBA prepared for the first time in India by the Tata Memorial Hospital (TMH) Tissue Bank. The DFDBA was used in the treatment of osseous defects after removal of periapical lesions associated with devitalised teeth in 10 healthy patients. At the 6-month recall visit all the patients showed a remarkable decrease in the grades of mobility, and 9 out of the 10 patients showed radiographic evidence of complete healing of the osseous defects with evidence of normal bony trabeculae.

These findings indicate that the indigenously prepared DFDBA is a cost effective, biocompatible material with osteogenic potential that can be used effectively in treating osseous defects of periapical lesions associated with non vital teeth.

Keywords Bone mineralisation · Demineralised freeze-dried bone allografts · Osteogenic potential · Periapical osseous defects · Tissue bank

Introduction

The efficacy of autogenous bone in the reconstruction of osseous defects in the jaw is well known. Being both osteoconductive and osteoinductive, healing with autograft is rapid with little or no inflammatory reactions. The procurement of the autograft however, requires an additional incision in the oral cavity, and in large and multiple defects, due to the unavailability of a sufficient quantity of bone from intraoral sites, grafts are frequently recovered from the iliac crest with the additional risk of donor site morbidity.

In the past few decades the establishment of well regulated tissue banks has enabled dental surgeons to avoid the trauma of autograft retrieval by using allograft bone materials to fill a variety of jaw bone defects (Libin et al. 1975; Marble 1968; Pearson et al. 1981; Quintero et al. 1982, Rummelhart

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et al. 1989). Freeze Dried Bone Allograft (FDBA) (Burwell 1996; Carr and Hyatt 1955; Chalmers 1950; Francis et al. 1995; Heiple et al. 1963; Kreuz et al. 1951; Lane et al. 1972; Pappas and Beisaw 1968) and Demineralised Freeze Dried Bone Allograft (DFDBA) have been successfully used to generate bone (Francis et al. 1995; Marble 1968; Pearson et al. 1981; Quintero et al. 1982; Rummelhart et al. 1989; Urist 1965).

However, while both provide an osteoconductive scaffold DFDBA also provides the osteoinductive factor (Pearson et al. 1981; Quintero et al. 1982), Bone Morphogenic Protein (BMP), a hydrophobic glycoprotein within the bone matrix which induces the differentiation of host mesenchymal cells into osteoblasts (Urist and Strates 1971).

Despite the proved efficacy of DFDBA its use in India has been limited by its lack of availability and the relatively high cost of imported grafts. In an attempt to fill this lacuna the Tissue Bank at the Tata Memorial Hospital started producing DFDBA, and in this paper we report the evaluation of this indigenously prepared bone material in the repair of human periapical osseous defects associated with devitalised teeth.

Materials and methods

All the patients in this study reported to the Dental OPD of the King Edward Memorial Hospital, a Municipal Hospital in the city of Mumbai. Ten patients (9 males and 1 female) with periapical osseous defects, between the ages of 22 and 46 years, the mean age being 35 years, were selected and monitored clinically and radiographically to assess the effectiveness of DFDBA in the healing of these defects.

All patients were in good health and an informed written consent for the use of human allograft tissue and the procedure was obtained. Of the 10 patients 6 had periapical pathological lesions in relation to maxillary anterior teeth and the remaining showed periapical pathologies of mandibular anterior teeth (Table 1). All the involved teeth failed to respond to stimulation by the electric pulp tester and appeared discoloured.

All patients were evaluated based on the following clinical and radiological parameters: mobility of the involved teeth was assessed and accordingly graded as under (Carranza and Newman 1996):

Grade I: Slightly more than normal

Grade II: Moderately more than normal

Grade III: Severe mobility faciolingually and/or mesiodistally, combined with vertical displacement

Pre-operative and post-operative intraoral periapical radiographs (IOPA) were assessed for osseous fill of the radiolucent periapical defects.

Teeth that exhibited acute or subacute clinical exacerbation of a periapical inflammation were not operated upon until all acute symptoms had subsided. The initial management in all cases included opening of root canals of the involved teeth and the establishment of drainage. The patients were administered oral Penicillin along with anti-inflammatory drugs and analgesics.

Demineralised freeze-dried bone allograft

DFDBA 500–1040 μ in size was obtained from the TMH Tissue Bank. The procurement, processing and sterilization of the bone grafts were done in compliance with the International Atomic Energy Agency (IAEA) Standards for Tissue Banking and have been described previously (Lobo Gajiwala 2003). The bone was obtained from the femoral heads of healthy donors (serologically negative for Hepatitis B and C, HIV and Syphilis) undergoing hip replacement surgery after obtaining the necessary consent.

