Osteoconductive and Osteoinductive Properties of Bone Fibers in Kore Strip

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INTRODUCTION AND BACKGROUND

Demineralized bone matrices (DBMs) are commonly utilized to treat bony defects as bone void filler grafts. The regenerative efficacy of these grafts is believed to arise from their ability to elicit new bone formation at the defect site, which is a biologic response known as osteoinduction. When characterizing and comparing DBM grafts, their osteoinductive properties are typically evaluated in an athymic mouse model that was first described by Marshall Urist[1]. The athymic nature of the mice allows a graft of non-mouse origin (DBMs are often derived from human bone) to be implanted and assessed without concern of a xenogenic immune response. The implant is placed at an ectopic site inside a muscle pouch with minimal bleeding and evidence of new bone formation is evaluated after 28 days. A semi-quantitative scoring scale has been established for measuring osteoinductivity (OI) based on the Urist model by Schwartz[2], which is now considered to be the industry standard for determining the OI of demineralized grafts.

Since DBMs are allografts generated from human donor bone, a degree of variability is expected from one donor lot to another due to possible differences in the inherent osteoinductive properties of the tissue. Additionally, the methods by which DBMs are processed may play an important role in maintaining the biologic activity of the tissue. For example, harsh chemical processing and terminal sterilization by gamma irradiation are two processing methods that can adversely affect tissue quality[3].

Kore Fiber® Strip (processed by MTF Biologics, Edison, NJ, and distributed by Kolosis BIO, Salt Lake City, UT) is a 100% bone allograft that is provided in a specific pre-formed shape and is primarily composed of demineralized cortical bone fibers. Kore Fiber Strip undergoes specific processing steps designed to allow the graft material to retain its shape once hydrated in a fluid such as blood or saline. In order to minimize any negative impact on OI during processing, Kore Fiber Strip is subjected only to minimal, aseptic chemical disinfection and does not undergo a terminal sterilization process. The purpose of this study was to demonstrate that the inherent biologic properties of the demineralized cortical bone fibers in Kore Fiber Strip have been preserved during processing. This study evaluated bone fibers equivalent to those used in the Kore Fiber Strip process for the presence of multiple growth factors known to be associated with new bone formation and their osteoinductive potential in the athymic mouse model. The bone fibers were also evaluated for their ability to support cell attachment, proliferation, and differentiation using an *in vitro* cell culture model.

MATERIALS AND METHODS

Growth Factor Characterization

In this study, demineralized cortical fibers, representative of those in Kore Fiber Strip, were aseptically processed from the cortical bone of three distinct donors using proprietary methods. At the time of sampling, the tissue was placed in a fixative solution, and shipped to an external lab (IHC World, LLC) to be embedded, sectioned, and stained for a panel of growth factors known to be relevant to bone healing (BMP-2, BMP-7, PDGF-BB, FGF-1, FGF-2, IGF-1, TGF-β, VEGF). These growth factors all contribute to the bone repair process and the expression of these growth factors can vary at different points during the bone healing cascade. After IHC staining, imaging was done using conventional microscopy, and the growth factors are visualized in the results section.

Cell Attachment and Differentiation

To evaluate cell attachment and responsiveness, fiber strip samples were generated from two distinct donors. Cryopreserved human mesenchymal stem cells (hMSCs) provided by Lonza were cultured and seeded onto the fiber scaffolds at a density of 200,000 cells per sample. The scaffolds were placed in Osteogenic Differentiation Media, with media changes occurring every 3-4 days. Samples were removed from the media for analysis at different time points throughout the study; cell count (performed at MTF Biologics R&D laboratories) and cell distribution (performed at the Rutgers Center for Confocal Imaging) were assessed using laser-scanning confocal microscopy at 30 minutes, 3 hours, and 24 hours. Cell differentiation, assessed using Von Kossa histological staining, was evaluated at 21 days (performed at Yale Orthopaedic Histology and Histomorphometry Laboratory). The histological images were analyzed for matrix deposition that would indicate the hMSCs are differentiating into bone forming cells within the samples.

