The Effect of Irradiation Sterilization on Demineralized Bone Matrix and Allograft Tissue

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Background

Bone regeneration is a multifaceted process involving the temporal and spatial coordination of biomolecule signaling factors (e.g. growth factors), cells, and other environmental factors. Bone grafting materials have been developed to augment and accelerate the bone healing process. These materials are designed to mimic the architecture and functionality of normal bone. Bone grafting options consist of allograft derived products (e.g. demineralized bone matrix and cancellous chips), synthetics, growth factors, and autologous tissues.

Commercial bone grafting options are characterized by their bone forming properties and mechanism of action. The terms osteoconductive, osteoinductive, and osteogenic are used to define the properties of bone grafting options that are potentially contributory to bone formation. These properties are influenced by the graft material composition and architecture. Osteoconductive materials provide a scaffolding that promotes cell attachment and bone formation. Material composition and architecture on macro-, micro-, and nano-scales have been shown to influence cell behavior and bone formation. Osteoinductive materials induce bone formation by stimulating undifferentiated cells to form bone cells that contribute to bone formation. This process is often mediated through potent bone forming growth factors, such as bone morphogenic proteins (BMPs). Osteogenic materials comprised of osteoprogenitors and other bone cells contribute to the healing and remodeling of bone. Autologous bone tissue from the iliac crest is still considered by many as the standard of care, due to its combination of all three bone forming properties and extensive clinical experience. Despite its properties and widespread use, autologous bone is limited by availability and patient donor site morbidity. This has led to decades of research developing bone graft substitutes and extenders that can promote bone healing.

To date, there are hundreds of commercial bone grafting options that span synthetic and allograft derived materials. Demineralized bone matrices (DBMs) make up a substantial portion of the bone graft market due to its bone forming properties, handling characteristics, and long history of use. DBMs are processed allograft bone that consist of structural proteins (e.g. collagen) and non-collagenous proteins that include BMPs and other growth factors known to be osteoinductive. It is well established that BMPs play a critical role in the osteoinductivity of DBMs. In addition to DBMs providing osteoinductivity, it has been shown that the demineralized bone form (e.g. particulate or fiber form) and architecture can impact the osteoconductivity and bone forming potential of DBM.[16] First generation DBMs traditionally consisted of particulate demineralized bone powder combined with a carrier. Later advancements in DBMs led to cortical fiber architecture that eliminated the need for a carrier, improved osteoconductive properties, and optimized graft containment characteristics. The resulting composition and architecture of cortical fibers may therefore improve bone forming potential over traditional particulate DBMs combined with a carrier.[19] Martin et al. demonstrated that demineralized material consisting of fiber architecture can enhance the bone formation when compared to a particulate form.[16]

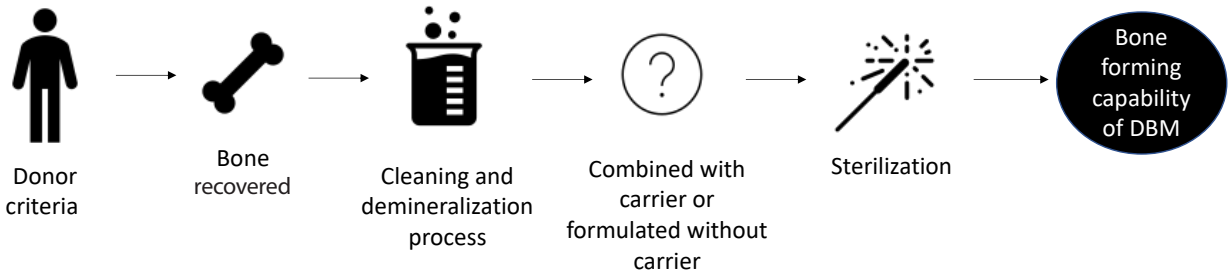

Figure 1: Donor variation, processing methods, and sterilization technique will influence properties of DBM.

Regardless of the particulate or fiber-based form, DBM variability can be attributed to donor factors, different processing techniques, the combination with carriers, and sterilization methods (Figure 1). In fact, osteoinductivity has been shown to be variable between DBM products and even within donor lots of the same product. This variability can be impacted by differences in manufacturer criteria for eligible donors (e.g. allowable donor age range may vary), the processes used for cleaning and demineralizing the tissue, the formulation and architecture, and methods of sterilization (Table 1). Donors, processing, and sterilization can ultimately impact the bone forming potential of the tissue. **This comprehensive review will evaluate the effects of sterilization on (i) the biological properties of allograft tissue and (ii) clinical outcomes**.