The donated bones were washed well under bio-filtered water and pasteurised at 60°C for 3 h in a waterbath shaker. They were then cleaned of all soft tissue and cut into smaller sizes to increase the efficiency of the subsequent cleaning and defatting processes. The bone pieces were washed free of blood and bone marrow using jet lavage, defatted with 70% ethanol, washed thoroughly, freeze-dried to remove 95% of the moisture and subsequently morsellised. The ground bone was sieved in the laminar airflow cabinet using standards sieves to give particle sizes ranging from 250 to 1,000 μ . The processed, lyophilised bone

Table 1 Clinical presentation of cases

Patient	Site	No. of teeth involved	Causative factor	Presenting symptoms
1	Maxillary anterior region (left)	3	Trauma (external)	Pain, palatal swelling and pus discharge
2	Maxillary anterior region(left)	2	Residual Cyst	Pain and labial swelling
3	Mandibular anterior region(midline)	2	Trauma (external)	Intra oral draining sinus
4	Mandibular anterior region (left)	3	Trauma (form occlusion)	Severe attrition and intra oral draining sinus
5	Mandibular anterior region (right)	3	Trauma (external)	Extra oral draining sinus
6	Maxillary anterior region (left)	2	Trauma (external)	Pain and extra oral swelling
7	Maxillary anterior region (midline)	2	Trauma (external)	Asymptomatic (incidental radiographic finding)
8	Maxillary anterior region (left)	2	Trauma (external)	Draining through labial gingival sulcus
9	Maxillary anterior region (left)	1	Trauma (external)	Intra oral draining sinus
10	Maxillary anterior region (left)	2	Trauma (external)	Intra oral draining sinus

particles were demineralised with 0.6 N hydrochloric acid and then thoroughly washed with neutral buffered solution till the pH was neutralised. The bone particles were recovered by centrifugation, frozen and freeze-dried again. The DFDBA was distributed in 0.5 cc quantities in polyethylene packets, appropriately labelled, and double packed in the laminar airflow cabinet. Validation studies were done to ensure a bio-burden of less than 1000 c.f.u. per packet. The grafts were terminally sterilised with 25 kGy of gamma radiation using a Gamma Chamber 1200 with a Cobalt⁶⁰ source.

Clinical procedure

A one stage periapical curettage and apicoectomy procedure was performed under nerve block and infiltration anaesthesia. The contents of the periapical areas proved microscopically to be radicular cysts or periapical granulomas. After thorough debridement of the periapical bony lesion the DFDBA was rehydrated with physiologic saline. The reconstituted graft was then gently packed into the cavity with no effort made to over pack the defect. Maximum wound closure was attempted, replacing the mucogingival tissue flaps to their original positions. After placing 4–0 silk sutures, the patients received post-operative instructions. At 1-week post-surgery patients returned for suture removal and another introral periapical radiograph was obtained of each defect

to document graft retention. The patients were then recalled at intervals of 1, 3, and 6 months for evaluation of tissue response to the therapy and IOPA radiographs were taken at each recall visit.

Observation and results

All the patients responded well to the therapy and no evidence of post-operative infection and signs of immunologic rejection were seen. No post-operative sequestration of graft material was observed at any of the recall visits. After institution of the therapy all draining sinuses healed completely with no evidence of pus discharge.

At the 1-month recall visit one of the patients reported with recession of gingival margin at the site of the crevicular incision with exposure of the root surface of one of the involved teeth. Desquamative changes on the gingival surface were observed in another patient in the first post-operative week. The patient responded well to local treatment and oral hygiene maintenance.

Clinically all patients showed remarkable decrease in the grades of mobility at the end of the 6-month period (Table 2). No recurrence of any pre-operative symptoms was seen in any of the patients at the end of 6 months. Radiographically the initial radiolucent appearance was replaced by mild radio-opacity at the end of 1-month. At the end of the 3-month recall visit 9 out of the 10 cases showed more than 50% mineralization of

