Bio-Integrative Soft Tissue Fixation Using Fiber-Reinforced Screw in an Anterior Cruciate Ligament Reconstruction Model in Sheep

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INTRODUCTION: ACL reconstruction (ACLR) using a bone-tendon-bone or an all soft-tissue graft is one of the most common procedures performed with more than 500,000 reconstructions performed annually in the USA alone and perhaps the most widely studied construct in the orthopedic literature[1]. These injuries usually require designated fixation devices that promote integration between soft and hard tissues which is essential to optimize surgical outcomes[2,3]. Interference screw (IS) fixation is a reliable and frequently used method as it allows rigid, direct fixation within the bone tunnel and facilitates osseous healing or tendon-bone incorporation allowing early range of motion and physiologic load[4,5]. With high initial fixation strength and load-to-failure mechanics, metal screws were considered the gold standard for many years. However, distortion of postoperative MRIs, potential laceration of the graft as screws are inserted or removed, and the trending efforts to minimize the presence of permanent implants, there is a clinical shift to their bioabsorbable counterparts[6,7]. Polymer-based implants overcome the limitations of their metal counterparts but present other limitations including intra- and postoperatively screw breakage or migration and material-related complications such as adverse inflammation, cyst formation, synovitis, or systematic allergic response appearing even years after implantation[8,9]. Therefore, there remains an unmet need to solve these present limitations. The bio-integrative fiber-reinforced screw evaluated in this study may offer a favorable solution. The safety and performance of these implants has previously been shown in various models to provide higher mechanical fixation strength than conventional bioabsorbable implants while supporting a balanced pH environment[10] and undergoing a gradual integration with bone avoiding a prolonged or late-stage adverse inflammatory response[11,12]. This present study evaluates the soft tissue fixation of this newly introduced bio-integrative screw in an established sheep model.

METHODS: Nine female sheep (Ovis Aries) were subjected to intraarticular implantation of the right knee with three 4.75 mm fiber-reinforced screws made of continuous 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 a degradable polymer [poly (L-lactide-co- D,L-lactide), PLDLA] (70:30 L:DL ratio), in 50% w/w ratio[12] (OSSIO*fiber*®, Ossio Ltd.). Screws at implantation sites 1 & 2 were implanted in an interference fashion, utilizing an autologous lateral digital extensor tendon while site 3 was implanted directly in bone (Fig. 1). The native ACL tendon was not compromised and left intact. Animals were closely monitored and clinically evaluated throughout the course of the study over a time frame of 30 months (132 weeks) following implantation. Histopathology was performed at 28, 52, 104, and 132-weeks (W). Viability of the tendon graft was evaluated at the relevant sites by its cellularity and ossification at the anchored sites. Overall cellular response and cell types, bioabsorption (i.e., phagocytosis, M1/M2-like macrophages/giant cell infiltration), and mesenchymal tissue ingrowth and new bone formation at the implantation sites were semi-quantitatively assessed and graded (on a scale 0-4) according to ISO-10993-6, Annex E.

RESULTS: All implantation procedures were completed successfully with no intra-operative implant breakage or failure recorded. Furthermore, there were no implant migrations reported in any of the animals. No adverse clinical observations were noted throughout the course of the study and no adverse macroscopic findings were found upon gross examination at necropsy. At 28W, both soft tissue and in bone implanted sites demonstrated mesenchymal ingrowth into the device wall (Fig. 2a&e) which significantly increased by 52W (Fig. 2b&e). As bio-integration response progressed, mesenchymal tissue ingrowth continued to increase, reflected by a score of 4 (±0) at 104W (Fig. 2c&e). At 104 and 132W the implant was completely or nearly completely replaced by new bone formation and connective tissue ingrowth, respective of whether it was a soft tissue fixation site or direct implantation in bone (Fig. 2c&d). Graft viability was evident at the earliest time point and the trend continued through 132W with tissue integration observed along the graft-tendon interface with increasing graft cellularity at 104W (3.44 ± 0.18) (Fig. 3a). At 132W the graft showed substantial bone integration with areas that were largely ossified (score of 3.29 ±0.34), namely deeper in the canal supporting bone anchoring due to definitive integration (Fig. 3b&c). Anti-inflammatory M2 macrophages and giant cells remained low through all time points, with minor increase between 52-104W which is attributed to an acceptable non-adverse phagocytic response. All other inflammatory cells (i.e., M1 macrophages, polymorphonuclears etc.) were absent through the entire study course.

