Effects of SC-CO_2 Sterilization and Storage on Osteoinductivity of DBM

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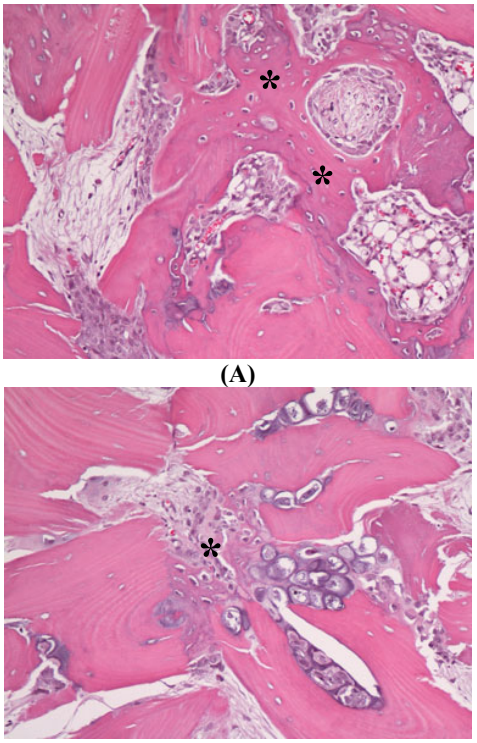
Statement of Purpose: Sterilization of demineralized bone matrix (DBM) remains a great challenge as current commonly used sterilization methods are found to be detrimental to osteoinductivity of DBM. Gamma-irradiation and e-beam are known to alter the structure and characteristics of biological materials through crosslinking and degradation of collagen matrix, and can eliminate or significantly reduce osteoinductivity of DBM. EO sterilization requires certain temperature and moisture level to be effective, which also affect DBM osteoinductivity. EO is also a recognized carcinogen, and its residual in biological materials can cause hemolysis and other toxic responses. In this study, an alternative sterilization method, supercritical carbon dioxide (SC-CO_2) was evaluated for DBM sterilization. SC-CO_2 was found to inactivate a variety of microorganisms since 1950s (1). When SC-CO_2 is used with additives such as peracetic acid (PAA), it can achieve terminal sterilization level required for medical devices. It has been reported that SC-CO_2 treatment is compatible with biological materials, including bone and acellular dermal matrix. The purpose of this study was to evaluate if this sterilization method could be used for DBM without significantly affecting its osteoinductivity. In addition, the effect of ambient storage on osteoinductivity of SC-CO_2-PAA sterilized DBM was also investigated. The osteoinductivity evaluation was carried out using an athymic rat model.

Methods: Dry DBM particulates were packaged in Tyvek pouches (1.1-1.2g/pouch), and then treated with SC-CO_2-PAA for 30min (super-critical state run time). After treatment, the samples were put in a nitrogen box for 24 hours before sealed in foil pouches. DBMs that were not treated were used as controls. The test and control samples were implanted into rats at 0, 6 and 12-month ambient storage time points to evaluate their osteoinductivity. At each time point, the test samples were implanted into four rats with one test and one control site per rat. Approximately 250mg of the hydrated DBM was placed into the pocket created intramuscularly, and then the muscle pocket and skin were closed. After 28 days, the animals were sacrificed and the implant sites removed. The tissues were fixed in 10% neutral buffered formalin and then went through routine histology processing. The sections were cut and stained with hematoxylin and eosin (H&E). Slides were examined under a microscope by a pathologist evaluating the percentage of new bone formation.

Results: Explants were processed and evaluated by a pathologist. Table I includes the average percent of field showing new bone formation for the three time points. A 20X objective lens was used, and four fields on each slide were examined. The evaluator was blinded to the treatments. At time zero, both control and test samples showed comparable amount of new bone formation, indicating the SC-CO_2-PAA sterilization did not affect the osteoinductivity of DBM. After storing the DBM for 6 or 12 months after sterilization, all the samples demonstrated new bone formation. Most of the samples showed ~30% new bone growth at 6 month and ~25% at 12 month. Generally, the evidence of the new bone formation or potential new bone formation found in the samples included the presence of osteoid, osteoblasts/ osteoclasts, calcified cartilage matrix/hypertrophic chondrocytes, bone marrow, and clusters of chondroblasts/condrorocytes (Figure 1).

Table I Summary of % of New Bone Area Formed in Control and Treatment Groups

Treatment	0-month Average±SD	6-month Average±SD	12-month Average±SD
Control	25(±1)%	34(±10)%	24(±3)%
Treated	24(±1)%	30(±1)%	24(±4)%



(A)

(B)

Figure 1. Micrographs of H&E staining of explants of DBM sterilized with SC-CO_2 (A) and DBM control (B) stored at ambient condition for 12 month. Note the new bone formation (*).

Conclusions: DBM can retain its osteoinductivity after sterilization with super-critical carbon dioxide and PAA sterilant, and no significant change in osteoinductivity was evident after 12-months ambient storage.

References: 1.Fraser D. 1951. Nature 167:33-34.