Table 2 Clinical results

Patient	Post-operative radiographic osseous repair		Post-operative wound healing			Mobility	
	>50%	<50%	Normal	Delayed	Infection	Pre-operative	Post-operative
1	Yes	–	–	Delayed	Nil	No mobility	No mobility
2	Yes	–	Normal	–	Nil	–	–
3	–	–	Normal	–	Nil	Grade II	Grade I
4	Yes	Yes	–	Delayed	Desquamative changes in gingiva	Grade III	No mobility
5	Yes	–	Normal	–	Nil	No mobility	No mobility
6	Yes	–	Normal	–	Nil	No mobility	No mobility
7	Yes	–	Normal	–	Nil	Grade II	No mobility
8	Yes	–	Normal	–	Nil	Grade I	No mobility
9	Yes	–	Normal	–	Nil	No mobility	No mobility
10	Yes	–	Normal	–	Nil	Grade II	No mobility

the periapical defects. One patient showed less than 50% mineralization. However, there was a definite reduction in the size of the defect in comparison to the pre-operative radiographs in all the patients. At the end of 6 months, 9 out of the 10 patients showed radiographic evidence of complete healing of the osseous defects with evidence of normal bony trabeculae. The patient with less than 50% osseous fill at the end of 3 months showed no radiographic improvement at the end of 6 months. Clinically the patient was asymptomatic. Root resorption and ankylosis were not observed in any of the patients.

Illustrative case report

Case 1

A 45-year-old male presented with a complaint of swelling in relation to the maxillary left front teeth. The patient had a history of being operated in the same region 6 years ago for a cystic lesion. No details of the previous surgery were available. His medical history was unremarkable.

Clinical examination revealed a soft, fluctuant swelling in the labial vestibule of anterior left maxilla (Fig. 1A). The left lateral incisor (22) (Federation Dentaire Internationale Nomenclature) was missing and a fixed partial denture was present. The pre-operative IOPA radiograph showed a well defined radiolucent lesion in the alveolar bone between the central incisor (21)

and canine (23) teeth (Fig. 1E). A diagnosis of a residual cyst was made and the lesion was completely enucleated under local anaesthesia. The resultant defect (Fig. 1B) was packed with DFDBA particles (Fig. 1C).

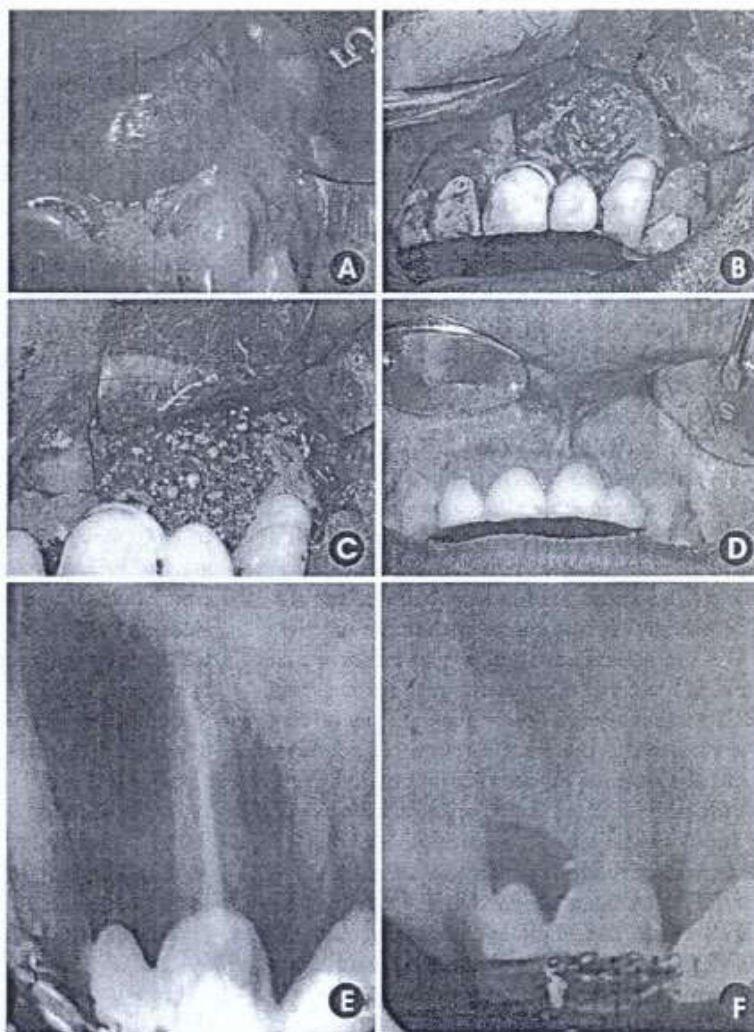
At the 3 months post-operative recall visit there was no clinical recurrence of the cystic lesion (Fig. 1D) and the IOPA radiograph showed complete ossification of the defect (Fig. 1F).

