Mineral Fiber-Reinforced Plate Implantation in a Sheep Model: Long Term Safety and Bio-Integration In-Vivo Study

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Introduction

Historically, most orthopedic implants were made of metal as it provides mechanical strength and durability, essential for stable bone fixation[1,2]. However, complications such as irritation, implant migration, stress concentrations and bone tissue loss, infections and sensitivity reactions to metal alloys, all pose the frequent need for hardware removal procedures, and drove the development of various biomaterials for bone fixation[2,3]. Biomaterials, traditionally polymer-based implants, carry their own risks, such as adverse inflammatory reactions to the polymer degradation by-products[4,5]. A new class of mineral fiber-reinforced implants used in this study offers strong bio-integrative bone fixation, enabling complete restoration to native anatomy while avoiding adverse inflammation. The implants are composed of continuous reinforcing mineral fibers comprised of elements found in native bone (SiO_2, Na_2O, CaO, MgO, B_2O_3, and P_2O_5) and bound together by PLDLA [poly (L-lactide-co- D,L-lactide)] (70:30 L:DL ratio) (50% w/w)[6]. In a previous implantation study in rabbits, fiber-reinforced nails were shown to completely bio-integrate by 104 weeks following in-bone implantation[7]. In a first-in-human 2-year multi-center study, mineral fiber-reinforced hammertoe implants were proven to provide safe and effective bone fixation following proximal interphalangeal joint arthrodesis, with high scores for bio-integration and joint fusion rate[8].

Study Objective

The purpose of this double-arm animal study was to evaluate the safety and bio-integration profile of mineral fiber-reinforced plates, following *in vivo* implantation over the surface of the bone. The study serves as a translational model of over-the-bone fixation techniques commonly used with various orthopedic devices (i.e., bone plates or fixation staples).

Materials and Methods

Bio-integrative mineral fiber-reinforced plates (OSSIOfiber®, OSSIO Ltd., Caesarea, Israel) were implanted bilaterally on the medial surface of the tibia of eight female sheep (Ovis Aries) (**Fig. 1**). Troughs or decortication were not performed in order to maintain the initial prominence of the plates over the surface of the bone. The left tibias underwent periosteal elevation (PE), allowing for plate implantation directly over the cortical surface, while right tibias were implanted over intact periosteum (Non-PE) (**Fig. 2**). Animal care and surgical procedures were performed in accordance with the local Research Unit Ethics regulations. Animals were closely monitored and clinically evaluated throughout the course of the study, over a time frame of 30 months (134 weeks) following implantation. Microcomputed tomography and histopathology were performed at 13, 26, 52, 78, 104, and 134-weeks post-implantation. Overall cellular response and cell types, bioabsorption (i.e., phagocytosis, M1/M2-like macrophages/giant cell infiltration), necrosis, hemorrhage, foreign debris (other than implant) and mineralization were semi-quantitatively assessed and graded according to ISO-10993-6, Annex E (with modifications). Mesenchymal tissue ingrowth into the implant site and new bone formation at the implanted region were also evaluated.

