Effect of Terminal Gamma Sterilization on Osteoinductivity

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Introduction

RTI Biologics, Inc. (RTI) currently produces allograft demineralized bone matrix (DBM) paste products that are manufactured under strict aseptic conditions using single donors. All donors are subjected to rigorous screening, prior to processing, to ensure that no transmissible pathogens are present in donor material. In addition, a terminal sterilization procedure has been introduced for all allograft DBM paste products.

Gamma irradiation is an accepted means of achieving terminal sterilization in a variety of products including biologics. Previous reports indicate the use of this method for decades in the allograft transplant arena.[1-3] Gamma irradiation at relatively low doses (~1 Mrad) is capable of killing most classes of microorganisms, with small viruses being the exception. Sterility by irradiation is accomplished primarily by the alteration of nucleic acids (RNA and DNA) rendering life forms incapable of reproducing. A secondary effect of irradiation is the generation of free radicals. While these are capable of contributing to the sterilization process, free radicals have also been shown to alter the mechanical and biological quality of bone allografts.[4-7]

Previous studies have shown mixed results with respect to the effects of gamma irradiation on the biological and biomechanical properties of bone.[4-7] In general, gamma irradiation of bone allografts at low temperatures appears to preserve both mechanical and biological properties. In the present study, the effects of gamma sterilization on the osteoinductivity of allograft bone paste products were investigated in a rat ectopic pouch model of bone formation.

Methods

In order to ensure that gamma irradiation at sterilization doses (2.5-3.1 Mrad) does not compromise the biological efficacy of DBM paste products, we assessed its effect on the osteoinductive property of demineralized bone matrix (DBM). A series of studies was performed that compared the biological activity of both precursor DBM powder and DBM plus a porcine-derived collagen carrier (RTI Paste) before and after gamma irradiation. Biological activity was assessed (qualitatively and quantitatively) using the Urist athymic nude rat model.[8] This *in vivo* model is utilized by RTI to identify DBM powder with acceptable osteoinductivity for use in all DBM paste products.

Results

In a comparative study, DBM powder and RTI Paste samples prepared across different donors were tested. Sample preparations were either irradiated in an environmentally controlled method or left untreated, after which they were implanted in abdominal muscle pouches of athymic nude rats. Explants were retrieved 4-weeks post-implantation, processed, and evaluated histologically for evidence of bone formation and inflammation. For data analysis, the overall osteoinductivity (OI) score obtained for the pre-gamma irradiation group was used as a base line of 100% and scores given to their irradiated counterparts expressed as a percent thereof.

Table 1 depicts the biological activity seen for each implant group (DBM powder or RTI Paste) before and after gamma irradiation.

Histological analysis of the test implants failed to reveal qualitative changes in samples following irradiation. Comparable remodeling features that are associated with new bone formation (cells associated with bone and marrow formation) were observed. Additionally there were no signs of inflammation [usually associated with the generation of free radical associated toxins[9]] in the explants as well. Representative low power and high power images of explants from matched donors before and after gamma irradiation are shown here. (Figure 1)

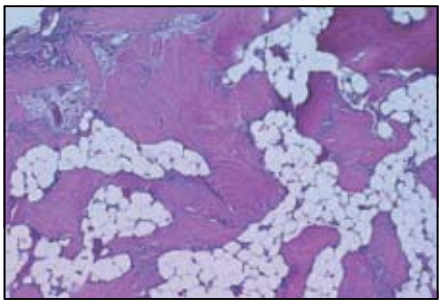
Table 1.

	Sample Type	No. Implants	Pre-Gamma OI	Post-Gamma OI	P value (t-test)
1	RTI Paste	24	100 ± 7.8	92.5 ± 8.8	0.3**
2	DBM Powder	24	100 ± 7.3	103 ±- 4.1	0.39**

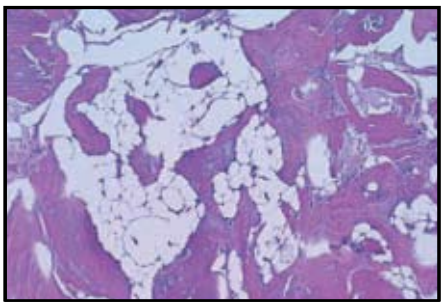
***When OI scores were compared between the groups using the t-test, statistically significant differences were NOT seen between the groups.*

Conclusion

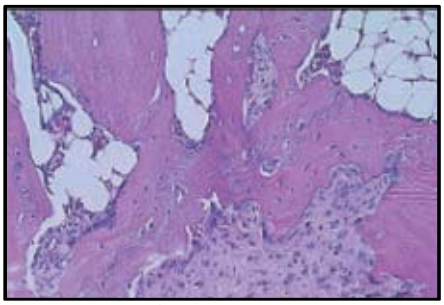
Previous studies have suggested a possible negative effect of gamma irradiation on the osteoinductivity and osteoconductivity of DBM.[5,10] In the present study, a direct comparison of non-gamma irradiated and gamma irradiated (2.5-3.1 Mrad) DBM was conducted in an *in vivo* rat ectopic pouch model. The data clearly indicate that gamma irradiation has no significant effect on the osteoinductivity of DBM powder or DBM-based products with porcine-derived collagen carrier when tested in this model. Histological evaluation of the test samples also demonstrated that gamma irradiation had no negative effects on the remodeling features associated with new bone formation. In addition, the gamma irradiation of the DBM did not cause an increase in inflammation. RTI routinely tests each donor lot material in this animal model to ensure that gamma irradiation, as well as our other processing protocols, does not have a negative effect on the osteoinductive potential of its DBM paste products.



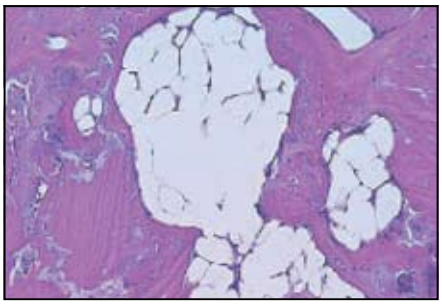
Pre-Gamma (Low Mag)



Post-Gamma (Low Mag)



Pre-Gamma (High Mag)



Post-Gamma (High Mag)

Figure 1

Both low and high magnification images of RTI Paste explants at 4 weeks, stained with Hematoxylin-Eosin showing comparable cellular events associated with new bone formation, without an inflammatory response.

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