Osteoinductivity in an Athymic Mouse Model

In order to assess osteoinduction, the study was designed to test 4 donor lots of samples that are representative of Kore Fiber Strip. Prior to implantation, fiber samples (N=8 for each lot) were hydrated in saline and then 25mg of wet fibers were transferred to a 1cc syringe. Next, samples were implanted bilaterally in the hamstring muscles of athymic mice at the testing lab (RWJMS, New Brunswick, NJ). The hamstring muscle group (*biceps femoris* muscle) was used since it is a large, easily accessible muscle, which is commonly used as an implant site to evaluate heterotopic bone formation[4]. Animals were sacrificed at 28 days post-implantation. Decalcified histology was then performed on the explanted samples. Subsequently, slides were stained with hematoxylin and eosin, and tissue sections were evaluated for osteoinductivity.

The relative amount of osteoinduction was evaluated semi-quantitatively by the study investigators using the scoring system described below (Table 1), to be consistent with standard in the industry[4]. Osteoinductive scores were based on the degree to which new bone, bone cells, osteoid, calcified

cartilage remnants, and marrow elements were present. The overall score for the test group was determined by averaging the scores from the samples. The results of semi-quantitative scoring are presented as a mean ± standard deviation. Images of histological slides from each test group were also captured and stored using a digital camera and computer system.

Score	Criteria
0	No evidence of new bone formation
1	1-25% of the section is covered by new bone
2	26%-50% of the section is covered by new bone
3	51%-75% of the section is covered by new bone
4	>75% of the section is covered by new bone

Table 1: Osteoinductivity Scoring Scale and Criteria

RESULTS

Growth Factor Characterization

Results of IHC staining were visualized in Figure 1 using conventional microscopy imaging. In these images, growth factor presence in demineralized cortical fibers is represented by brown coloration, which can be seen throughout the fibers. A description of the different growth factors that were evaluated in this study as well as whether they were present in the fibers is seen in Table 2 below. Representative images are below in Figure 1.

Growth Factor	Role in Bone Healing Cascade	Present
BMP-2	Differentiation of MSCs into osteoprogenitor cells, chondrocytes and osteoblasts	✓
BMP-7	Differentiation of osteoprogenitor cells into osteoblasts	✓
PDGF-BB	Mitogenic for MSCs and osteoblasts and responsible for macrophage chemotaxis	✓
FGF-1	Mitogenic for MSC, chondrocytes, and osteoblasts. Promotes vascularization	✓
IGF-1	Promotes proliferation and differentiation of osteoprogenitor cells	✓

TGF-β	Pleiotropic growth factor responsible for simulation of undifferentiated MSCs	✓
VEGF	Promotes migration and proliferation of osteoblasts. Promotes angiogenesis	✓

Table 2: Presence of growth factors in demineralized bone fibers of Kore Strip.

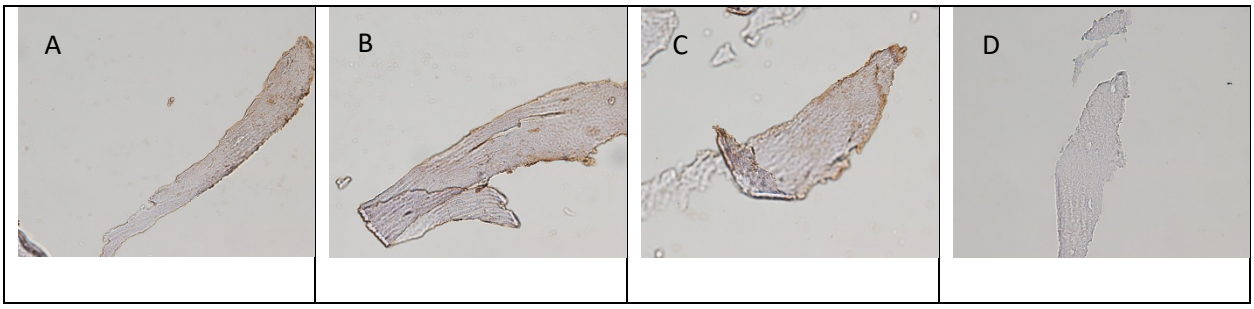

Figure 1: Representative immunohistochemical staining images of endogenous growth factors in Kore Strip fibers. Brown coloration indicates the presence of the targeted growth factor, BMP-2 (A), BMP-7 (B), PDGF-BB (C), and a negative control (D).