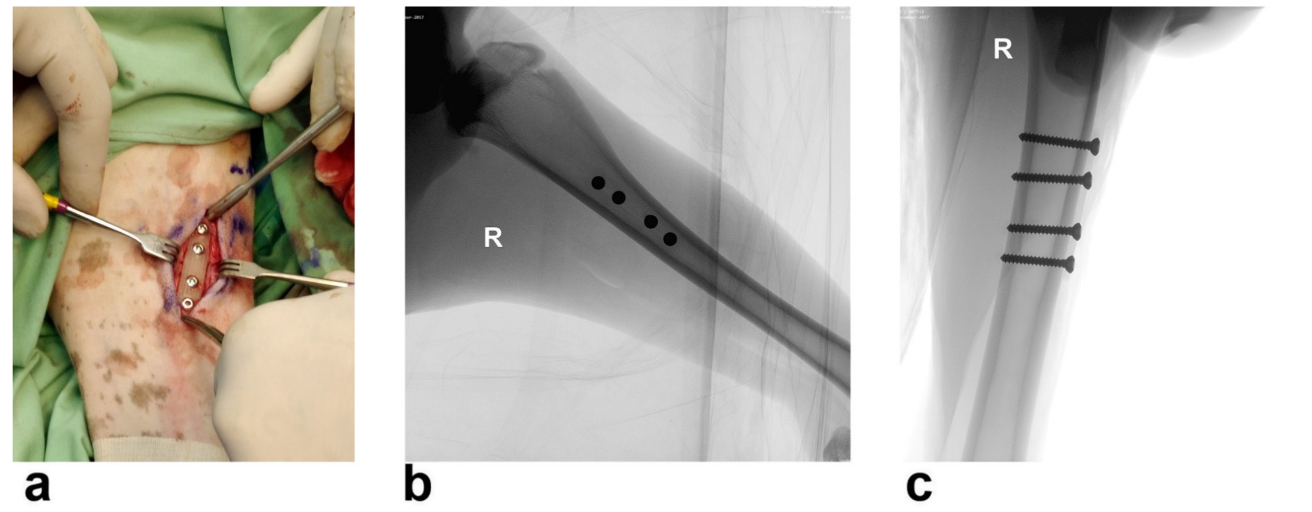

Figure 1: **(a)** Intraoperative photograph of mineral fiber-reinforced plate implantation. Four 3.5 mm stainless-steel screws were used to fixate each plate to the bone. **(b)** Lateral view and **(c)** anteroposterior view radiographs of plate implanted over the medial shaft of the proximal tibia, immediately postoperative. Note the fiber-reinforced plates are barely visible due to the material radiodensity, similar to that of the adjacent native bone.

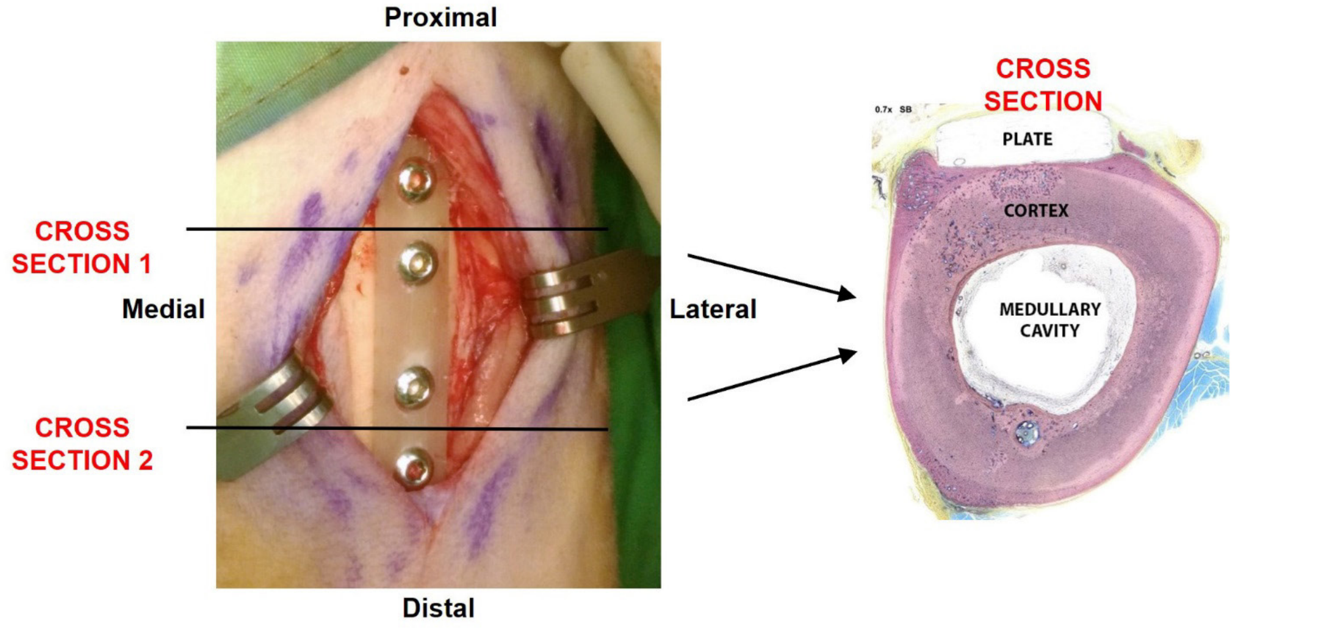

Figure 2: Sectioning planes of tibial bone and plate samples; the proximal cross-section (Cross Section 1) was stained with hematoxylin and eosin, and the distal cross-section (Cross Section 2) was stained with Stevenel's blue.

