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Augmentation of tendon graft anterior cruciate ligament reconstruction outcome using a silk based osteoconductive sheath

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Background: With increased longevity in the global aging population, joint health remains critical as the quality of life is increasingly associated with human mobility. Coupled with the growing focus on an active lifestyle, intervention procedures performed on the knee have been gaining popularity, particularly the Anterior Cruciate Ligament (ACL) reconstruction. Such surgical intervention often involves the use of tendon autografts, with semitendinosus and gracilis tendons being popular choices. Nevertheless, less than optimal healing of the tendon graft within the surgically created bone tunnel remains a fundamental problem in this procedure. The proposed solution involves the application of a novel silk fibroin (SF) based sheath, embedded with nanoparticles of low crystallinity hydroxyapatite (nHA), to complement the use of tendon autografts and promote enthesis formation. This SF-nHA sheath configuration is tested against SF sheaths with incorporation of bone morphogenetic protein 2 (BMP-2) only (SF-BMP2), a blend of BMP-2 and nHA (SF-BMP2-nHA) and pure silk sheaths (pure SF) to evaluate their potential in osteointegration of tendon grafts. The optimal sheath type was subsequently put through an in depth assessment for biocompatibility and also *in vivo* study in a small and large animal model for up to 9 months to evaluate the efficacy of the sheath in promoting osteointegration of tendon grafts.
Materials and methods: Knitted SF scaffolds (240 fibroins, L30 × Ø5 mm) were first fabricated from raw *Bombyx mori* silk (Chul Thai Silk Co. Ltd) and subsequently degummed. The nHA precipitates were synthesized by a co-participation method of aqueous $(NH_4)_2HPO_4$ with aqueous $Ca(NO_3)_2$. Hydrothermal treatment of the precipitates' aqueous solution was carried out at 140 °C under a pressure 0.3 MPa for 2 h in an autoclave to form the nanograde rod HA crystals. SF sponges, made from a blend of aqueous SF solution (2% w/v) and synthesized nHA (12.9 mg/ml), were incorporated to the knitted structures via a lyophilization process in a customized mould. This SF-nHA group was compared with pure SF, SF-BMP2 (19.3 μg/ml BMP-2) and SF-BMP2-nHA (19.3 μg/ml BMP-2 and 12.9 mg/ml nHA) via static culture of 10 × 10 mm specimens with seeded porcine bone marrow derived MSCs (P2, 0.5 × 10^6/scaffold) over 28 days. *Ex vivo* static culture of the four groups of SF sheaths with tendon autografts (porcine Flexor Digitorum Profundus sections, L10 × Ø5 mm) and porcine bone marrow derived MSCs (P2, 2 × 10^6/scaffold) was then conducted over 28 days. In these assessments, the cellular viability, proliferation, gene expression, collagen deposition levels, scanning electron microscopy (SEM) and histological analyses were performed (n = 3). Consequently, the most efficacious group for osteointegration of tendon grafts was selected for the in depth biocompatibility assessments and *in vivo* tests.
The complete optimized sheath was then tested through a series of biocompatibility assessment for cytotoxicity, sensitization, intracutaneous reactivity, acute systemic and 14 days sub-chronic repeated dose toxicity, genotoxicity (Ames test, chromosome aberration assay, mouse lymphoma assay) and 12-weeks implantation in rabbit femur. A preclinical trial was conducted in the porcine ACL reconstruction model using tendon autograft harvested from the flexor digitorium profundus and SF-based sheath sutured onto both ends of the graft. Graft integration in the presence of SF-based sheaths within the bone tunnels and tendon integrity within the intra-articular space were assessed via imaging (CT and MRI), histomorphometrical and histopathological methods at 1, 3, 6 and 9 months time points. All animal experiments were approved by the respective institutional IACUC.
Results: The SF sheaths were observed to be porous with interconnected pores. nHA and BMP-2 were observed to be securely incorporated in the lyophilized SF sponges. BMP-2 bioactivity was ascertained after the fabrication process and was shown to be eluting with an initial burst release, followed by a lowered sustained release.
MSCs were observed to be viable and proliferative in all four groups of the *in vitro* study. Increased proliferations were observed in SF-nHA, SF-BMP2 and SF-BMP2-nHA at the early phase (days 1-7) with an accelerated differentiation phase beginning from day 14. Consequently, there was an upregulation of osteogenic related genes (Collagen I (Coll I), Collagen III (Coll III), osteonectin (ON) and osteopontin (OPN)), leading to a significantly increased deposition of collagen by day 21. However, it was further noted that between SF-nHA, SF-BMP2, and SF-BMP2-nHA, the presence BMP-2 did not improve upon a persistent (beyond 21 days) upregulation of osteogenic genes and increase in collagen production.
When cultured with excised tendon sections, it was observed that cells from within the tendon tend not to participate in interfacial tissue regeneration, while seeded MSCs were viable and produced ECM for bridging the tendon-scaffold interface after 2 weeks in SF-nHA, SF-BMP2, and SF-BMP2-nHA as observed via SEM and H&E staining images. SF sheaths with nHA stimulated osteogenesis from seeded MSCs as observed by the presence of calcium deposits in SF-nHA and SF-BMP2-nHA via alizarin red staining. Ossification was thus observed with the presence of nHA, with or without the presence of BMP-2.
