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

T.K.H. Teh1, P. Shi1, X. Ren1, J.H.P. Hui1, W.L.B. Tan1, J. Li1,2, J.C.H. Goh1,2
1Department of Biomedical Engineering, National University of Singapore, Singapore
2Department of Orthopedic Surgery, National University of Singapore, Singapore

Institute of Materials Research and Engineering, Agency for Science, Technology and Research, Singapore

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 interest in 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 fibrin (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 fibres, L0 x 35 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 (NH4)HPO4 with aqueous Ca(NO3)2. Hydrothermal treatment of the precipitates’ aqueous solution was carried out at 140 °C under pressure 0.3 MPa for 2 h in an autoclave to form the nanocrystalline HA crystals. SF was knitted 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 × 106/scaffold) over 28 days. In vitro static culture of the four groups of SF sheaths with tendon autografts (porcine Flexor Digitorum Profundus sections, L10 × 05 mm) and porcine bone marrow derived MSCs (P2, 2 × 105/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 found to be the SF sheath with BMP-2 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 toxicity. Apoptotic cells were stained by a TUNEL assay, and 12-2 days implantation in rabbit femur. A preclinical trial was conducted in the porcine ACL reconstruction model using tendon autograft harvested from the flexor digitorum profundus and SF-based sheath sutured onto both ends of the graft. Graft integration in the presence of SF-based sheath within the bone tunnels and tendon integrity within the intra-articular space were evaluated 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 scaffolds. 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 II (Coll II), osteocalcin (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 as observed from the growth 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 intratendonastic 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 the cartilage surfaces of 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 interfascial 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 scaffolded 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 with 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 integration 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 the 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-SF-based 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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B0865
Review of diagnostic parameters in patellofemoral instability — How to best predict risk of patella dislocation?

J. Lee, S. Tan, J. Hui
National University Hospital, Singapore

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, flexion contracture and malalignment of the patellofemoral joint. The predominant symptom was anterior knee pain (65%) followed by patella subluxation and dislocation, 190 (45.2%) had right-sided symptoms and 160 (38.1%) had left-sided symptoms of patellofemoral instability (anterior knee pain, patella subluxation and dislocation). We retrospectively evaluated the radiographs (CT patella tracking) of 120 patients with symptoms of patellofemoral instability (anterior knee pain, patella subluxation and dislocation) to develop a more reliable radiographic assessment of bilateral patellar instability.

Among 420 patients with clinical patellofemoral instability, 70 (16.7%) had bilateral patellofemoral instability. Among 240 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 of patellofemoral instability (anterior knee pain, patella subluxation and dislocation). The predominant symptom was anterior knee pain (65%) followed by patella subluxation and dislocation, 190 (45.2%) had right-sided symptoms and 160 (38.1%) had left-sided symptoms of patellofemoral instability (anterior knee pain, patella subluxation and dislocation).

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J. Lee, S. Tan, J. Hui
National University Hospital, Singapore
dysplasia defined as sulcus angle >145° with 74% having high-grade dysplasia (Dejour B-D) and 26% having low grade dysplasia (Dejour A). There were 236 (56.2%) patients with Wiberg 2 patella, 100 (23.8%) patients with Wiberg 3 patellas and 84 (35.6%) patients with Wiberg 1 patella.

The sulcus angle measured at the patella equator was 33% greater than the angle measured at the Roman arch. Subjects with Wiberg type 3 patellas were 34% more likely than type \( \frac{1}{2} \) to have a sulcus angle > 160° and more likely to have high-grade trochlea dysplasia (Dejour B-D). The sulcus angle had minimal correlation with the posterior condylar and anterior trochlea angles. Lastly, the sulcus angle had a 37% reduction post-trochleoplasty and 94% of post-operative patients had a post-operative sulcus angle of < 145°. In conclusion, the sulcus angle classically measured at the Roman arch is significantly smaller than that measured at the equator and this underestimates the true incidence of trochlea dysplasia. Also, Wiberg classification of the patella predicts likelihood and severity of trochlea dysplasia and should be employed in radiographic assessments of patellofemoral instability.

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Arthroscopic meniscoplasty for discoid lateral meniscus in children and adolescents — Long term results
Y.H. Ng, J.Z.J. Lee, A.K.S. Lim, J.H.P. Hui
Department of Orthopaedic Surgery, National University Hospital, Singapore

Background: The discoid meniscus is an uncommon condition mostly seen in adolescents, and they usually affect the lateral meniscus. Multiple studies have demonstrated a greater prevalence of discoid meniscus in the Asian population. The optimal mode of treatment of discoid lateral meniscus (DLM) is currently still not well established.

Materials and Methods: All consecutive patients less than 21 years old who underwent arthroscopic treatment for symptomatic DLM over a 10-year period, from 2002 to 2012, were retrospectively reviewed.

Results: A total of 29 patients were reviewed (35 DLMs), made up of 17 males and 12 females. There were 16 right-sided, 7 left-sided DLMs, with 6 patients presenting with bilateral DLMs. The mean age at diagnosis was 15.9 years old and the mean duration of follow-up was 84.8 months. The most common presenting complaints were mechanical knee pain (91.7%), knee clicking or snapping (50.0%) and locking the knee (45.8%). The incomplete type DLM (Watanabe type II) was the most common arthroscopic subtype of DLM. All patients were treated arthroscopically by (i) meniscoplasty alone, (ii) meniscal repair and meniscoplasty, (iii) partial meniscectomy and meniscoplasty, or (iv) total meniscectomy. Excellent outcome scores were reported in 21 out of 22 patients at 2 years, and there was significant improvement in Lysholm scores at all follow-up timepoints for patients in all 4 treatment groups. However, there was no significant difference in functional outcome scores between the 4 treatment groups, and the functional outcome scores of the patients were maintained to the time of final follow-up.

Conclusions: Our study demonstrated that arthroscopic meniscoplasty is an effective treatment modality for symptomatic DLM and yields excellent functional outcomes when performed alone or in conjunction with partial meniscectomy and meniscal repairs.

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Rotational comparison between double bundle ACL reconstruction and single bundle ACL reconstruction combine with antero lateral ligament (ALL) reconstruction
L.A. Pontoh, R. Gatam, T. Suryo
University of Indonesia, Indonesia

Purpose: The purpose of this study was to compare the knee rotation of the ACL reconstructed knee between double bundle ACL reconstruction and Single bundle ACL reconstruction combine with ALL reconstruction.

Methods: Ten single bundle ACL reconstructions combine with ALL reconstructions and ten double bundle ACL reconstructions were performed randomized. Magnetic Resonance Imaging TTTG was measured in all cases before surgery and three months after surgery.

Results: On average changes in TTTG before and after surgery were 3.8 mm in single bundle ACL reconstruction with ALL reconstruction group and 3.3 mm in double bundle ACL reconstruction group, there was no significantly statistic difference.

Conclusions: Double bundle ACL reconstruction might be superior to prevent internal rotation of the knee. Improved quality of future study would allow better outcome.

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