

A Comparative Study of the Osteoinductivity of the Kolosis BIO Kore Fiber™ and Other Commercially Available Demineralized Bone Grafts

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INTRODUCTION AND BACKGROUND

Demineralized bone grafts derived from allogeneic bone are often utilized to treat bony defects. The regenerative efficacy of these grafts are due to the presence of endogenous growth factors such as bone morphogenetic proteins (BMPs) which give rise to the ability of the grafts to elicit and support new bone formation at the defect site.¹ This phenomenon is a biologic response known as osteoinduction. In addition, if the allograft can provide a scaffold that supports cellular attachment, survival and osteogenic differentiation, the allograft may also be called osteoconductive.

Kore Fiber (processed by MTF Biologics, Edison, NJ; distributed by Kolosis BIO, Salt Lake City, UT) are a 100% bone allograft that consists of demineralized cortical bone fibers and has been processed to be moldable and putty-like once hydrated in a fluid such as blood or saline. In order to minimize any negative impact on the biologic activity during processing, Kore Fiber are subjected only to gentle, 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 Kore Fiber have been preserved during processing and compare it to leading demineralized bone grafts in the market. This study determined osteoinductive potential of Kore Fiber in an athymic mouse model and compared the results to that of other commercially-available DBMs and demineralized bone fiber allografts.

MATERIALS AND METHODS

The study design evaluated multiple donor lots for each graft material (Table 1). Prior to implantation, 25mg of each sample (N=8 for each lot) were prepared and/or transferred to a 1cc syringe. Pre-hydrated samples were placed directly in the syringe, while freeze-dried tissue was hydrated in saline and then transferred to the syringe. Samples were implanted bilaterally in the hamstring muscles of athymic mice at the testing lab (Apptec, St. Paul, MN or Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ). The hamstring muscle group (*biceps femoris* muscle) is a commonly used implant site to evaluate heterotopic bone formation, given its size and ease of accessibility⁴. Animals were sacrificed at 28 days post-implantation and histology was performed on the explanted samples, with 10 to 15 sections per sample. Subsequently, slides were stained with hematoxylin and eosin, and tissue sections were evaluated for osteoinductivity.

Tissue type	# of lots
Kolosis BIO Kore Fiber	3 lots
Zimmer Biomet InterGro Putty	3 lots
Sea Spine/Integra Accell Connexus	4 lots
LifeNet BLX Fibers	3 lots
Medtronic Grafton	3 lots

Table 1: Number of Lots Tested for Each Tissue Form

The relative amount of osteoinduction was evaluated semi-quantitatively by the study investigators using the scoring system described in Table 2, which is standard in the industry⁴; the observer was blinded to the identification of the implant. 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 5 highest scores from the histological slides; scores from each experimental group were determined by pooling the overall scores of the individual samples. The results of semi-quantitative scoring are presented as a mean \pm 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 2: Osteoinductivity Scoring Scale and Criteria

RESULTS

Following explantation after 28 days, samples from all of the lots that were tested were evaluated using histology for evidence of new bone growth. Histological findings of the Kolosis BIO Kore Fiber samples included the observation of newly formed bone that bridged particles of original bone and the formation of marrow elements (Figure 1). The Kore Fiber samples were also noted to be consistently osteoinductive in this model with 100% of the samples exhibiting evidence of osteoinduction, with an average OI score of 1.79 ± 0.73 (Table 3). The other demineralized bone grafts showed significantly lower OI scores and inconsistent osteoinduction throughout the lots for each other type of graft tested (Table 3). A comparison of the Kore Fiber to other bone grafts, in both the score and percent of samples that are osteoinductive, can be seen in figures 2 and 3 below.

<p><u>Kolosis BIO Kore Fiber:</u> Explant of demineralized cortical bone fibers demonstrating multiple regions of new bone formation (yellow arrows) and the presence of bone marrow (green arrows). H&E stain; 200X magnification.</p>	<p><u>Zimmer Biomet InterGro:</u> H&E stain; 40X magnification; BAR = 250 μm. New bone formation with marrow (arrows) and residual DBM associated with new bone.</p>	<p><u>Sea Spine/Integra Accell Connexus.</u> H&E stain; 100X magnification; BAR = 100 μm. This images demonstrates an inflammatory response with no new bone formation.</p>
<p><u>LifeNet Redigraft® BLX Demineralized Fibers:</u> H&E stain; 40X magnification; BAR = 250 μm. Demonstrates <25% area of new bone formation (yellow arrows) among the residual original bone fibers of the implant.</p>	<p><u>Medtronic Grafton® Putty:</u> H&E stain; 100X magnification; BAR = 100 μm. Demonstrates a limited area of new bone formation (yellow arrows) among the residual original bone fibers of the implant.</p>	

Figure 1: Histology images of explants from each test article

Summary Statistics	Osteoinduction Score (0-4 Scale)		# of Samples with Osteoinduction	Percentage of Osteoinductive Samples
	Mean	Std Dev		
Kolosis BIO Kore Fiber	1.79	0.73	24/24	100% (24/24)
Zimmer Biomet InterGro Putty	0.85	0.79	23/24	61% (14/23)
Sea Spine/Integra Accell Connexus	0.77	0.95	29/32	59% (17/29)
LifeNet BLX Fibers	0.57	0.59	23/24	48% (11/23)
Medtronic Grafton	0.52	0.73	23/24	43% (10/23)

Table 3: Osteoinductivity Results Including OI Scores and the Percentage of Samples Found to Be Osteoinductive.