For samples from both donors, there was a high degree of cell attachment, and the cells that attached remained adherent over time, as seen in the cell distribution images (Figure 2). Here, hMSCs, which are visible as green dots, can be observed across the fiber scaffold at each time point from 3 hours to 24 hours.

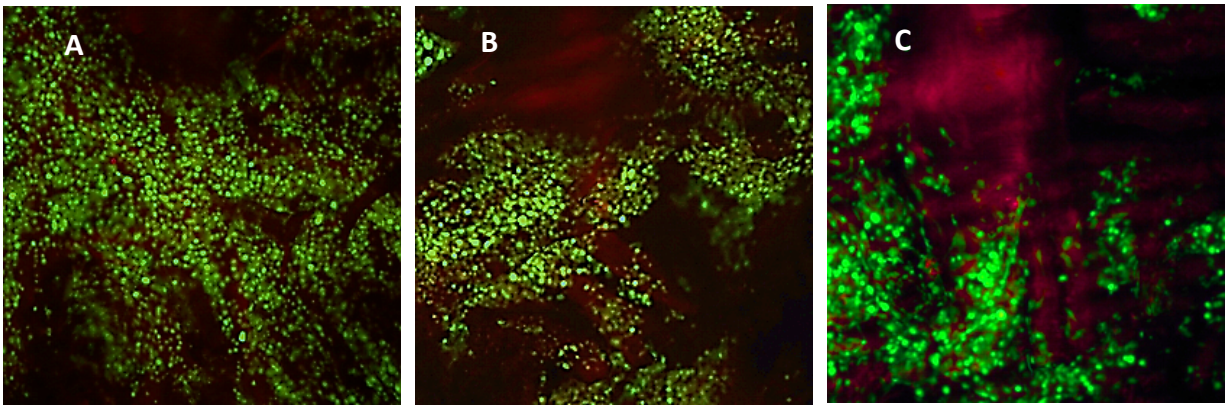

Figure 2: Time sequential confocal images of hMSCs seeded onto Kore Strip fibers that were obtained during the first day of culture. The cells were fluorescently stained green using CellTracker™ Green CMFDA while the scaffolds were stained red using Alexa Fluor 633. The time points were 30 minutes (A), 3 hours (B), and 24 hours(C).

For the cell differentiation portion of the study, adherent cells were observed on the surface of both donors at each timepoint for the H&E staining. At the day 21 timepoint, new extracellular matrix was visible near the surface of the scaffold, where cells were present. For both test articles, positive Von Kossa staining was observed at day 21. Representative pictures of each of the staining types can be seen in Figure 3.