Consequently, the SF-nHA sheath configuration was selected for further biocompatibility assessment and *in vivo* testing. The SF-nHA sheath was found to induce discrete intracytoplasmic granules with no cell lysis or reduction in cell growth in the tested mouse connective tissue cell line (NCTC clone 929), indicating non-cytotoxicity. Sensitization tests indicated limited erythema and oedema at challenged skin site over the 48 hours. There were also no significant biological reactivity findings compared to the respective negative control groups in the acute systemic and sub-chronic repeated dose toxicity assessments. Genotoxicity (Ames test) showed that the sheath was non mutagenic in the tested bacterial strains of *Salmonella typhimurium* and *Escherichia coli*, while *in vitro* chromosome aberration test indicated that the sheath did not induce structural chromosome aberration in the cultured mammalian somatic cells. Histopathological assessments of the extracted femurs of the 12 weeks implantation study indicated absence of inflammatory cells with presence of neovascularization and bone ingrowth.
At 1 month post ACL reconstruction using the SF-based sheath with tendon autografts in the preclinical porcine model, the reconstructed ACL became taut, when initially the tendon graft was implanted slack. This indicated that there was simultaneous growth and graft remodeling during this period. Regenerated epiligament was also formed, which provided vascularization to the graft. It should be noted that the cartilage of the ACL reconstructed knee remained pristine and clear of cartilage erosion, which was indicative of accelerated and enhanced joint stability soon after the ACL reconstruction. The enhancement in osteointegration of tendon autograft was evident as multiple small foci of mineralization were identified within the femoral and tibial ends of the graft from as early as one month post ACL reconstruction. By 3 months, bone tissue infiltration into the interfacial space was evident from the increase in bone mineralization and vascularized neotissue formation, indicating improved graft to bone integration comparing to control (tendon autograft ACL reconstruction without SF-based sheath).
Discussion: Prompt osteointegration of the tendon graft within the bone tunnel post ACL reconstruction was difficult with conventional therapies due to the lack of biochemical precursors. These precursors are provided by the SF-based sheath, which also includes a temporary scaffolding material that helps to provide a snug fit to the bone interface. It will prevent micromotion resulting in early inflammatory reactions, which can lead to the onset of fibrosis. The sheath will also serve a delivery platform for cellular and bioactive components. Progenitor cells, either seeded or attracted from the host into the porous sheath, will reconstitute the native cellular environment of the enthesis by differentiating into chondrocytes and osteoblasts. They will not only deposit the necessary ECM but also cytokines that elicit regenerative responses at the integration site. The delivery of osteogenic factors via the SF-based sheath will accelerate tissue restoration by triggering a migration of host reparative cells. These bioactive agents will also induce cellular differentiation required for the formation of fibrocartilage and bony tissue at the anchorage site.
Results from the *in vitro* study indicated that although BMP-2 led to earlier upregulation of osteogenic genes, the expression of Coll I and ON were not significantly higher in SF-BMP2-nHA by day 28 when compared with SF- nHA. In terms of protein production, collagen synthesis between SF-nHA and SF-BMP2-nHA were not significant throughout the study, indicating that even though SF-BMP2-nHA might have stimulated the targeted genotypic behavior, the phenotypic outcome was not significantly improved. This was further substantiated when the silk sheaths were cultured with excised porcine tendons, whereby mineralized ECM could be found after 4 weeks of culture in SF-nHA and SF-BMP2-nHA.
Balancing clinical needs and our *in vitro* and *ex vivo* findings, the SF-nHA sheath was selected for further development. It was found that nHA stimulated tissue infiltration of host bone tissue, resulting in bone tunnel narrowing with new mineralized tissues observed in both the small and large animal models. Consequently, there was enhanced graft-to-host integration progressively over the 9 months implantation period, which potentially resulted in overall mechanical properties closer to that of the native bone-ACL-bone construct.
Conclusion: Based on our knowledge, this study is the first to investigate a SF-based device to augment ACL reconstruction with tendon autografts. With minimal disruption to current surgical practice, the SF-based sheath exhibits clinical potential in accelerating healing to allow earlier and more aggressive rehabilitation.
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Review of radiographic parameters in patellofemoral instability – How to best predict risk of patella dislocation?

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Patello-femoral instability refers to any functional and/or anatomical abnormality within structures surrounding the patella and the femoral trochlea leading to knee pain, patellar subluxation, dislocation and symptoms of patellar hypermobility.
There are four factors implied in patellofemoral instability: trochlear dysplasia, patella alta, abnormal tibial tubercle-trochlear groove distance (TT-TG), and patellar tilt. Previous literature has shown that trochlear dysplasia is the single most important factor implied in the genesis of patellar instability, especially for habitual and recurrent dislocation (ref). However, the measurement parameters used to define trochlea dysplasia show poor-to-fair inter and intra-observer correlation. We aim to refine these parameters to develop a more reliable radiographic assessment of patellofemoral instability.
We retrospectively evaluated the radiographs (CT patella tracking) of 120 patients with symptoms of patellofemoral instability (anterior knee pain, patella subluxation and dislocation), looking specifically at trochlea dysplasia and how the sulcus angle varied with different degrees of knee flexion and when measured at different parts of the patella. We also looked at how the sulcus angle was influenced by other factors such as the Wiberg classification of the patella and the posterior condylar and transepicondylar lines. Lastly, we looked at the changes in the sulcus angle after trochleoplasty and how the Dejour type influenced the type of trochleoplasty used.
Among 420 patients with clinical patellofemoral instability, 70 (16.7%) had bilateral patellofemoral instability, 190 (45.2%) had right-sided symptoms and 160 (38.1%) had left-sided symptoms. The predominant symptom was anterior knee pain (65%) followed by patella subluxation (43%).
The average sulcus angle (per knee) measured at 0° flexion at the patella equator was 165.3° and the average patella tilt at 0° flexion was 20.5°. 92% of subjects had radiographic trochlea