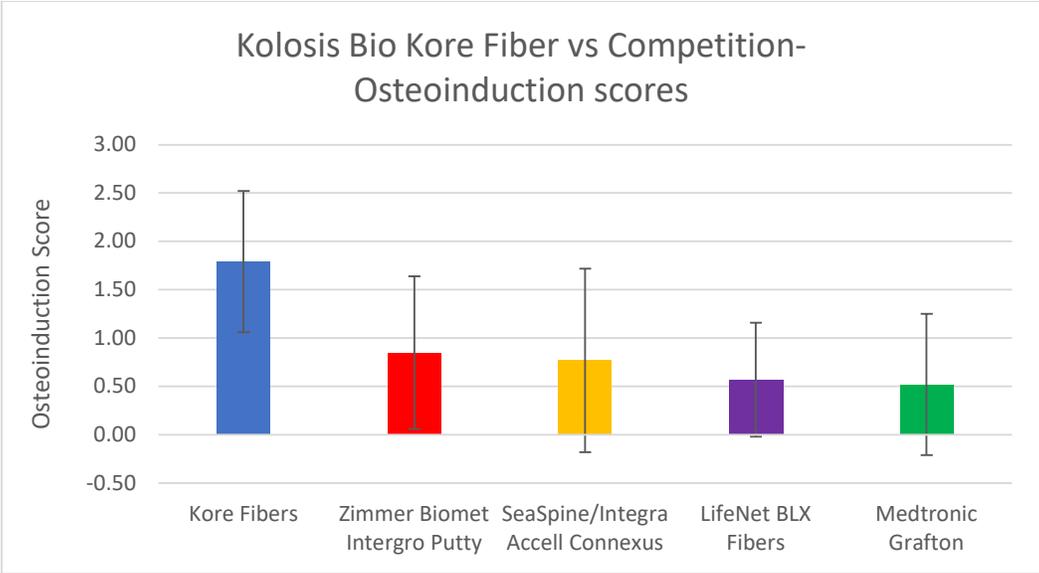


Figure 2: Osteoinductivity Comparison Among Graft Types - Osteoinduction Scores

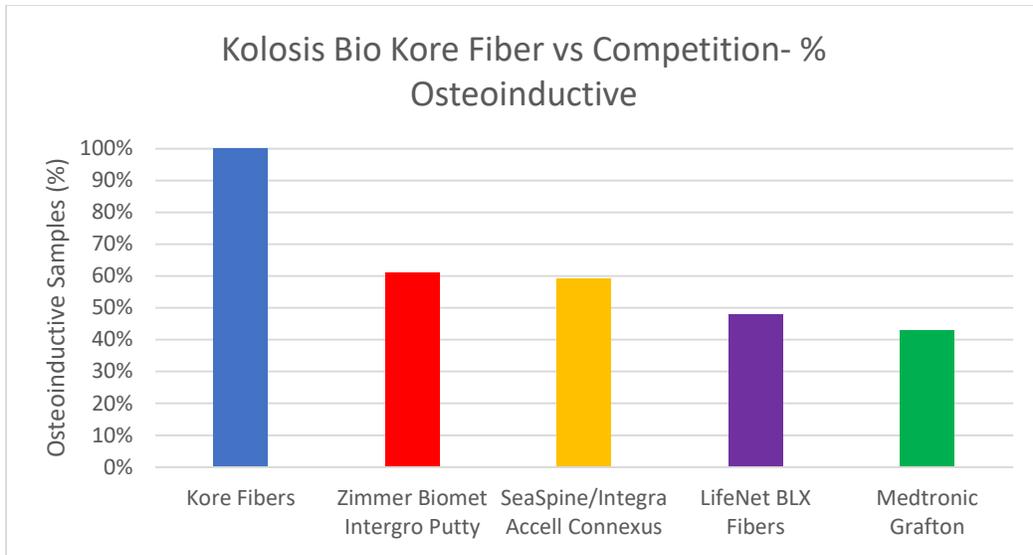


Figure 3: Osteoinductivity Comparison Among Graft Types - % Osteoinductivity

DISCUSSION & CONCLUSIONS

The efficacy of demineralized bone allografts has been previously shown to vary widely from samples that originate from one tissue processor to another⁵. 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, 6}. Kore Fiber are a moldable, demineralized cortical bone allograft that is aseptically processed in a gentle manner to maintain its biologic activity. This study was conducted in order to demonstrate that the osteoinductive properties of the Kore Fiber are preserved during the preparation of these grafts, as well as the consistency of OI in the model between donors. In addition, commercially-available bone grafts were also evaluated for their osteoinductive potential.

Kore Fiber consistently elicited bone formation in an ectopic site using the athymic mouse model in 100% of the implants. This highlights the consistency in the inherent osteoinductive property of the Kolosis tissue although a certain level donor to donor variability is expected in natural tissue². Robust bone formation with adequate marrow in the defect site can be observed from all 24 samples of Kore Fiber in contrast with the other grafts. The observations of this study suggest that the superior aseptic tissue processing methods of Kore Fiber preserve the endogenous biologic activity of natural tissue more effectively and consistently.

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