Tissue sterilization techniques

To reduce the bioburden risk of allografts, various terminal sterilization methods have been established including irradiation, ethylene oxide, and steam. The most common terminal sterilization method is ionizing irradiation - gamma irradiation and electron beam (e-beam) irradiation.[27] Irradiation can inactivate and kill various pathogens reducing bioburden, but also can generate heat, energy, and free radicals that can adversely affect the tissue architecture and biological and mechanical properties.[27] The ability of irradiation to kill pathogens and the extent of tissue damage are both dose dependent. This review will investigate the effects of terminal sterilization with irradiation on the biological properties of DBM as well as other allograft tissue types and review clinical comparative data to determine if irradiated tissue is comparable to non-irradiated or aseptically processed allograft tissue.

Pre-clinical findings

A number pre-clinical studies have investigated the effects of gamma irradiation on the osteoinductive potential of DBM or BMPs.[1–3,12,18] Studies report conflicting outcomes on the effects of gamma irradiation on DBM bioactivity. Munting et al. reported gamma-sterilization had maintained the osteoinductivity of demineralized bone.[18] A later study by Ijiri et al. found gamma irradiation significantly reduced the osteoinductivity of BMPs.[11] Despite the controversy around the effects of gamma irradiation on DBM, a number of the studies report a reduction or dose dependent effect on BMP concentration, osteoinductivity, or bone formation due to gamma irradiation (Table 1).[2,3,5,9,11,12] It is also established

Table 1: Types of processing and sterilization techniques

Processing techniques to reduce contamination	Description	Limitations
Aseptic Processing	Involves sterile retrieval and processing with stringent quality control testing. Aseptic processing may vary between manufacturer and incorporate various wash steps and chemicals.	More costly due to rigorous selection and regulated processes to control microbial and particulate contamination
Terminal Sterilization with e-beam	Type of ionizing irradiation that uses an accelerated beam of electrons to kill pathogens[6]	Can generate heat and free radicals that may alter tissue properties.[17] Has lower penetrability compared to gamma irradiation.
Terminal Sterilization with Gamma Irradiation	A type of terminal sterilization using gamma rays typically produced by 60CO sources to target nucleic acid components and inactivate pathogens[2,27]	The process can generate heat and free radicals that can alter the tissue properties[6]

that gamma irradiation can impact collagen and extracellular matrix proteins in allograft tissues.[9,17] Buton et al. reported negative effects of gamma irradiation on allograft bone embrittlement due to disruption of the collagen network.[4] Another study demonstrated the negative effects of e-beam and gamma irradiation on the extracellular matrix of dermal grafts (Figure 2). Both types of irradiation techniques led to significant damage of the basement membrane.[17] Damage to extracellular matrix proteins such as collagen can impact the osteoconductivity of the graft and influence cell binding, attachment, and function. A study by Hofmann et al. investigated the effects of various sterilization methods on bone marrow stromal cell attachment to demineralized bone matrices. Their findings suggest that that BMSC adhesion and function were influenced by sterilization methods.[10] Cell seeding and attachment are critical factors that can influence the performance of a graft. DBMs are commonly augmented by bone marrow aspirate (BMA). The ability of progenitor cells from BMA or bone forming cells from the surrounding tissue to adhere to the graft may be altered by the processing and sterilization of the demineralized tissue. Further studies are needed to understand the effect of sterilization on DBM matrix proteins that influence cell seeding and repopulation.

Most of the studies identified by this review investigate the impact of gamma irradiation on allograft tissue. The effects of e-beam irradiation on the biological properties of DBM is less established. Qiu et al. found that e-beam irradiated DBM lost osteoinductivity after 12-months of storage.[20] Another study reported a dose-dependent reduction of BMPs in both e-beam and gamma irradiated demineralized bone tissue.[2] To date, there are limited published high quality peer-reviewed studies to understand the effects of e-beam irradiation on the biological properties of DBM. Furthermore, the clinical impact of both e-beam and gamma irradiated DBM has not be investigated.

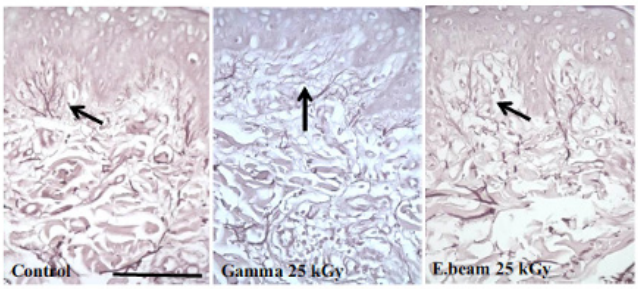

Figure 2: Histological staining of dermal grafts following gamma and e-beam irradiation. Both irradiation techniques showed disintegration of elastic fibers. (From Mrazova et al. 2016 Cell Tissue Bank 17:255-260)

Table 2: Summary of pre-clinical studies investigating the effects of irradiation sterilization on the biological properties of demineralized bone matrices.

