Review Article

Are Bone Allografts Safe and Effective for Today's Dental Practitioner?

Brian Samsell[1], **Mark Moore**[1], **Giampietro Bertasi**[2], **Sergio Spinato**[3], **Fabio Bernardello**[4], **Alberto Rebaudi**[5], **Gian Luca Sfasciotti**[6] and **Ralph Powers**[1*]

[1]*Institute of Regenerative Medicine, LifeNet Health, 1864 Concert Drive, Virginia Beach, VA 23453, USA*

[2]*University of Padua, Padua, Italy*

[3]*Department of Biomedical and Neuromotor Sciences, School of Dentistry, Unit of Periodontology and Implantology, University of Bologna, Bologna, Italy*

[4]*Private Practice, Terranegra di Legnago, Verona, Italy*

[5]*Private Practice,Genoa, Italy*

[6]*Department of Dentistry and Maxillo-facial Sciences, Sapienza University of Rome, Rome, Italy*

*Corresponding author: Ralph Powers, DDS, Institute of Regenerative Medicine, LifeNet Health, 1864 Concert Drive, Virginia Beach, VA 23453, USA, Tel: 1-757-609-4533; E-mail: ralph_powers@lifenethealth.org

Abstract

A wide variety of dental procedures, including ridge and sinus augmentation, treatment of bony defects, and extraction socket preservation, may require a bone grafting material. To meet this need, there are many choices available including alloplasts, xenografts, autografts, and allografts. In particular, allografts, being a natural, human biological matrix and readily available have proven clinically reliable. However, not all allografts are equal in terms of processing, sterility, and proven clinical performance for dental applications. Here, we review the use of disinfected and terminally sterilized bone allografts for dental applications.

Keywords: Allograft; Defect; Preservation; Implant; Augmentation; Bone regeneration; OraGraft

Introduction

Many dental procedures, including ridge augmentation, implant placement, or intrabony periodontal defect treatment, require the growth of new bone to be successful. Growth of new bone has become a critical element for procedures performed by the modern dental specialist. New bone growth is facilitated by osteogenesis, osteoinduction, or osteoconduction [1]. Osteogenesis utilizes osteoblasts to generate new bone. Osteoinduction involves signaling molecules or growth factors, inducing local cells into osteoblastic or odontoblastic activity. Osteoconduction is a process whereby a graft acts as a scaffold for new bone formation, but requires the presence of bone forming cells, typically from the host. When using common bone void fillers, it is important to be aware of the method of bone growth when comparing the four major graft types: alloplastic, xenogeneic, autogenous, and allogeneic grafts. Alloplasts, commonly made from hydroxyapatite (HA)or β-tricalcium phosphate (β –TCP), provide an osteoconductive scaffold. They lack both osteogenenic and osteoinductive properties, so they are often supplemented with autograft bone, bone marrow aspirate (BMA), or bone morphogenetic proteins (BMP) to provide better functionality [2].

Xenografts for human implantation are typically derived from bovine, porcine, equine or coralline apatite matrices. In a similar way to alloplastic grafts, xenografts may experience immunogenic reactions to the foreign substance and complications can occur [3]. In addition, xenografts often require aggressive treatment with cytotoxic chemicals, such glutaraldehyde and formaldehyde, in order to prevent immunogenic reactions [4]. These treatments can affect mechanical properties and behavior [4,5]. Finally, when using xenografts, surgeons need to be aware of religious or ethical considerations of their patients.

Autogenous grafts are taken from a donor site within the patient, often from an intra-oral site such as the ramus of the mandible, the chin, or the tuber maxillae or sometimes from an extra-oral site as the hip, tibia, rib, or calvarium for larger grafts. Favorably, autografts possess osteogenic properties and no immunogenic issues [1]; however, there are disadvantages. There can be patient pain and morbidity at the harvest site [6-10] as well as insufficient usable graft material procured due to donor site atrophy or an underestimate of availability. The associated costs and time involved with donor site surgery and resultant complications may be significant. The surgeon must spend more surgical time by first harvesting the autograft and then trimming it into the correct size for implantation, in addition to risk of donor site infection [11-13].

Allografts come from tissue recovered from qualified deceased human donors. Allograft bone used in dental applications may be demineralized, which exposes osteoinductive growth factors, thus promoting new bone formation [14]. In addition, the natural osteoconductive property of human bone facilitates the generation of more new bone formation and cellular proliferation than either xenografts or alloplasts alone [15]. Furthermore, the use of an allograft enables the surgeon to avoid the immunogenic reaction possible with alloplasts and xenografts, and also the donor site morbidity and associated complications when using autografts. These advantages position allografts as a favorable option for both surgeon and patient, although variability exists in allograft processing and sterility. Here, we review sterility considerations in choosing an allograft and report on clinical evidence using a particular manufactured bone allograft for dental applications.