Discussion

Deminerallised freeze-dried bone allograft (DFDBA) is widely used in periodontal therapy (Libin et al. 1975; Piattelli et al. 1996; Rummelhart et al. 1989) and has been demonstrated to be safe and capable of inducing new bone formation (Mellonig et al. 2001). Several reports have indicated however, that a wide variability exists in the ability of commercial preparations of DFDBA to induce new bone (Jergesen et al. 1991; Schwartz et al. 1996, Zhang et al. 1997b). The present study evaluated the efficacy of indigenously produced DFDBA in the treatment of osseous defects after removal of periapical lesions associated with non-vital teeth.

The DFDBA was issued by the TMH Tissue Bank. The grafts were found to be well tolerated by all patients in this study with no noticeable immunologic complication, graft rejection or infection. The clinical response to treatment was favourable, with almost all patients showing near

Fig. 1 (A) Preoperative swelling in anterior left maxillary region. (B) Cystic lesion after reflection of mucoperiosteal flap. (C) Osseous defect packed with DFDBA following cyst enucleation. (D) 3 months post-operative clinical presentation. (E) Pre-operative IOPA radiograph showing radiolucent defect between 21 and 23. (F) 3 months post-operative IOPA radiograph showing complete ossification



complete ossification of the periapical defect. Only one patient showed less than 50% osseous fill at the end of the 6 months post-operative visit. He was a case of bruxism, and the decreased osseous fill could be attributed to the persistent trauma from occlusion due his clinical condition. The clinical and radiological results were comparable to those of Saad and Abdellatief who used FDDBA in similar defects (Saad and Abdellatief 1991).

The graft donors in our study ranged in age from 31 to 90 years and included both male and female donors. According to some recent studies donor age and gender may play an important role in inducing bone formation (Jergesen et al. 1991)

with grafts from female donors in the 0–25 years age group demonstrating the maximum BMP-4 levels, and grafts from male donors over 26 years possessing more BMP-4 than those from female donors in the same age group (Honsawek et al. 2005). The sample population in our study was too small to assess the role of graft donor age and gender in new bone formation.

Studies have shown that DFDBA derived from a cortical source may have greater resident osteogenic potential than a similar preparation derived from a cancellous bone as it contains more collagenous matrix than cancellous bone (Urist et al. 1970). The DFDBA in the present study was

obtained from femoral heads, and thus were cortico-cancellous in nature similar to the autogenous iliac crest or mandibular symphysis bone grafts routinely used. While autogenous bone grafts are the gold standard for reconstruction of osseous defects, the disadvantages of limited quantities of autografts from intraoral sites, donor site morbidity and possible hospitalisation were obviated using the allografts.

Bone particle size is another factor to be considered in the use of DFDBA. Bone particle size in the range of 550–710 μ provided for maximum osteoinductive potential in *in vitro* and *in vivo* bioassays (Rummelhart et al. 1989; Zhang et al. 1997a). The particle size of DFDBA used in our patients ranged from 500 to 1040 μ . This particle size permitted easy clinical handling and avoided the macrophage response and resorption elicited by particle sizes of less than 125 μ (James et al. 1976). At the same time the problem of sequestration usually encountered with large particle size was not observed. Studies also indicate that implanted bone chips by virtue of their mass and surface area may serve to prevent the overlying soft tissues of the mucoperiosteum from collapsing into the osseous defect. The implanted particles thus provide a potential space within which new bone can develop (Boyne et al. 1961).

Osteoinductive performance of DFDBA has been shown to be influenced by the processing methods used. These include the choice of demineralising agent (Boyce et al. 1999) and the use of sonication and chelating agents both of which are detrimental to DFDBA performance (Urist et al. 1968). The present study used hydrochloric acid (0.5–0.6 M), the most commonly used demineralising agent. (Boyce et al. 1999). Initial processing included treatment with ethanol to remove fat, a step which may enhance osteoinductivity (Harakas 1984; Urist et al. 1967).