DISCUSSION: The success of ACLR depends largely on solid and stable healing of the graft in a bone tunnel. Enhancing graft viability and integration with host bone is critical to facilitate early and aggressive rehabilitation for a more reliable and predictable return to full activity. Bone remodeling also plays an important role in the graft-to-bone fixation during the healing process[13]. Different studies have reported that bone ingrowth promotes graft healing[14]. This is well reflected in the current study which demonstrates the safe and advantageous use of the fiber-reinforced screw in an *in vivo* animal model analogous to a commonly performed human orthopedic application. This bio-integrative screw provides secure fixation of the graft and a favorable local environment enabling increased graft cellularization i.e., graft mid-substance viability through the ligamentization process, with mesenchymal ingrowth and new bone formation, while gradually and securely integrating with the surrounding bone.

SIGNIFICANCE: The present study demonstrates the safe and successful bio-integrative soft tissue fixation within a bone tunnel in an ACLR model with no long-term adverse effects.

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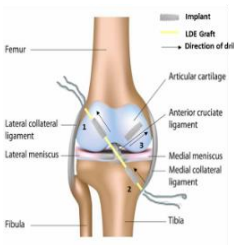

Figure 1: Anatomical illustration of the sheep knee and implantation model. LDE- Lateral Digital Extensor Tendon. **Site 1**- interference implantation in femur. **Site 2**– interference implantation in tibia. **Site 3**- femur implantation directly in bone

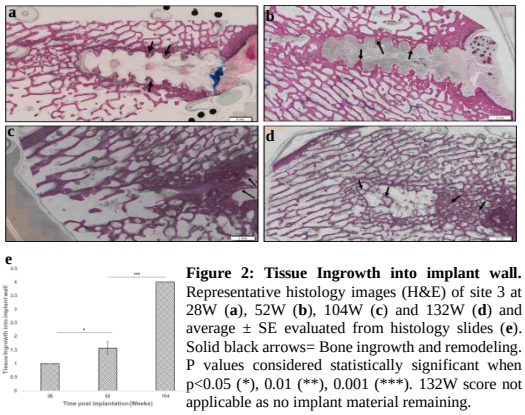

Figure 2: Tissue Ingrowth into implant wall. Representative histology images (H&E) of site 3 at 28W (**a**), 52W (**b**), 104W (**c**) and 132W (**d**) and average ± SE evaluated from histology slides (**e**). Solid black arrows= Bone ingrowth and remodeling. P values considered statistically significant when p<0.05 (*), 0.01 (**), 0.001 (***). 132W score not applicable as no implant material remaining.

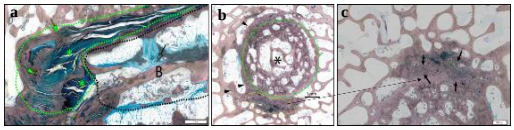

Figure 3: Graft Viability: Cellularity and Ossification. Representative histology images (SB) of site 1 at 104W (**a**), site 2 at 132W (**b**), and magnification of b (**c**). Black dotted line=implantation tract showing regeneration of trabecular bone (B) with no residual implant material remaining and minimal residual phagocytic response (clear arrow). Green dotted line=area of tendon graft showing new bone integration. Green asterisks=recellularized graft with areas of ossification (green arrows). Green circle=implantation site in cross section, showing complete bio-integration and replacement by new trabecular bone (asterisk); Solid arrowheads=new bone along the edges of implanted site and graft. Solid black arrows= advanced new bone formation within implantation site.