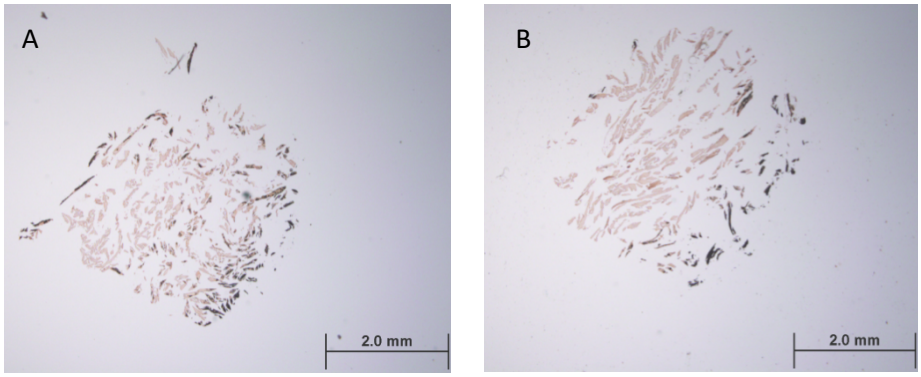

Figure 3: Histological image of hMSCs seeded onto Kore Strip fibers at 21 days after cell seeding. Sections were stained Von Kossa (A and B). Histological images of the von Kossa staining of sections (black color) depict evidence of mineralization through the deposition of calcium phosphate by differentiated hMSCs on the fibers.

Athymic Mouse Study

Following explantation after 28 days, samples from all 4 lots of cortical bone fibers were found to show evidence of OI during histological examination. Histological findings included the observation of newly formed bone that bridged particles of original bone and the formation of marrow elements (Figure 4).

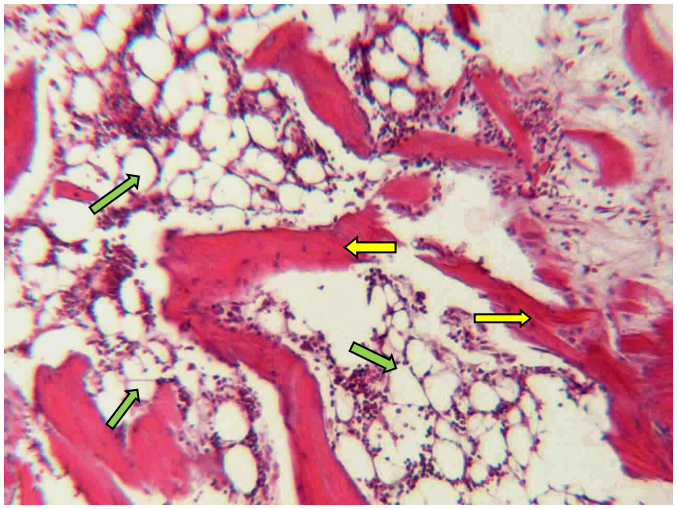

Figure 4: Explant of Kore Strip fibers demonstrating multiple regions of new bone formation (yellow arrows) and the presence of bone marrow (green arrows). H&E stain; 100X magnification.

Kore Strip fiber samples were also noted to be consistently osteoinductive, with an average OI score of 1.58 ± 1.12 was determined. (Table 3)

Summary Statistics	Osteoinduction Score (0-4 Scale)	
	Mean	Std Dev
Kore Strip fibers	1.58	1.12

Table 3: Osteoinductivity results including OI scores and the percentage of samples found to be osteoinductive.

DISCUSSION & CONCLUSIONS

The efficacy of demineralized bone allografts has been previously shown to vary widely from samples that originate from one tissue processor to another[2]. While inherent donor variability may contribute to these differences, it has been recognized that tissue processing methods can significantly impact the osteoinductive potential of demineralized allograft bone[3,4]. Kore Fiber Strip is a pre-shaped, demineralized bone allograft that is primarily composed of cortical bone fibers. It is processed in a careful aseptic manner without extended exposure to harsh chemical reagents or being subjected to terminal sterilization by gamma irradiation in order to maintain its inherent biological characteristics.

Overall, the results generated by the characterization studies indicate that the inherent osteoconductive and osteoinductive properties of Kore Strip fibers are well-preserved following processing. Using immunohistochemical staining, an array of growth factors related to bone healing were found to be present in the fiber samples. When human MSCs were added to fibers, a high degree of cell attachment was observed, and evidence of osteogenic differentiation was detected following culture in osteogenic medium. In addition, samples of Kore Strip fibers elicited new bone formation in an ectopic site using the athymic mouse model. Based on the collective findings from the pre-clinical studies, Kore Strip fibers provide a cell-friendly environment along with preserved endogenous osteoinductive signals and can act as a suitable scaffold for bone repair.

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