Allograft Safety through Terminal Sterilization—the Effect of Gamma Irradiation on the Osteoinductivity of Bone Matrix

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Grafting with ground human bone matrix is performed widely in oral and maxillofacial procedures, in particular in the correction of ridge defects, sinus augmentation, extraction socket maintenance, and periodontal reconstruction. The bone scaffold formed by ground cortical bone and cancellous chips creates the favorable environment required for bone-forming cells to be able to generate new bone. The demineralization of bone matrix exposes bone morphogenetic protein (BMP) and other bone growth promoting factors. Because of this, demineralized bone matrix (DBM) not only provides a scaffold for bone formation, it also promotes differentiation of stem cells into viable bone-forming cells, a process termed as osteoinductivity.

Even as the use of allograft tissue for oral surgery continues to increase, dental professionals remain concerned about the safety and efficacy of allograft implantation. Several allograft providers have begun terminal sterilization of allograft tissue by gamma irradiation, prompting a discussion about the effect of irradiation on the osteoinductive properties of bone matrix.

Bone Remodeling and Healing

Healthy adult human bone is made up of two components: cortical bone (also called lamellar or compact bone) and cancellous (or trabecular) bone. Cortical bone forms the dense outer layer of shaft and flat bones and makes up 75 to 80% of the body’s bone mass. In contrast, cancellous bone fills the central cavity (medullary cavity) of the bone and has a structure that resembles a honeycomb. This network of spaces called interstices contains the hematopoietic, or blood-building, elements.

Bone tissue continuously undergoes remodeling that is tightly regulated by biochemical, biomechanical, cellular, and hormonal mechanisms as part of a naturally occurring cycle of bone renewal. These same steps of bone resorption and deposition are also part of the bone healing process and are made use of when bone is transplanted in the form of a bone graft.1

Three physiological properties play a role in the remodeling and healing of bone; these are osteogenesis, osteoconduction, and osteoinduction. Osteogenesis, which is dependent on the presence of viable cells with the ability to form new bone, is a property found only in fresh autograft bone and in bone marrow cells. Osteoconduction is the property of bone to serve as a favorable environment for bone-forming cells at the recipient site to infiltrate, proliferate, and form new bone. A number of scaffold materials can provide this favorable environment including autografts, ground cortical bone and cancellous chip allografts, and synthetic matrices such as hydroxyapatite and calcium phosphate. Osteoinduction is the ability of bone material to promote stem cells to differentiate into bone-forming cells upon stimulation by a matrix and associated protein factors such as bone morphogenetic protein (BMP). During the demineralization of bone matrix, BMP isoforms and other bone growth promoting factors are exposed, enabling them to carry out the process of osteoinductive new bone formation.1,2

Demineralized bone matrix, also known as demineralized freeze-dried bone allograft or DFDBA, is the most frequently used osteoinductive allograft tissue available on the market.
Why Demineralization Matters

Ever since Urist et al recognized that new bone formation is not generated by a single factor, but rather is the result of a large number of molecules interacting in a systematic cascade, DBM has been accepted as the most optimal natural solution for clinical purposes. Residual mineral levels of DBM can be seen as a measure of the reproducibility of the demineralization process as well as a marker for the increased availability of active growth and differentiation factors. Although comprehensive discussion of the impact of demineralization of allograft tissue on new bone formation is beyond the scope of this article, studies have shown that DBM of a particle size of 250 to 710 micron with a residual calcium content of 2% exhibited the highest osteoinductivity.

In the early 1990s, LifeNet introduced a patented demineralization process that involves solubilization of the mineral phase of the ground bone in a closed continuous-flow system to minimize the risk of contamination. This process was further enhanced through the development of LifeNet’s patented Pulsatile Acid Demineralization (PAD™) technology. Rapid “pulsing” of the ground bone with acid improves the precision and speed of the demineralization process. The PAD™ process results in controlled residual calcium levels of 0.5% to 4.0%.

Osteoinductivity of DBM and the Impact of Gamma Irradiation

An advantage of allograft over autograft bone is that it is readily available in the desired quantity and preparation and circumvents the need for a second surgery site, consequently reducing post-operative pain. Allograft tissue should ideally be osteoconductive or, better yet, osteoinductive, contain a minimum of antigenic factors, and be free of any microbial contaminants. It should be remembered that human bone tissue for grafting is procured from deceased donors, and despite stringent processing methods, human tissue might harbor microbial contaminants that are present in the tissue at the time of recovery. As there is no completely infallible way to exclude such donors, conscientious tissue banks are adopting sterilization procedures that do not adversely affect the performance of implanted allograft tissue.

The effect of a variety of sterilization methods on the osteoinductivity of demineralized bone matrix has been extensively studied in a variety of animal models implanted at both heterotopic and orthotopic sites. These methods included treatment with glutaraldehyde solution, formaldehyde gas, and ethylene oxide, as well as autoclaving and gamma irradiation. Of all sterilization methods examined, gamma irradiation demonstrated the most consistent results and appeared to be the most appropriate sterilizing method for demineralized bone in clinical use.