Demineralization time, which affects final calcium content, is another variable to control in DFDBA processing (Glowacki et al. 1984; Guo et al. 1991; Hosny et al. 1985). It is generally accepted that bone morphogenetic proteins (BMPs) present in DFDBA are responsible for the induction of new bone. BMP-4 (Honsawek et al. 2005; Shigeyama et al. 1995), BMP-2 and BMP-7 have all been demonstrated in the extracts of

DFDBA, and shown to be effective osteoinductive proteins (Wozney and Rosen 1998). The demineralization process exposes the BMPs which are trapped by minerals, and the amount of BMP exposed is proportional to the degree of demineralization (Honsawek et al. 2005). The DFDBA used in the current study was demineralised to give a residual calcium content of 5–8%. According to one study less than 40% of residual calcium is required for a strong osteoinductive response result (Guo et al. 1991). Demineralization of bone matrix to less than 2% residual calcium however, decreases its osteoinductivity (Zhang et al. 1997b), probably due to the acid lability of BMPs (Honsawek et al. 2005).

Since allograft associated disease transmission is a critical concern in clinical practice, a number of strategies were used to reduce this risk. These included processing the grafts in environmentally controlled rooms, thorough debridement and cleansing of the bone, pasteurisation, use of treatment solutions (alcohol) and quality control using the ISO 9001:2000 Quality Management System. The use of a demineralising acid further eliminated the risk of viral (Hepatitis B and C virus, and HIV) contamination (Boyce et al. 1999), a primary clinical concern because of the lethal nature of the diseases. Terminal sterilisation with 25 kGy of gamma irradiation was employed. In some studies this dose has been shown to reduce the osteoinductive response (Boyce et al. 1999; Ijiri et al. 1994; Urist and Hernandez 1974), but in our study it did not appear to interfere with the clinical results. Lyophilisation (freeze-drying) was used to extend the graft shelf life. It also permitted the DFDBA to be available off the shelf in ready-to-use packs that could be conveniently transported and stored at room temperature.

One of the major advantages of the DFDBA used was its affordable cost. Being a municipal hospital most of the patients treated belonged to the lower economic group for whom imported DFDBA would have been out of reach. In the absence of the indigenously produced grafts routine surgery would have involved increased hospital and medical costs and risks of complications due to autograft retrieval, or unpredictable results if the lesions were allowed to heal without the use of grafts.

The present study made no attempt to distinguish between the osteoinductive and osteoconductive capacity of DFDBA. A comparative study between FDBA and DFDBA would need to be undertaken to greater appreciate this difference.

Conclusion

Indigenously prepared DFDBA obtained from the Tata Memorial Hospital Tissue Bank is a cost effective, biocompatible material of osteogenic potential and can be used effectively in treating osseous defects after removal of periapical lesions associated with devitalised teeth.