Study	Purpose	Sterilization method	Outcome measures	Summary of results	Effect of irradiation on allograft tissue
Kayal et al. 2015[12]	Effect of irradiation on BMPs from DBM and the physical-chemical properties of Pluronic® F-127	Gamma irradiation (25 kGy)	BMP-2 and BMP-7 amount and bone formation in rat model	Gamma irradiation reduced the amount of BMPs but maintained bone formation	Negative effect
Antebi et al. 2015[2]	Study the effects of ionizing radiation (gamma and e-beam) on lyophilized and frozen DBM	Gamma and e-beam irradiation (15kGy, 25kGy, 50kGy)	BMP-2 and -7 protein concentrations	Dose-dependent reduction of BMPs. Higher reduction was observed with e-beam at 50kGy	Negative effect
Munting et al 1988[18]	Determine the effect of different sterilization techniques on osteoinductivity of DBM	Gamma irradiation (2.5Mrad)	Bone formation in rat muscle implantation model	Gamma irradiation did not significantly impact osteoinductivity	No effect
Ijiri et al. 19941[1]	Determine the influence of sterilization on DBM osteoinductivity	Gamma irradiation (2.5Mrad) or ethylene oxide	Bone formation and osteoinductivity in subcutaneous rat model	Gamma irradiation significantly reduced bone formation capability of BMP	Negative effect
Alanay et al. 2008[1]	Evaluate the effect of hydrogen peroxide exposure with or without the controlled high-dose gamma irradiation on fusion rates of DBM in rat model	Gamma irradiation (50kGy)	Fusion rate in athymic rat spinal model	Gamma irradiation showed no significant difference in fusion rate	No effect

Arjmand et al. 2013[3]	Effects of gamma irradiation on osteoinductive properties of DBM	Gamma irradiation (25kGy)	Osteoinductivity in ectopic rat model	Gamma irradiation reduced bone formation	Negative effect
Chen et al. 2007[5]	Investigate changes to biological properties of irradiated and non-irradiated DBM	Gamma irradiation (0kGy, 15kGy, 25kGy)	Osteoclast-like cells and protein expression	Reduction of bone formation in 25 kGy irradiated samples and low dose (15 and 25 kGy) reduced protein expression	Negative effect
Han et al. 2008[9]	Investigate changes to DBM functionality after gamma irradiation	Gamma irradiation (0kGy, 12kGy, 18kGy, 25kGy)	Osteoinductivity in vitro and in vivo, and collagen solubility	Gamma irradiation reduced osteoinductivity of DBM – highest level of 25kGy showed almost no osteoinductivity compared to non-treated controls	Negative effect

Interestingly, in the tendon allograft field, there has been a significant amount of work around pre-clinical and clinical studies to evaluate the method of sterilization or aseptic processing. While more work needs to be completed to elucidate clinical outcomes for DBMs that are sterilized or aseptically processed, similar attributes of allograft tissues between DBMs and tendons provides a foundation for potential impact to be considered and studied.

There are multiple tendon allograft pre-clinical studies investigating the effects of gamma irradiation on the biomechanical and biological properties of tendon allografts. Similar to DBMs, there are considerably less studies investigating the effects of e-beam irradiation on tendon allograft biological and mechanical properties.[22] Seto et al. compared the e-beam and gamma irradiation effects on the mechanical properties and enzyme resistance of tendons. Both gamma and e-beam irradiation caused similar degenerative effects with reductions in tensile strength, elastic modulus, strain, and toughness of tendons.[22] Another study by Gut et al. reports conflicting outcomes. The data from this study suggests that e-beam sterilization did not influence the mechanical properties of bone-tendon-bone allografts.[8] Notably, a study by Schmidt et al. showed that e-beam sterilized allograft showed significantly worse outcomes compared to fresh frozen allografts and autografts in a sheep ACL replacement model. The authors concluded that e-beam sterilization cannot be recommended for soft tissue allograft sterilization.[21]

Clinical findings

To the extent of our search, there are no clinical studies investigating the effects of irradiation sterilization on the clinical performance of DBMs. However, a substantial body of literature has investigated the effects of irradiated tendon allografts on patient outcomes.[7,13,15,25,26] Table 2 highlights the comparative studies investigating the effects of irradiated and non-irradiated tendon allografts on ACL reconstruction. A prospective randomized trial compared anterior cruciate ligament (ACL) reconstruction outcomes with irradiated and non-irradiated allograft. The study reported significantly higher laxity and greater arthritic progression in the irradiated group compared to patients who received non-irradiated allograft.[25] Other cohort studies (LOE II,III) investigating irradiated tendon allografts found higher failure rates and higher risk of revision compared to non-irradiated tissue or autograft.[13,24,26] In response to the literature suggesting inferior outcomes with irradiated tendon allografts, there has been a shift away from irradiated tendons to using fresh-frozen, non-irradiated, aseptically processed tendon allograft tissue.[23] This transition is further supported by a recent systematic review that found no significant difference in graft failure rate, postoperative laxity, or patient-reported outcome scores between non-irradiated allograft and autograft for ACL reconstruction.[15]

Overall, this comprehensive review supports that terminal sterilization with irradiation may negatively influence the biological properties and clinical outcomes of allograft tissue. [2,3,5,9,11,12,26] Further work is needed to understand the biological impact of sterilization with irradiation on DBMs, particularly e-beam irradiation. To date, there are no clinical outcome studies investigating the impact of irradiation on DBMs used for bone repair or fusions. High quality randomized controlled trials are needed to understand the clinical impact of irradiation on DBM clinical performance.

