Evaluation of the integration of silk fibroin ligament-like tissue into the bone after ACL reconstruction of the sheep model, using 7Tesla MR imaging.

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Target audience: Musculoskeletal radiologists and physicists

Purpose: Currently, the incidence of partial or complete cruciate ligament rupture is very common and represents a major reason of instability of the knee. Anterior cruciate ligament (ACL) reconstruction has become an approved clinical procedure for injuries of the ACL. It is typically performed using either tendon tissue from the same subject or using various tissue engineering approaches. In current studies, the most promising material for tissue engineering is silk fibroin (SF) as scaffold, which demonstrated the superior mechanical and biological properties compared to other alternatives. Such a scaffold serves as substrate for cells, which migrate into the SF and form new ligament-like tissue. Surprisingly, there is still a lack of knowledge on the integration of SF scaffolds into bone. Thus, the goal of this study was to evaluate the integration of the newly developing ligament in a sheep model 6 and 12 months post-surgery by using in-vitro MRI analysis.

Methods: In a sheep model, the ACL of the right leg was excised and the tibial and femoral bone tunnels were created. Both ends of a custom-made SF scaffold (diameter 6.0 mm) were sutured with 1–0 polyester suture (Ethibond, Johnson & Johnson, USA) in a whip-stitch style. Then the scaffold was passed through the bone tunnel and joint cavity. Finally, both ends were fixed by sutures tied over "endobuttons" in femur and tibia. After defined time-points at 6 months, 6 sheeps were sacrified and after 12 months another 5 sheeps were sacrified. The osteointegration was analyzed using 7 Tesla MR scanner (Siemens Medical Solution, Erlangen, Germany). The dedicated 28 channel Tx/Rx knee coil (QED, OH, USA) was used. The ultrashort echo time (UTE) technique [1-3] was used for the imaging. The measurement parameters were next: TR/TE = 13/5.12 ms, FOV = 133 x 159 mm, resolution = 0.24 x 0.24 x 0.8 mm, bandwidth = 233 Hz/pix, flip angle = 10º, total measurement time was 9min 10sec. Images were calculated online, using built-in SYNGO software. ROIs were drawn on the ACL in femur and tibia, as well as in the alcohol which was surrounding the sample for fixation. Mean values and standard deviations for femur, tibia as well as alcohol were recorded. ROI values were afterwards normalized to the alcohol. A two tailed independent t-test was performed. Histology of the bone samples were performed and compared to the MR images.

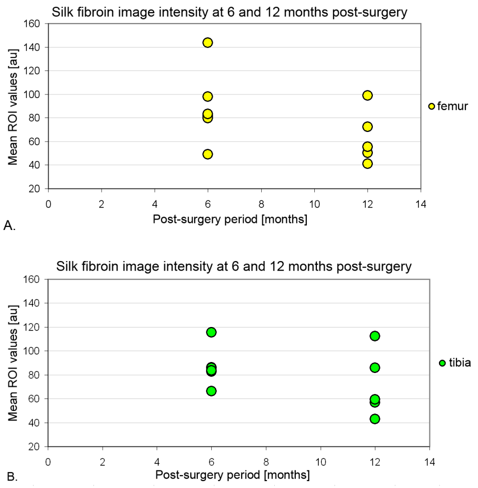

Fig.1: show the 6 months vs 12 months post-op UTE image intensity in the femur (A.) and the tibia (B.)

Fig.2: Example of the sagital oriented UTE image of the sheep-knee.

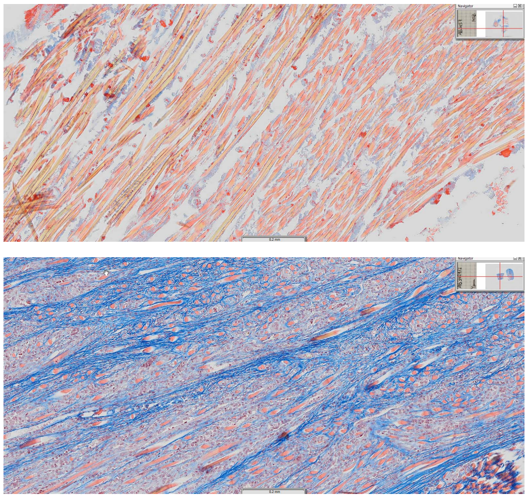

Fig.3: histological image of the SF ligament-like tissue:(top) after 6 months, (bottom) after 12 months

Results: The fig.1 show UTE image intensity at early (6 months) and late (12 months) post operative follow up for the femur (fig 1.A) and tibia (fig.1B). Mean value of the UTE image intensity for the femur at 6 and 12 months post-op was 89.25 ± 31.13 and 63.61 ± 22.82 respectively. Similarly, mean value of the UTE image intensity for the tibia at 6 and 12 months post-op was 86.71 ± 15.93 and 71.54 ± 27.57 respectively. A two tailed independent t-test was performed. We were not able to find a statistical significant difference for femur (p= 0.161) and for tibia (p=0.282). Fig. 2 show example of UTE image. Yellow arrow show the sagital oriented silk fibroin ligament-like ACL reconstruction tissue. Outer ring provide high intensity signal. Green arrow show native healthy ligament, which is fixed into the femur and detached from tibia on the bottom end. Figure 3 show histology of the SF ligament-like tissue 6 months (top) and 12 months (bottom) post-op.

Discussion: The results show decreasing MRI image intensity of the newly formed ligament-like tissue. MR analysis demonstrated that after 6 months the silk scaffolds were surrounded by soft tissue that further developed to a tight osteointegration. In addition, our study show that UTE is the technique of choice for MR imaging of the very short T2* structures, like a silk fibroin based ligament. Small number of samples could be one possible explanation of the results of statistical significance.

Conclusion: The integration process of the silk fibroin based ligament-like tissue in in-vitro animal models described in the presented study seems to be a promising treatment. The UTE MRI technique has proven its feasibility for application in either native or newly developed ligament-like tissue. Advantage of UTE is its applicability on the clinical MR scanners.

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References

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