A biomimetic mechanically reinforced collagen-based scaffold promotes the repair of large cartilage defects in goats

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INTRODUCTION: The ability of traditional treatments to repair large-area of articular cartilage defects, providing functional recovery with minimal risk of revision surgery, remains an unmet clinical challenge[1]. Our lab has previously developed biomimetic porous collagen hyaluronic acid biomaterials which have shown significant potential for chondral tissue regeneration; however, due to their poor mechanical properties and low compressive moduli (0.5-1.5kPa), their use has largely been restricted to repair of small articular defects [2,3]. In order to enhance their clincial use, we have designed a novel 3D printed polycaprolactone (PCL) framework to reinforce the biomimetic collagen-based scaffolds[4], thus providing the appropriate mechanical properties to support physiological loads while restoring function in larger cartilage defects. Following their successful *in vitro* evaluation[4], this study aimed to evaluate the regenerative capacity of these mechanically reinforced collagen-based scaffolds in large load-bearing articular cartilage defects in a well-stablished *in vivo* goat stifle/knee model. Additionally, this study also aimed to investigate the effectiveness of a newly developed (in our lab)[4] suture fixation technique used to secure the scaffold to the subchondral bone, thus addressing the major challenge of biomaterial fixation to the adjacent native bone tissue and to the articular cartilage joint surfaces.

METHODS: 3D printed polycaprolactone (PCL) framework: an innovative gyroid design was 3D printed with an Allevi II (3D Systems) as previously shown in our lab[4], having a compressive modulus mimicking the physiological range; scaffold: 0.67MPa, healthy cartilage: 0.5-2.0 MPa.

Incorporation of collagen-based matrix: a previously designed pro-chondrogenic scaffold composed of type I/II collagen-hyaluronic acid (CI/CII-HyA)[3] was prepared and incorporated onto the PCL framework before being freeze dried to produce a biomimetic mechanically reinforced robust scaffold (Fig.1).

In vivo evaluation: cell free reinforced scaffolds (Ø8mm; height 1-1.25mm) were implanted in large cartilage defects (Ø8mm) in the goat medial femoral condyle. To secure the scaffold to the chondral defect, a commercially available osteo-suture screw (Smith&Nephew Twinfix Ti) was used to implant subchondral fixation anchors (cylindrical Ø1.8mm with a height of 2.8mm) which were connected to the scaffolds by resorbable (PDS II 3-0, Ethicon) or nor-resorbable (Prolene 4-0, Ethicon) suture thread. Each animal, with a total number of 3, was treated with 2 scaffolds (one per joint) which were fixed by using resorbable (n=3) or non-resorbable (n=3) suture thread per joint. Following 6 months implantation, animals (2-3 years old) were sacrificed followed by macroscopic and histological evaluation of tissue integration and cartilage regeneration. µCT analysis was used to assess the integration in the subchondral bone of the anchors with the effective fixation of the biomaterial to the articular cartilage defects. Ethical approval (University College Dublin AREC 12-17).

RESULTS: Following the successful *in vitro* assessment of the enhanced mechanical properties and the pro-chondrogenic regenerative capacity of the mechanically reinforced scaffolds[4], we evaluated the regenerative capacity of these scaffolds *in vivo* (Fig. 2). Overall, the scaffold promoted the regeneration of the large chondral defects in all animals at early time point of only 6 months post-implantation. The macroscopic evaluation of the articular cartilage at the defect site revealed the formation of new cartilage covering mostly the full defect area in 5 out of 6 cartilage defects as shown in Fig. 3. Moreover, µCT analysis enabled us to validate the effectiveness of the suture fixation technique of the scaffold to the adjacent subchondral bone demonstrating to secure the scaffold to the chondral defect while avoiding any major damage to the subchondral region (Fig. 4). Regardless of using resorbable or nor-resorbable suture threads, the joints treated with scaffolds demonstrated to have a mean of BV/TV 40.57% (non-resorbable) and 37.32% (resorbable) approaching the BV/TV mean relative to the intact native tissue taken from the contralateral medial condyle which was 47.84%. Initial histological assessment by the mean of Safranin O (revealing sulphated glycosaminoglycans, typical of articular cartilage) and type II collagen (typical of healthy articular cartilage) has demonstrated the quality/quantity of the tissue formed within the chondral defects; thus supporting the regenerative capacity of the scaffolds.

