A Comparative Study of the Osteoinductivity of the Kolosis BIO® Kore Graft Fibers 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 is 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 Moldable Fiber® (processed by MTF Biologics, Edison, NJ; distributed by Kolosis BIO, Salt Lake City, UT) is a 100% bone allograft that is primarily composed of demineralized cortical bone fibers and is shapeable and putty-like once hydrated in a fluid such as blood or saline. In order to minimize any negative impact on its inherent biologic characteristics during processing, Kore Fiber is 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 native osteoinductive properties of the demineralized cortical bone fibers in Kore grafts have been preserved during processing 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 accessibility[4]. 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 Graft Fibers	3 lots
Zimmer Biomet InterGro® Putty	3 lots
SeaSpine/Integra Accell Connexus®	4 lots
LifeNet Readigraft® BLX Fibers	3 lots

Table 1: Number of lots tested for each graft type.

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 industry[4], and 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 ± 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 Kore Graft fiber samples included the observation of newly formed bone that bridged particles of original bone and the formation of marrow elements (Figure 1). These 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 performance of Kore Graft fibers to other bone commercially available grafts, in both the score and percent of samples that are osteoinductive, can be seen in Figures 2 and 3 below.

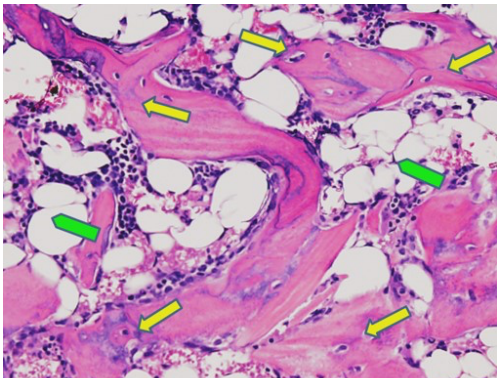
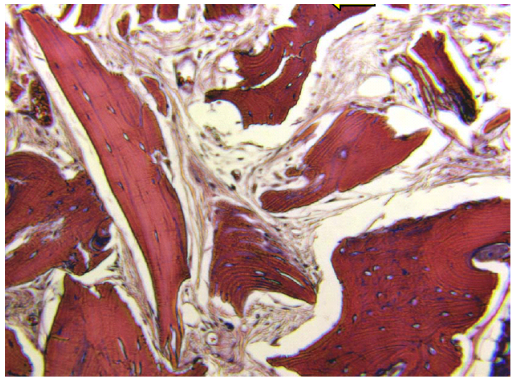
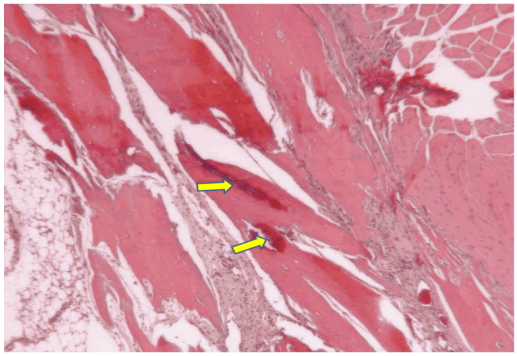
Kolosis BIO Kore Graft Fibers: *Explant of demineralized cortical bone fibers demonstrating multiple regions of new bone formation (yellow arrows) and the presence of bone marrow (green arrows). H&E stain.*	***Zimmer Biomet InterGro:*** *Limited areas of new bone formation with marrow (yellow arrow) dispersed between residual DBM particles. H&E stain.*
	
SeaSpine/Integra Accell Connexus: *Minimal evidence of new bone formation with fibrous tissue surrounding original DBM. H&E stain.*	***LifeNet Readigraft BLX Demineralized Fibers:*** *Sparse regions of new bone formation (yellow arrows) among the residual original bone fibers of the implant. H&E stain.*
	