Results

No adverse clinical observations were noted through the entire course of the study and no adverse macroscopic findings were found upon gross examination at necropsy. New bone growth was evident adjacent to the implant at 13W and 26W, forming a thin structural "tent" around the implant, somewhat more substantial in the PE compared to non-PE samples. This "tenting" remodeled down and was no longer visible at later timepoints (**Fig. 3**). Mesenchymal tissue ingrowth substantially increased within the site by 78W and reduced at 134W in both groups as the implant bio-integration completed. Cellular response was dominated by anti-inflammatory M2-like macrophages and multinucleated giant cells (MNGCs) corresponding to phagocytic activity. This cellular activity was similar for both groups (PE & Non-PE) at each timepoint. Inflammatory cells (i.e., eosinophils, lymphocytes, plasma cells, and M1-like macrophages) were not observed. The phagocytic cellular response had started at 13W (**Fig. 4a&b**), with the highest activity rate at 52W-78W (**Fig. 4c&d**). Low amounts of mineral fibers were evident at 78W and were no longer observed (fully remodeled) by 104W. At 104W, only residual polymer material was left, and cellular response subsided (**Fig. 4e&f**). By 134W, all implants were fully bio-integrated with no material remaining (**Fig. 4g&h**).

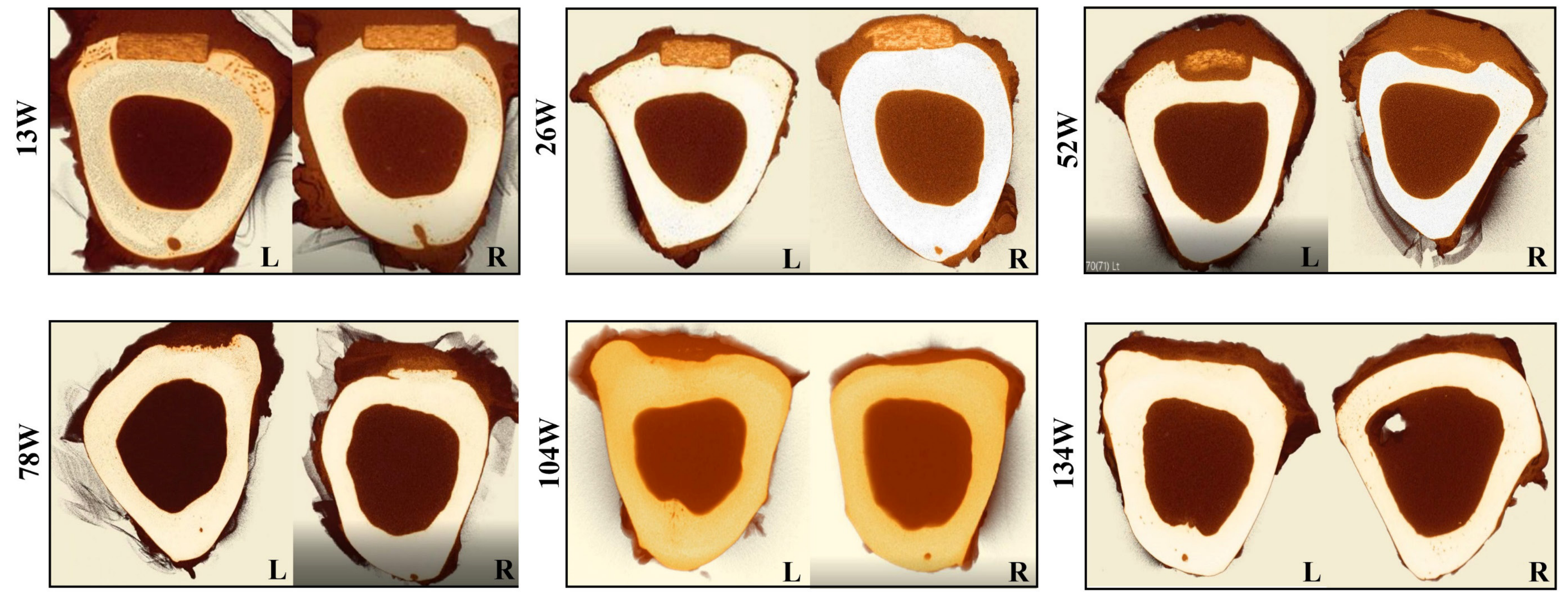

Figure 3: Micro-CT cross-sectional images of sheep tibias with mineral fiber-reinforced plates implanted directly over the cortex (PE) in the left (L) limb, and over the intact periosteum (non-PE) in the right (R) limb. **13W** and **26W**: Note periosteal new bone growth adjacent to the implant, forming a thin structural "tenting" around the implant. **52W**: The implant is less visible, and the "tent" surrounding the plate has remodeled. **78W**: The implant is barely visible and has mostly absorbed. **104W** and **134W**: The implant is no longer visible.