DISCUSSION: This study has demonstrated strong promise for the novel mechanically reinforced collagen-based scaffold to repair large clinically challenging load-bearing articular cartilage defects in the goat medial femoral condyle - after only 6 months of treatment. Macroscopic and histological evaluation shows the formation of new cartilage covering mostly the full defect area suggesting strong chondral regenerative potential of the scaffolds with ability to facilitate satisfactory filling of the large articular cartilage defects. Moreover, our study demonstrates the potential of an innovative suture fixation technique to secure the scaffolds to the subchondral bone region - addressing a major challenge of biomaterial fixation to the adjacent native bone tissue and to the articular cartilage joint surfaces. The µCT analysis revealed that regardless of using resorbable or nor-resorbable suture threads (i) the scaffolds were successfully secured to the underneath subchondral bone fully covering the area of defect and (ii) that the osteo-suture fixation anchors implanted to the subchondral bone region were well integrated to the original healthy bone without causing any significant damage to the tissue as shown by the BV/TV values.

SIGNIFICANCE/CLINICAL RELEVANCE: This novel reinforced collagen-based scaffold in combination with our innovative suture fixation method provide a promising and viable treatment approach to much larger chondral defects, becoming a promising alternative strategy to large cartilage defect repair.

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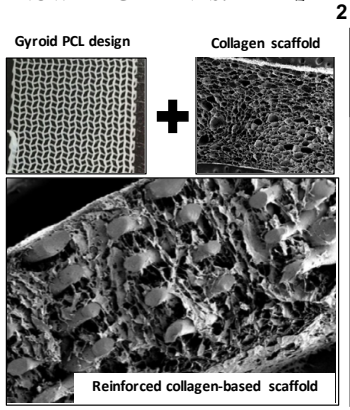

Fig.1 Representative SEM images of the biomimetic mechanically reinforced scaffold developed by combining an innovative 3D printed gyroid polycaprolactone (PCL) framework with a biomimetic collagen-based composition[4].

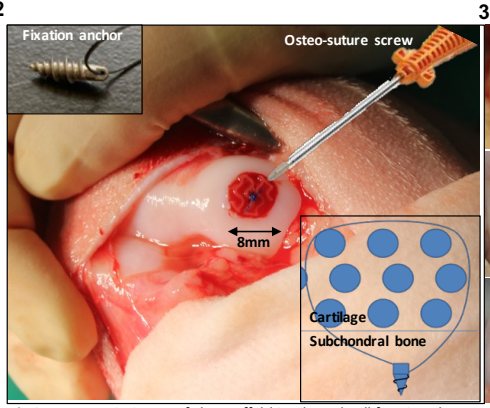

Fig.2 Macroscopic image of the scaffold implanted cell-free in a large cartilage defect (Ø8mm) in the goat medial femoral condyle. To secure the scaffold to the chondral defect, a commercially available osteo-suture screw was used to implant the subchondral fixation anchor which was connected to the scaffold by suture thread[4].

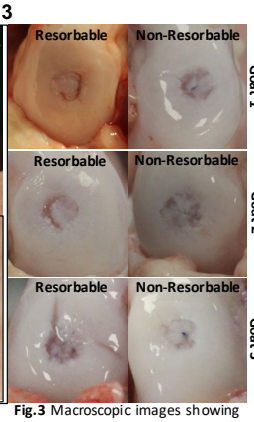

Fig.3 Macroscopic images showing that the scaffolds supports cartilage repair after only 6 months of treatment (regardless of the suture type).

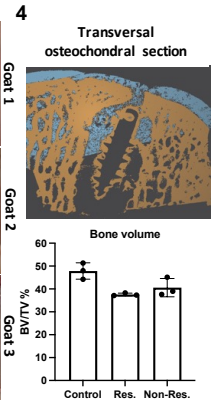

Fig.4 Representative µCT image of a osteochondral section treated with scaffold and quantification of the bone mineral fraction in the defects after 6 months.