

The Effects of E-Beam Sterilization on the Performance of ^{JRF}StimuBlast™ Demineralized Bone Matrix

Arthrex Research and Development

Introduction

Most implantable medical devices are sterilized as the final step in the manufacturing process prior to distribution and clinical use. However, many tissue-based products are not terminally sterilized. Instead, they are aseptically processed, which increases the risk for possible bacterial contamination. Most aseptically processed grafts have a sterility assurance level (SAL) value of 10^{-3} , while terminally sterilized grafts have a SAL value of 10^{-6} . This means that there is a 1 in 1,000 chance for contamination in an aseptically processed graft, while there is a 1 in 1,000,000 chance for contamination in a terminally sterilized graft. This paper discusses the sterilization technique used to process ^{JRF}StimuBlast, an allograft-based demineralized bone matrix (DBM).

Sterilization Techniques

Four terminal sterilization methods are currently used for medical devices: steam, ethylene oxide gas (EtO), gamma irradiation, and electron beam (e-beam) irradiation. Steam sterilization uses water vapor at very elevated temperatures and high humidity. It is used to sterilize surgical instruments, but is not recommended for tissue-based materials, as the hot steam may alter structural proteins and cause the material to not perform as expected postimplantation¹.

EtO sterilization exposes the material to a humid gas atmosphere and requires very long cycles for gas removal. The ability of DBM to induce new bone formation decreases with increased EtO exposure; the potential result is allograft resorption without adequate osseous development. One possible cause could be the inadequate removal of residual molecules such as ethylene chlorohydrin left on the tissue surface after sterilization^{2,3}, which may trigger an early resorption process. Another potential cause may be exposure of the bone graft to high temperatures that cause protein denaturation².

Gamma irradiation sterilization exposes the product to a Cobalt 60 radiation source⁴; this involves heat and extended radiation exposure. A higher dose of gamma irradiation is routinely used for plastics, metals, and ceramics; however, it is not recommended for tissue-based materials. A dose-dependent reduction in the osteoinductive potential of DBM has been shown using gamma irradiation doses between 10-40 kGy (1-4 Mrad)^{5,6}.

E-beam sterilization uses irradiation from a beam of high energy electrons for a short exposure interval, typically less than one minute, usually between 20-35 kGy (2-3.5 Mrad). It provides a sterilization method with shorter exposure time, far less heat, and a more uniform dose^{4,7}. E-beam doses less than 50 kGy have been shown to not affect the osteoinductivity of DBM-based graft materials⁸. This method of sterilization is commonly used for biologics within the tissue and medical device industries^{9,10}. E-beam sterilization is used to terminally sterilize all ^{JRF}StimuBlast DBM-based implants. To ensure DBM osteoinductivity, a final processed, sterilized sample of ^{JRF}StimuBlast from every donor lot is tested using the classic Urist *in vivo* model¹¹⁻¹⁴. This method involves implanting the test material in the muscle pouch of a nude athymic rodent. A material that is osteoinductive forms bone in this ectopic (nonbony) site.

Testing of E-Beam Effects

In order to further ensure that e-beam sterilization does not have negative effects on the osteoinductive potential of DBM, a dosage study was performed. Samples from a single donor were sterilized using an e-beam dosage of 30 kGy. DBM 0 was the positive control since it did not receive an e-beam dose. DBM 1 received a single dose of e-beam, DBM 2 was e-beamed twice, and DBM 3 received 3 e-beam doses. After e-beam, the DBM samples were tested for osteoinductive potential using an *in vitro* assay validated to the classic Urist *in vivo* method, which uses C2C12 murine myoblasts cultured with the test DBM sample to convert them to an osteoblastic phenotype¹³. Osteoinductive potential is determined by the amount of alkaline phosphatase (ALP) produced by the myoblasts in the presence of DBM, which is then compared to the positive and threshold (inactivated DBM) controls. DBM is inactivated by treating with guanidine to remove proteins^{13,15}. If the amount of ALP produced is at least 1.5X above the threshold control, the test material passes and is deemed to have osteoinductive potential. The study continued for 14 days with n=6 samples per group. Significance was found when $p < 0.05$.

Figure 1 graphically shows the amounts of ALP for each test group. Table 1 numerically shows the same values, as well as the percent increase of ALP over threshold, the percent decrease in ALP compared to DBM 0, and the p-values for each group. The amount of ALP produced by the threshold control was 7.84 nmol/mL/hr, and all four test groups easily passed the acceptance criteria, scoring at least 50% over the threshold control. T-tests showed that DBMs 1, 2, and 3 were not statistically different than DBM 0 positive control, even though DBM 3 had a lower amount of ALP than the other groups. This analysis shows that e-beam sterilization had no effect on the osteoinductive potential of DBM¹¹.

Figure 1:

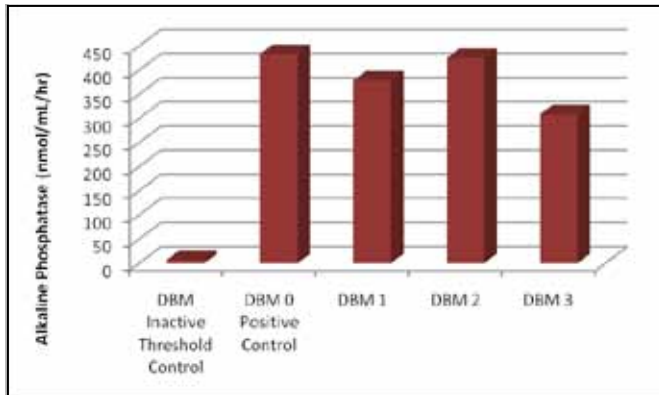


Table 1:

Group	ALP (nmol/mL/hr)	% Increase Over Threshold	% Decrease vs. DBM 0	P. Value vs. DBM 0
DBM 0	433.81	5533.99	n/a	n/a
DBM 1	379.00	4834.18	12.63	0.53
DBM 2	425.29	5424.62	1.96	0.92
DBM 3	308.76	3938.77	28.82	0.12

Conclusion:

Terminal sterilization for tissues and biologics has been demonstrated as an effective means of providing an additional measure of safety to the customer, as aseptic processing alone cannot significantly reduce the possibility of contamination to a SAL value of 10⁻⁶. Several methods to sterilize medical devices, including steam, EtO, and gamma irradiation, are not well-suited for biologics such as DBM because of the potential to adversely alter essential proteins present in the tissue. Published work and the results of this study, however, support the use of e-beam irradiation as the preferred sterilization method for DBM, as it does not have a negative impact on its osteoinductive potential.

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