Special note on allograft sterility

The possibility of disease transmission is an often cited risk of allograft use. Tissue banks accredited through the American

Association of Tissue Banks (AATB) have essentially negated this risk through stringent donor screening, recovery, and disinfection processes. However, some tissue banks, accredited or non-accredited, while using aseptic recovery and processing techniques, do not offer the additional safety margin of terminal sterilization. Allografts that are minimally processed, and then distributed frozen are at risk of transmitting viruses such as HIV and HCV [16]. To optimally eliminate bacterial agents and inactivate viruses, aseptic recovery and stringent disinfection procedures can be used in conjunction with a validated terminal sterilization technique.

Sterility is measured using a Sterility Assurance Level (SAL) as a measure of the probability of the presence of a viable microorganism. For example, a 10^{-3} SAL would mean no more than 1 in 1000 grafts would contain a viable microorganism. This 10^{-3} SAL corresponds to that assured by validated, aseptic transferring steps of already sterile product and is recommended by Food and Drug Administration (FDA) only for medical devices that do not cross the skin barrier [17]. In contrast, a 10^{-6} SAL indicates no more than 1 in 1,000,000 grafts are would contain a single viable microorganism. A SAL of at least 10^{-6} is considered sterile when achieved only when using a validated process [18] and Centers for Disease Control and Prevention (CDC) Guidelines assert implanted medical devices should be sterilized [19] to this level. This level of terminal sterilization can be achieved with alloplasts, xenografts, and allografts using gamma irradiation. A high level of sterility is expected in surgical settings for many operating room supplies, especially medical implants. However, some allografts do not achieve this level of sterility. While older studies have reported that high doses of gamma irradiation can damage tissue [20,21], proper irradiation conditions to ensure material sterility without impacting clinical performance take into account the following four criteria: target dose, dose range, temperature during irradiation, and tissue treatment prior to irradiation [22]. Keeping the graft at low temperature during irradiation minimizes the creation of free radicals that can negatively affect tissue properties [23] and should be considered a critical step. Under controlled conditions, terminal sterilization and viral inactivation can be achieved without negative impact on biomechanical properties or clinical performance of allograft tissues [20,24,25].

Thus, while many tissue banks distribute allografts for dental procedures, variability in disinfection and sterilization methods leads the clinician to consult published literature in chosing an allograft.

Properties of disinfected, terminally sterilized bone allograft for dental applications

This review will focus on highly disinfected, terminally sterilized bone allografts. In particular, OraGraft® (LifeNet Health, Virginia Beach, VA), hereafter referred to as OG bone allograft, is currently processed using a proprietary and patented method that removes greater than 99% of bone marrow and blood elements from the internal blood matrix [26]. This process utilizes detergents, isopropanol, and hydrogen peroxide to clean and disinfect the bone. In addition, the graft is subjected to a terminal sterilization process utilizing less than 2 Mrad of gamma irradiation at dry ice temperatures to render OG bone allografts sterile to a SAL of 10^{-6} [27]. It should be noted that this low dosage irradiation is sufficient to sterilize bone material if the manufacturer practices stringent processes related to the use of tissues deriving from only medically suitable and qualified donors and recovered in an aseptic environment using zone recovery techniques and then utilizing a battery of tests to detect bacteria (both aerobic and anaerobic), fungi, viruses and infectious diseases [28,29] including the use of Nucleic Acid Tests (NAT). NAT testing provides a 12 day window for HIV-1 on each donor instead of the longer 22-day window with traditional antibody tests [30]. In addition to testing, subsequent cleaning and disinfection further reduces the risk of disease transmission by removing blood and bone marrow. The terminal sterilization process is validated to ensure the microbial SAL of 10^{-6} and low dosage irradiation has demonstrated viral inactivation [25] (Table 1).