In further studies, the effect of gamma radiation at various temperatures on bone formation and remodeling were explored. In the experiments performed by Weintroub and Reddi, samples were maintained in ice water during irradiation. Preparations that had been irradiated with doses up to 25 kGy showed inductive properties that were similar to the non-irradiated control. Dziedzic-Goclaw ska and colleagues irradiated DBM at room temperature or on dry ice (-72°C). While samples that were irradiated at room temperature had been completely resorbed five weeks after implantation into the muscle pouch of a rat, DBM irradiated on dry ice were osteoinductive and were resorbed more slowly. DBM that had been treated with a dose of 35 kGy at -72°C demonstrated new bone formation that was comparable to non-irradiated control samples. These results lead both groups to hypothesize that
temperature may play a critical role in the protection of osteoinductive properties of DBM against radiation damage.

**Dependable Irradiation Practices**

To address the need to provide sterile tissue LifeNet performed a series of studies to determine the threshold for safely irradiating bone tissue while maintaining its original osteoinductivity. In one experimental series demineralized bone particles were irradiated at 15 and 30 kGy (absorbed dose) on dry ice (-20 to -50°C) or at ambient temperatures and compared to non-irradiated control samples. The preparations were implanted heterotopically into athymic mice for 28 days and were then assessed for percentage calcium deposition and new bone formation in the implanted samples. No statistically significant differences in calcium deposition and percent new bone formation were found between the experimental samples irradiated at 15 and 30 kGy and the non-irradiated control (dose independence). Similarly, the results showed no significant difference between the experimental samples irradiated on dry ice or at room temperature relative to the non-irradiated control (temperature independence).\(^{13}\)

**Figure 1.** Percent new bone formation determined by histomorphometric analysis of explanted DBM.\(^{13}\)

In light of the finding that demineralized bone particles that were irradiated at low temperatures were less susceptible to radiation damage\(^9,10\), LifeNet also undertook a study to examine the results of a range of absorbed gamma radiation doses on demineralized bone particles.\(^{13,14}\) In this assay, bone particles 250 to 710 micron (µm) in size were irradiated at 7 kGy, 14 kGy, 21 kGy, and 30 kGy. Experimental samples were implanted in muscle pouches of athymic mice for 28 days and were then compared to non-irradiated control samples in regard to percentage calcium deposition. The data demonstrated that gamma irradiation decreased the remineralization of the implanted DBM preparation to a point at which it allowed for greater resorption and replacement with new bone. This observation corroborates the results of experiments performed by Weintroub and Reddi, which suggested that irradiation of 30 to 50 kGy enhanced bone induction, leading to a higher level of mineralization than non-irradiated control samples in a heterotopic rat model.\(^9\) The experimental samples irradiated at each radiation dose and evaluated in the study did display significant osteoinductivity, however, regardless of the dose administered.\(^{14}\)
Safety through Sterility

With the increased concern about allograft safety in the medical community, allograft suppliers are focusing heavily on providing sterile grafts. Although there have been no reports of disease transmission through implanted demineralized bone products to date, dental practitioners should be knowledgeable about the practices of individual tissue banks when making the decision to purchase and use allograft tissue.\textsuperscript{15} Physicians also have the responsibility to inform their patients about the risks and benefits of using allograft tissue.

LifeNet has been a pioneer in allograft cleaning and disinfection since the introduction of the proprietary and patented Allowash\textsuperscript{®} technology in 1995. In a comprehensive and validated process, greater than 99\% of bone marrow and blood elements from the internal bone matrix are removed, and subsequent chemical disinfectant treatment showed a 4- to 20-log kill for bacteria and viruses depending on the microorganism. In 2005, LifeNet extended this process through the addition of a terminal sterilization step with the introduction of Allowash XG\textsuperscript{®}. Allowash XG\textsuperscript{®} results in sterile allograft tissue with a Sterility Assurance Level (SAL) of $10^{-6}$ without compromising the biochemical properties of the tissue needed for surgical applications. The following table provides an overview of the steps that make up the entire Allowash XG\textsuperscript{®} process.

| Table 1. Allowash XG®—six steps to sterile tissue |
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| **Steps to Sterilization** | **Description Summary** |
| 1. Bioburden Control | Meticulous and rigorous screening routine based on FDA and AATB guidelines with strict donor exclusion criteria |
| 2. Bioburden Assessment | Extensive serologic testing for microbiological contamination that includes bacteria, fungi, and infectious diseases |
| 3. Minimizing Contamination | State-of-the-art processing facilities to maintain cleanliness levels designed to eliminate the possibility of cross-contamination by |

\textbf{Figure 2.} Residual weight changes in percent residual calcium of DBM irradiated on dry ice.\textsuperscript{13,14}
4. Rigorous Cleaning, Blood and Marrow Removal
Flush, centrifugation, hypotonic processes, and ultrasonication to solubilize and remove blood elements, including marrow and lipids.

5. Disinfection and Rinsing Regimen
Intensive decontamination, disinfection, and scrubbing regimen designed to remove and eliminate viruses and bacteria, followed by centrifugation and/or microabsorption to remove residual water.

6. Terminal Sterilization
Low-level gamma irradiation at low temperatures resulting in sterile allograft tissue with an SAL of $10^{-6}$.

Data derived from animal models and clinical results indicate that the Allowash XG® process has no detrimental effect on the characteristics of allograft tissue. LifeNet’s validated Allowash XG® technology results in sterile allograft tissue at a $10^{-6}$ SAL without compromising the biomechanical and biochemical integrity of the tissue. By terminally sterilizing bone allograft, LifeNet now offers dental professionals and their patients an additional level of safety and confidence.

References


