An improved strategy of autologous chondrocyte implantation with stratified zonal chondrocytes delivery for repair of articular cartilage

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INTRODUCTION: The zonal properties of articular cartilage critically contribute to the mechanical support and lubrication of the tissue. Current treatments for articular cartilage have yet to regenerate this zonal architecture, thus compromising the functional efficacy of the repaired tissue and leading to tissue degeneration in the long term. Layer-by-layer stratified implantation of zonal chondrocytes was proposed to recapitulate the zonal properties of articular cartilage. However, the implementation of stratified zonal chondrocytes implantation faces two challenges; the lack of an efficient zonal chondrocyte isolation protocol, and an expansion protocol that retain the phenotypic function of the expanded cells. We have previously reported on the use of a spiral microfluidic device for label-free, high-through-put size-based segregation and enrichment of articular cartilage zonal chondrocytes [1], integrated to dynamic microcarrier culture for the generation of expanded zonal chondrocytes [2]. In this study, the repair efficacy of zonal cartilage regeneration through bilayered implantation of expanded autologous zonal chondrocytes was investigated in a clinically relevant porcine chondral defect model over a period of 12 months.

METHODS:
In vivo procedures were performed in accordance with the Institutional Animal Care and Use Committee at the National University of Singapore. Autologous chondrocytes extracted from articular cartilage from the non-weight bearing trochlea region of the knee were subjected to an expansion-sorting strategy, integrating dynamic microcarrier (dMC) culture and spiral microchannel size-based zonal chondrocyte separation [2]. Zonal chondrocytes were implanted as bilayered fibrin hydrogel construct (dMC-BL, n=6), comprised of superficial zone chondrocytes overlaying middle/deep zone chondrocytes, into defects at the femoral condyles of the porcine knee. Repair efficacy was compared with implantation with cell-free fibrin hydrogel (Control, n=6) and full thickness (FT) cartilage-derived heterogenous chondrocytes (TCP-FT, n=5 and dMC-FT, n=6). Cartilage repair was evaluated at 12 months including morphological, histology, immunohistochemistry, polarized light microscopy, micro-CT and biomechanical measurement. Lived magnetic resonance imaging (MRI) were performed at 1, 12, 24, 36 and 48 weeks to track the zonal cartilage regeneration progress. Statistical significance (set at $p<0.05$) between 3 or more groups was evaluated by one-way ANOVA with Tukey post-hoc test. Statistical significance between 2 groups was evaluated by independent-samples t-test.

RESULTS: At 12 months, the bilayer-treated defects (n=6) has significantly higher ICRS macroscopic and histological scores than Control (n=6), TCP-FT (n=5), and dMC-FT (n=6) treatments (Fig. 1). Histomorphometric analysis of type I and II staining intensities shows significant improvement in the hyaline cartilage quality. Overview of the MRI T2 relaxation time values of the zonal regions of the repaired tissues (Fig 3A) indicates significant improvement with dMC-BL treatment in both superficial and middle/deep layers as early as 3 months compared to Control, TCP-FT and dMC-FT groups. Notably, dMC-BL treatment attained a superficial zone T2 value similar to that of normal cartilage, starting from 9 months. T2 value of middle/deep zone of dMC-BL treatment reached healthy cartilage values as early as 3 months, 6 months ahead of dMC-FT, while both TCP-FT and Control group did not reached normalcy at 12 months. The dMC Bilayer group had the highest number of regenerated tissues showing distinct optical birefringence of collagen fibers among all the treatment groups. Micro-CT analysis indicates significant improvement of the subchondral bone repair (Fig. 2). The improved morphological, histological outcomes and zonal development of the Bilayer-treated defects was corroborated with the biomechanical competency of the repair tissues, in terms of surface lubrication and compressional strength, that were comparable to normal tissue at 12 months (Fig 3B). Treatment with TCP-FT and dMC-FT were significantly better that Control, but were significantly lower than normal tissue.

DISCUSSION: The proof-of-value 12 months animal studies provide unequivocal evidence on the efficacy of the stratified zonal chondrocytes implantation for enhanced cartilage repair. We speculate that the positioning of the defined layers of SZ over M/DZ chondrocytes has contributed to the immediate in situ surface lubrication of the implanted constructs, while established the compressive strength in the deeper zones. This would have facilitated the shear-compression induced zonal maturation of the regenerated tissues, providing better weight distribution in both the cartilage and subchondral bone, that could be important for the long term integrity of the regenerated tissue.

SIGNIFICANCE/CLINICAL RELEVANCE: The results demonstrate that with appropriate expansion and isolation of zonal chondrocytes, the strategy of stratified zonal chondrocyte implantation can be implemented to facilitate the recapitulation of the zonal characteristic and consequently improved the quality and mechanical function of the repaired articular cartilage. Our approach could represent a significant advancement to current Autologous Chondrocyte Implantation-based cartilage regeneration, with the potential to improve the long-term integrity of the regenerated tissues.

REFERENCES:
1. Yin L, Wu Y, Yang Z, Denslin V, Ren X, Tee CA, Lai Z, Lim CT, Han J, Lee EH. Characterization and application of size-sorted zonal chondrocytes for articular cartilage regeneration. *Biomaterials*. 2018 May;165:66-78.
2. Tee CA, Yang Z, Yin L, Wu Y, Han J, Lee EH. Improved zonal chondrocyte production protocol integrating size-based inertial spiral microchannel separation and dynamic microcarrier culture for clinical application. *Biomaterials*. 2019 Nov;220:119409.

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IMAGES AND TABLES:

Figure 1

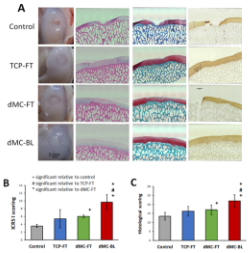

Figure 2

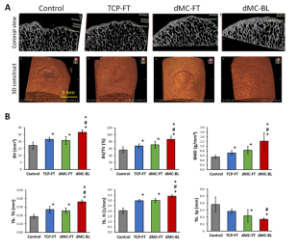

Figure 3