Table 3: Clinical studies investigating the effects of irradiated tendon allograft on outcomes.

Author	Level of Evidence (LOE)	Study Design	Outcome Measures	Findings
Tian et al. 2017[25]	I	Prospectively randomized trial evaluating double-bundle ACL reconstruction with irradiated (2.5Mrad) and non-irradiated hamstring allograft	Functional tests and subjective clinical outcomes scores	Significant increase in laxity and greater arthritic progression in patients of irradiated tendon group. No difference in activity level or IKDC clinical outcome scores. Authors do not advocate to use irradiated hamstring tendon allograft for ACL
Maletis et al. 2017[14]	II	Cohort study comparing risk of revision after ACL reconstruction with soft tissue allograft (processed chemically, terminally sterilized, or aseptically processed) and autologous bone-patellar tendon bone or hamstring grafts	Rate of revision	Soft tissue tendon allografts that were irradiated with greater than or equal to 1.8Mrad were found to have higher risk of revision when compared to autografts. Grafts irradiated with less than 1.8Mrad still had higher risk of revision compared to autograft. Non-irradiated tissue allografts did not have higher risk of revision compared to autograft
Maletis et al. 2017[13]	II	Cohort study compared risk of revision after ACL reconstruction with bone-patellar tendon bone (BPTB) autografts and BPTB allografts	Rate of revision	BPTB allografts had significantly higher risk of revision than autograft. Processing methods and irradiation did not affect the risk of revision
Tian et al. 2016[26]	II	Randomized controlled trial to compare autograft hamstring tendon and irradiated allograft ACL reconstruction	Functional tests, activity levels, and clinical outcomes scores	No difference in clinical outcome scores. Report significant difference in functional measurements and rate of laxity. The failure rate of irradiated group was significantly higher
Tejwani et al. 2015[24]	III	Cohort study that evaluated the effect of graft processing, patient characteristics, and graft type on revision surgery after allograft ACL reconstruction	Rate of revision	Grafts with irradiation greater than 1.8Mrad and grafts processed with BioCleanse showed significantly higher risk of revision compared to other processing
Grassi et al. 2017[7]	IV	Meta-analysis of outcomes of revision ACL reconstruction with different types of grafts	Clinical outcome measures of ACL reconstructions following different graft types	Overall, autografts had better outcomes than allografts with respect to laxity, rate of complications, and re-operations. Non-irradiated allografts had significantly smaller rate of re-operation compared to autografts and no significant difference in clinical scores. Irradiated allografts showed inferior results to non-irradiated and autograft
Mariscalco et al. 2014 AJSM[15]	IV	Systematic review comparing ACL reconstruction outcomes with autograft and non-irradiated allograft from prospective or retrospective comparative studies	Reported outcome data including graft failure, postoperative laxity, and patient-reported outcome scores	No significant differences were found in graft failure rate, postoperative laxity, or patient-reported outcome scores between autograft and non-irradiated allograft

Summary

- Despite the conflicting reports investigating the effects of irradiation on DBM, a majority of the studies included in this review suggest a reduction or dose dependent effect of gamma irradiation on BMP concentration, osteoinductivity, or bone formation[2,3,5,9,11,12]
- A number of studies show gamma and e-beam irradiation may disrupt extracellular matrix proteins and collagen in allograft tissues[9,17]
- Additional high quality pre-clinical studies are needed to confirm the impact of gamma and e-beam irradiation on BMP concentration, osteoinductivity, cell binding, extracellular matrix proteins, and collagen.
- The clinical impact of altered DBM due to irradiation has not been investigated – high quality clinical studies are needed to determine if sterilization by e-beam or gamma can impact clinical outcomes and fusion rates
- The effect of irradiation on tendon allograft performance for ACL reconstruction has been extensively studied. [7,13,15,25,26] A number of clinical studies have reported inferior outcomes with irradiated tendon grafts compared to non-irradiated (aseptic) or autologous grafts.[13,24,26]
- Due to the clinical impact observed with tendon allografts it is critical to investigate the clinical impact of irradiated DBM compared to non-irradiated (aseptic) or autologous bone.

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