	Aseptic Processing	EtO	Gamma Irradiation	Chemical Sterilants	Sterilization Process used for OG Bone Allograft
Kills bacteria	No	√	√	√	√
Kills fungi	No	√	√	√	√
Kills spores	No	√	√	No	√
Kills viruses	No	No	√ (dose-dependent)	No	√
Removes blood and lipids	Surface Only	No	No	Surface Only	√
Preserves strength	√	√	Decreases(dose-dependent)	√	√
Preserves biocompatibility	√	√ (dose-dependent)	√	√	√

Table 1: Comparison of different allograft processing methods [29,30]

Regarding potential impact of irradiation on important allograft properties, numerous studies have found no significant difference in the biomechanical properties [31-36] or clinical outcomes [37-39] between non-irradiated allografts and properly treated irradiated allografts. Additional studies have shown that irradiated demineralized bone matrices (DBM) retain comparable levels of osteoinductivity to DBMs that were not irradiated. Of particular interest, Diziedic-Goclawska et al. [40] found DBM samples that were irradiated at room temperature were reabsorbed within five weeks while samples irradiated on dry ice had comparable results to non-irradiated samples. Wientroub and Reddi [41] noted similar osteoinductive properties of DBM between non–irradiated rat bone allografts and bone allografts irradiated at low temperatures.

Many clinicians prefer the use of a demineralized bone matrix, especially if desiring a more osteoinductive material. A bone graft can be labeled demineralized if it meets the American Association of Tissue Banks (AATB) standard definition of containing no greater than than 8% residual calcium. However, several reports indicate the optimal residual calcium range is approximately 1%-4% [42-45]. Bone containing residual calcium levels of >4% are considered under-demineralized since osteoinductive growth factors remain trapped in the matrix, muting their clinical impact. Conversely, bone containing residual calcium levels of <1% may be considered over-demineralized since osteoinductive growth factors may be either denatured by overexposure to the acidic demineralization solution or physically removed from the matrix in the solution, both conditions leading to diminished osteoinductive potential. To prepare demineralized bone matrix, OG bone allografts undergo a patented [46] technology to target an optimal calcium residual level of 1%-4%. This method utilizes controlled pulses of acid solution to carefully demineralize bone to target levels without excessive acid exposure.

Clinical studies using OG bone allografts

While achieving an adequate degree of safety through advanced processing techniques is essential, clinical efficacy must be demonstrated. The following clinical studies all utilized OG bone allografts, including those grafts terminally sterilized since 2004. Aichelmann-Reidy et al. [47] used OG demineralized freeze-dried bone allograft (DFDBA) in a 20 patient, randomized study comparing calcium sulfate (CS) and polytetraflouroeythlyene. After 6 months follow-up, the authors reported "achiev[ing] 50% or better resolution in both in 95% (18/19) of the sites" in both treatment groups and concluded with support for using CS combined with DFDBA for treating intrabony defects. Callan [48] used both DFDBA and freeze-dried fascia lata femoris to, respectively, fill and protect an osseous defect in a case series. The author recommended both allograft types after finding this technique "provide[d] an increased amount of alveolar bone for better implant placement." In a randomized, single-masked study involving 40 patients, Gurinsky et al. [49] compared enamel derived matrix (ECM) only and ECM combined with DFDBA to treat intrabony periodontal defects. They concluded both treatments significantly improved the defects with the combined DFDBA treatment "yield[ing] statistically significant improvements in bone fill, crestal resorption, and percentage of site gaining greater than 50% and 90% bone fill when compared to ECM alone." Landi and Sabatucci [50] published a technique report which described utilizing DFDBA to successfully treat defects in the mandibular ridge to prepare the location for implantation. The authors note "keratinized tissue was fully recognizable around all of the implants" at six weeks post-operative. Wood and Mealey [51] also compared the efficacy of demineralization in a randomized, comparative study involving 40 patients implanted with either DFDBA or freeze dried bone allografts (FDBA). After 19 weeks follow-up, biopsies showed patients implanted with DFDBA had significantly greater amounts of new vital bone formation (38.42%) and a lower mean percentage of residual grafts particles (8.88%) than patients in the FDBA group (24.63% and 25.42%, respectively). Numerous other clinical studies have reported using processed DFDBA for repairing periodontal intrabony defects, inducing bone regeneration around implants, and maxillary sinus augmentation [52-56].