Figure 1: Histology images of explants at 20x magnification

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

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

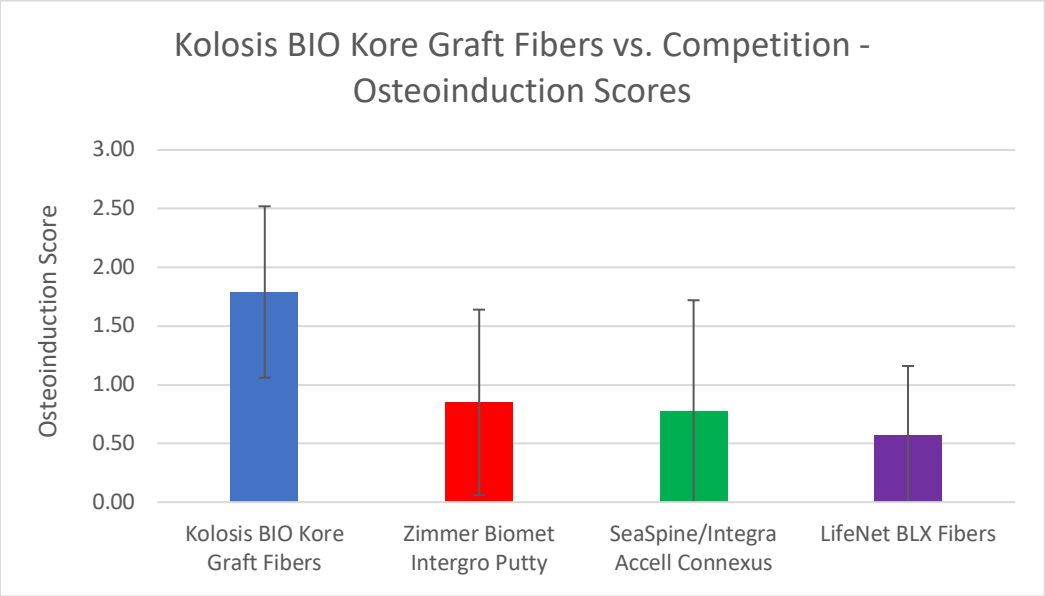


Figure 2: Osteoinductivity comparison among graft types - osteoinduction scores.

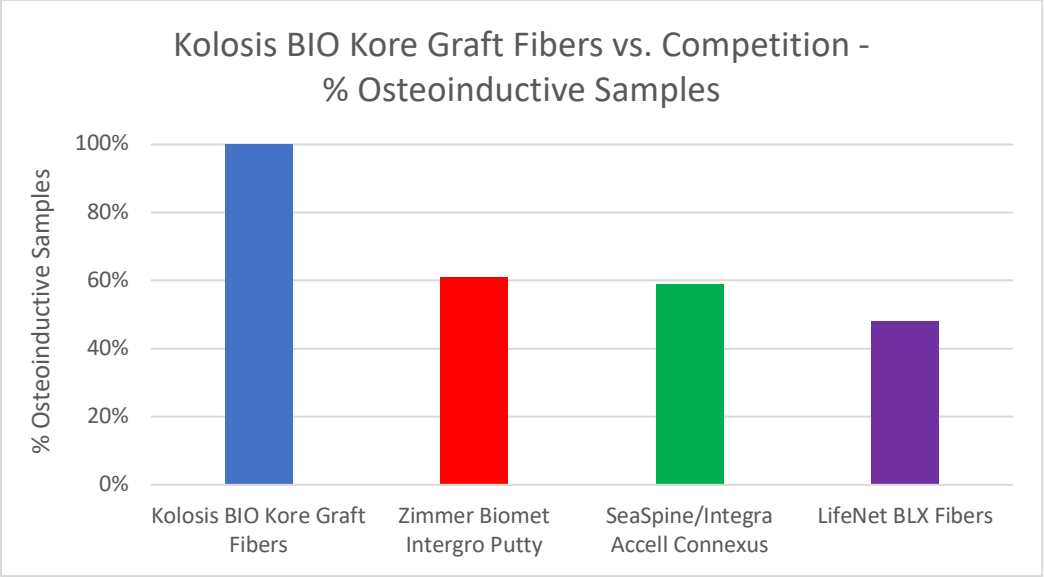


Figure 3: Osteoinductivity comparison among graft types – percentage of samples that exhibited evidence of osteoinduction.

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[5]. 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 Moldable Fiber is a shapeable, putty-like demineralized cortical bone allograft that is primarily composed of cortical bone fibers and is aseptically processed in a gentle manner to maintain its inherent biological characteristics. This study was conducted in order to demonstrate that the native osteoinductive properties of Kore Graft fibers are preserved during the preparation of these grafts, as well as to show the consistency of their performance in the athymic mouse model between donors. In addition, commercially available bone grafts were also evaluated for their osteoinductive potential.

Kore Graft fibers elicited bone formation in an ectopic site for 100% of the implants using the athymic mouse model. These findings highlight the consistency in the preservation of the inherent osteoinductive properties of the fibers although a certain level of donor-to-donor variability is expected in natural tissue[2]. More specifically, robust bone formation with adequate marrow in the defect site was observed from all 24 samples of Kore Graft fibers in contrast with the other grafts where only 61% or less of the samples were osteoinductive. The observations of this study suggest that the specific aseptic tissue processing methods used for the bone fibers in Kore grafts preserve their inherent biologic properties more effectively and reproducibly than in other competitive allogeneic DBM and fiber grafts.

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