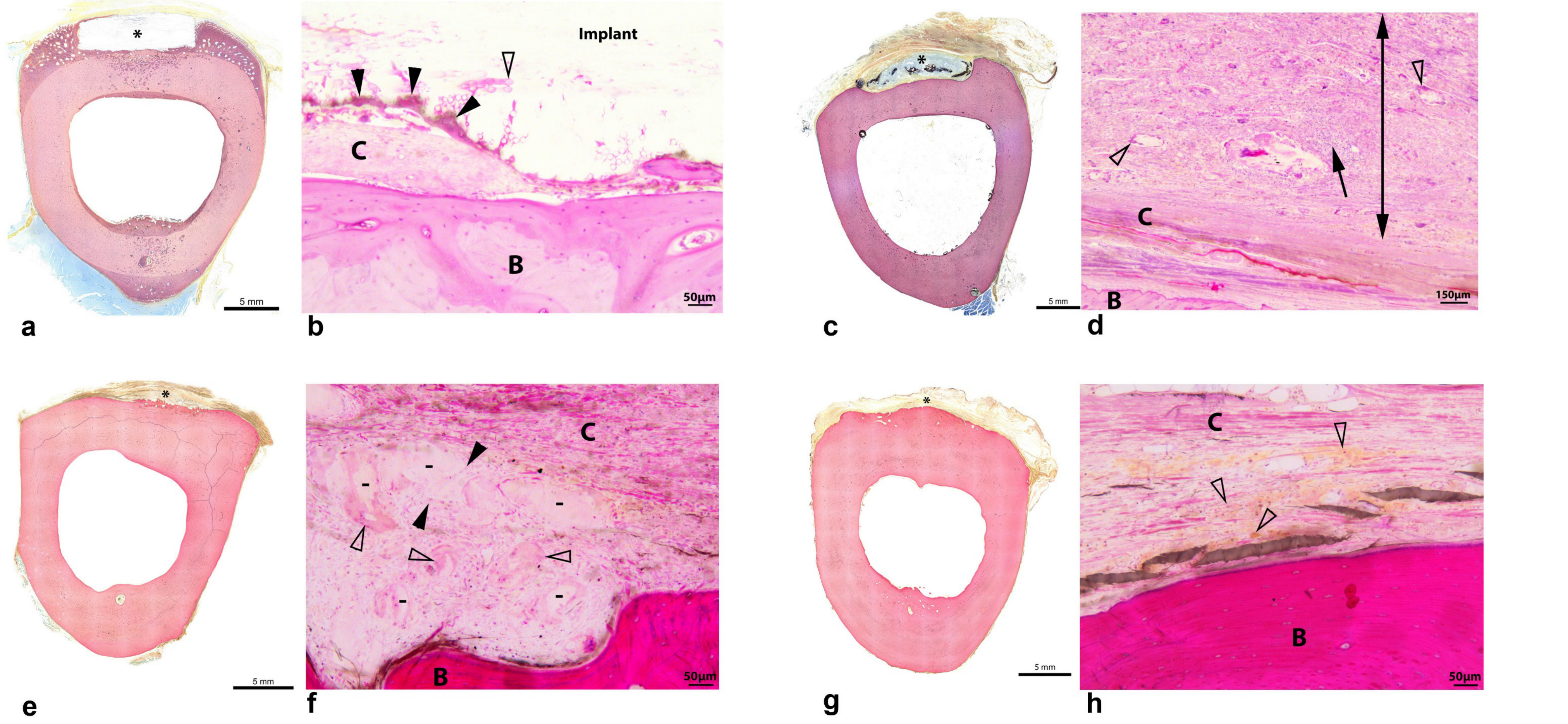

Figure 4: Cross-sections of sheep tibias with mineral fiber-reinforced plate implanted directly over the cortex (periosteal elevation, PE), and over intact periosteum (non-PE), stained with Stevenel's blue (overview of slide; **a,c,e,g**) and H&E (higher magnification demonstrating edge of plate and bone; **b,d,f,h**). **13W** (**a-b**): Plate is fully present due to the early stage of bio-integration (**a**). The mineral fibers are evident (open arrowhead), and the polymer component is undergoing a very early stage of bioabsorption, represented by occasional multinucleated giant cells (filled arrowheads). There is a thin layer of connective tissue (C) residing between the tibial bone (B) and overlying implant (**b**). **52W** (**c-d**): Implant (asterisk) has decreased substantially in size compared to 13W (**c**). A layer of connective tissue (C) is present between the bone (B) and the residual implant material; the implant contains mesenchymal tissue ingrowth (double arrow) and exhibits a peak cellular response, composed of numerous multinucleated giant cells (open arrowheads) and macrophages (arrow) (**d**). **104W** (**e-f**): Plate is nearly completely bio-integrated without residual polymer evident (asterisk) (**e**). Small amount of residual polymer implant material (dashes) surrounded by low numbers of macrophages (solid arrowheads) and multinucleated giant cells (open arrowheads), consistent with the final stages of bio-integration. The implant site contains connective tissue (C) and a small amount of bone (B) (**f**). **134W** (**g-h**): implant is completely bio-integrated without residual polymer evident (asterisk) (**g**). No residual polymer remaining, with only few macrophages (open arrowheads) noted within the tissue where the plate was originally located. The implant site contains connective tissue (C) and a small amount of bone (B) (**h**).

Conclusions