Several more clinical studies have been published that used non-demineralized OG FDBA for similar treatments [57-66]. In a prospective study comparing allograft only with allograft and autograft combination treatment, Beitlitum et al. [62] used FDBA to augment the alveolar ridge deficiencies of 50 patients. The authors found that not only did the FDBA alone yield good clinical results but concluded "there was no added clinical effect of the application of a layer of autogenous bone," indicating the autograft treatment was essentially equivalent to the allograft treatment alone. Kassolis et al. [58] concluded their study of 15 patients with support for using FDBA for maxillary sinus grafting noting that "FDBA in combination with PRP [platelet-rich plasma] provides a viable therapeutic alternative for implant site preparation." In a study designed to test for donor-specific HLA antibodies, Quattlebaum et al. [57] used FDBA to treat periodontal osseous defects in 20 patients. The authors were unable to detect any antibodies at intervals over a 3 month time period. Schwartz et al. [59] reported "excellent bone fill of the osseous defect" at six month follow-up in a case report where FDBA was mixed with an enamel matrix derivative to fill a palatal bony defect located on the maxillary incisor. In a recent case series, Spinato and Galindo-Moreno [66] saw "good preservation of soft and hard tissue architecture" after a one year follow-up in eight patients who were treated with a mixture of FDBA and DFDBA to prepare the maxillary extraction site for implant placement. Vidal et al. [63] reported a 100% success rate of implant placement (defined as grade 3 or >1 mm of bone loss) after one year follow-up in a study consisting of 51 patients who had immediate implant placements. While the total number of patients who received FDBA was not specified, FDBA along with a collagen membrane was grafted onto sockets that had >1 mm distance to the implant surface.

OG cancellous blocks are often used for maxillary and alveolar ridge augmentations [67-73]. Lyford et al. [67] used cancellous blocks to augment the alveolar ridge in a case series of 3 patients. The authors believe their work is the first published study of such treatment and concluded with support for allograft use stating "the cancellous block allograft may provide one such alternative treatment that meets the clinical requirements while satisfying the patient's expectations [reduced extent, time, costs of the surgical procedure]." Nissan et al. [68] published a study where they augmented deficient alveolar ridges for single-tooth implants in 9 patients with cancellous blocks. By 18 month follow-up, there was no bone loss below the first thread and all implants were still functional and in good condition. The authors noted that this technique has the advantages of "minimizing post-operative morbidity; elimination of second-stage implant placement surgery, reduced surgical trauma; minimal use of a provisional removable restoration; and the ability to satisfy esthetic demands in the shortest time possible." In 2011, Nissan et al. [69] published another similar, but larger study where they used 46 cancellous blocks to treat alveolar ridge deficiencies in 31 patients who required implants. They found 95.6% graft success (two failed bone grafts) and 98% immediate implant success with the single failed implant due to an automobile accident. The implant was reinserted and all implants had a 100% success rate (defined as clinical osseointegration) after a mean 34 month follow-up. Chaushu et al. [70] used cancellous blocks for maxilla sinus floor augmentation along with simultaneous implant placement for 28 patients. After a 27 months follow-up, the authors found a 94.9% success rate (defined as clinical osseointegration), although all implants considered failed were reinserted and osseointegrated. The histologic analysis revealed new bone formation "containing viable osteocytes merged with residual grafted bone." In a case study of a single patient with a 21 month follow-up, Wallace and Gellin [71] used cancellous blocks to augment the maxillary ridge for implant placement. Not only did the authors find the graft successful

(allowed implant placement) but they suggested "cancellous block allografts could be a promising alternative to autogenous block grafts." Wallace and Gellin [72] reached the same conclusion when they followed this initial study up with a published 12 patient case series in 2010 that also had a 100% graft success rate (no grafts were lost by 5 months following placement). The authors also had a 100% implant success rate as "no implants failed during the 4 month integration period." Wallace [73] recently published a study utilizing cancellous particulate FDBA in combination with a membrane composed of decellularized dermis to preserve extraction sites for implant placement in six patients. Histologic analysis showed a "significant percentage [average 12.8%] of new bone regeneration after 12 weeks in molar extraction sites."

Very recently Bernardello et al. [74] performed the first human histologic evaluation of a two-stage crestal sinus elevation technique, utilizing β-TCP as radiographic tracer and mineralized human bone allograft as grafting material, in a severely atrophic maxilla. After a six month healing period, a core biopsy was taken and the histology highlighted a wide mineralized composite network of allograft granules connected by newly formed bone and osteoblast activities [75]. However, β-TCP resulted in poor contact with bone. The histologic outcomes of this report demonstrated significantly better behavior outcomes of FDBA than β-TCP.

Conclusion

While many different graft types are available for dental procedures, there are numerous advantages of allografts, as described here. However, allograft processing varies by manufacturer, and resulting product intended for dental applications may differ in sterilization assurance, osteoinductive potential, and proven clinical performance. One allograft option, referred to here as "OG bone allograft", is provided sterile to a SAL of 10^{-6} and with an extensive history of published studies to support clinical efficacy, makes this type of graft a valid option for the dental practitioner to consider.

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