References

- Burwell RG (1996) Studies in the transplantation of bone. *J Bone Joint Surg* 48B:532
- Boyce T, Edwards J, Scarborough N (1999) Allograft bone – The influence of processing on safety and performance. *Orthop Clin N Am* 30(4):571–581
- Boyne PJ, Lyon HW, Captain DC et al (1961) The effects of osseous implant materials on regeneration of alveolar cortex. *Oral Surg Oral Med Oral Pathol* 14 (3):369–378
- Carr CR, Hyatt GW (1955) Clinical evaluation of freeze-dried bone grafts. *J Bone Joint Surg* 37A:549
- Carranza SA, Newman MJ (1996) *Clinical Periodontology*, 8th edn. Harcourt Brace Company, Singapore, p 349
- Chalmers J (1950) Transplantation immunity in bone homografting. *J Bone Joint Surg* 41-B:160
- Francis J, Brunsvold M, Mellonig J (1995) Clinical evaluation of an allogenic bone matrix in the treatment of periodontal osseous defects. *J Periodontol* 66:1074–1079
- Glowacki J, Kaban LB, Sonis ST (1984) Physiological aspects of bone repair using demineralised bone. In: Hurt TK, Heppinstall RB et al (eds) *Soft and hard tissue repair*. Praeger, New York pp 265–280
- Guo MZ, Xia ZS, Lin LB (1991) The mechanical and biological properties of demineralised cortical bone allografts in animals. *J Bone Joint Surg Br* 73:791–794
- Harakas NK (1984) Demineralized bone matrix induced osteogenesis. *Clin Orthop* 188:239–251
- Heiple KG, Chase SW, Hernden CH (1963) A comparative study of the healing process following different types of bone transplantation. *J Bone Joint Surg* 45A:1593
- Honsawek S, Powers RM, Wolfenbarger L (2005) Extractable bone morphogenetic protein and correlation with induced new bone formation in an in vivo assay in the athymic mouse model. *Cell Tissue Bank* 6:13–23
- Hosny M, Sharawy M (1985) Osteoinduction in rhesus monkeys using demineralised bone powder allografts. *J Oral Maxillofac Surg* 43:837–844
- Ijiri S, Yamamuro T, Nakamura T et al (1994) Effect of sterilization on bone morphogenetic protein. *J Orthop Res* 12:628–636
- James TM, Gerald MB, Robert WB, Joseph JL (1976) Clinical evaluation of freeze dried Bone allografts in Periodontal Osseous defects. *J Periodontol* 47(3):125–131
- Jergesen HE, Chua J, Kao RT et al (1991) Age effects on Bone Induction by demineralised bone powder. *Clin Orthop* 268:253–259
- Kreuz FB, Hyatt GW, Turner TC (1951) Preservation and Clinical use of freeze dried bone. *J Bone Joint Surg* 33A:863
- Lane SW, Guggenheim G, Egyedi P (1972) Comparison of homologous freeze dried and fresh autogenous bone grafts in the monkey mandible. *J Oral Surg* 30:649
- Libin BM, Ward HL, Fisherman L (1975) Decalcified, lyophilized bone allografts for use in human periodontal defects. *J Periodontol* 46:51–56
- Lobo Gajiwala A (2003) Tissue Banking in India: Gamma-irradiated allografts. *Cell and Tissue Banking* 4(2–4): 203–211
- Marble HB (1968) Homografts of freeze dried bone in cystic defects of the jaws. *Oral Surg* 26:118
- Mellonig JT et al (2001) Tissue banking of bone allografts used in periodontal regeneration. *J Periodontol* 72:834–838
- Pappas AM, Beisaw NE (1968) Bone Transplantation, correlation of physical and histologic aspects of graft incorporation. *Clin Orthop* 61:79
- Pearson GE, Rosen S, Deporter PA (1981) Preliminary observations on the usefulness of a decalcified, freeze dried cancellous bone allograft material in periodontal surgery. *J Periodontol* 52:55
- Piattelli JD, Scarano A, Corigliano M, Piattelli M (1996) Comparison of bone regeneration with use of mineralized and demineralised freeze dried bone allografts. A histological and histochemical study in man. *Biomaterials* 17:1127–1131
- Quintero G, Mellonig JT, Gambill VM (1982) Clinical evaluation of decalcified freeze dried bone allografts in periodontal osseous defects. *J Periodontol* 53:726
- Rummelhart JM, Mellonig JT, Gray JL, Towle HJ (1989) Comparison of freeze dried bone allografts and demineralised freeze dried bone allografts in human periodontal osseous defects. *J Periodontol* 60:655–663
- Saad YA, Abdellatif EM (1991) Healing assessment of osseous defects of periapical lesions associated with failed endodontically treated teeth with use of freeze-dried bone allograft. *Oral Surg Oral Med Oral Pathol* 71:612–617
- Schwartz Z, Mellonig JT, Carnes DL et al (1996) Ability of commercial demineralised freeze dried bone allograft to induce new bone formation. *J Periodontol* 67:918–926
- Shigeyama Y, D'Errico JA, Stone R, Somerman MJ (1995) Commercially-prepared allograft material has biological activity in vitro. *J Periodontol* 66:478–487
- Urist MR (1965) Formation by autoinduction. *Science* 150(698):893–899

- Urist MR, Dowell TA, Hay PH et al (1968) Inductive substrates for bone formation. *Clin Orthop* 59:59–96
- Urist MR, Hernandez A (1974) Excitation transfer in bone: deleterious effects of cobalt 60 radiation sterilization of bone bank. *Arch Surg* 109:486–493
- Urist MR, Jurist JM, Dubuc FL, Strates BS (1970) Quantitation of new bone formation in intramuscular implants of bone matrix. *Clin Orthop* 68:279
- Urist MR, Silverman BF, Buring K et al (1967) The bone induction principle. *Clin Orthop* 53:243–283
- Urist MR, Strates BS (1971) Bone morphogenetic protein. *J Dent Res* 50:1392
- Wozney JM, Rosen V (1998) Bone morphogenetic protein and bone morphogenetic protein gene family in bone formation and repair. *Clin Orthop* 346:26–37
- Zhang M, Powers RM, Wolfinbarger L (1997a) A quantitative assessment of osteoinductivity of human demineralised bone matrix. *J Periodontol* 68(11):1076–1084
- Zhang M, Powers RM, Wolfinbarger L (1997b) Effect(s) of the demineralization process on the osteoinductivity of demineralised bone matrix. *J Periodontol* 68(11):1085–1092