- This study demonstrated the safe application of mineral fiber-reinforced plates over the bone surface in a sheep model, with complete bio-integration into the surrounding tissue, and with no adverse tissue response.
- The implants demonstrated a desirable biocompatibility response. A gradual increase in cellular activity was demonstrated via phagocytosis of the polymer portion, and this subsided as the polymer was eliminated.
- The healing response at 134W was optimal and essentially equivalent, whether the periosteum was originally elevated or remained intact.

Impact Statement and Future Directions

- Large animal models are considered benchmark systems for the assessment of long-term effects of orthopedic implants due to their similarity to humans with respect to bone composition, dimensions, physiology, biomechanics, and remodeling characteristics[9].
- This newly developed bone fixation technology prevents the inconvenience and possible complications of both hardware removal procedures common to metal implants as well as the risk of adverse inflammation and tissue reactions characteristic of conventional polymeric implants.
- In this double-arm study, we compared periosteal elevation (PE), which mimics an Open Reduction Internal Fixation (ORIF) surgical approach and/or potential traumatic injury disrupting the periosteum, to implantation over intact periosteum (non-PE), simulating Minimally Invasive Surgical (MIS) techniques. Notably, in all cohorts similar optimal results were observed with respect to bio-integration and an anti-inflammatory profile (i.e., absence of M1-like macrophages, eosinophils, lymphocytes, and plasma cells).
- This study suggests the feasibility and versatility of these fiber-reinforced implants and their potential use for a wide range of orthopedic fixation devices and applications.
- Future clinical studies are planned for these bio-integrative implants' performance in specific orthopedic indications.

References

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