Understanding of collagen type II organization in different shapes of cartilage microtissues

Aylin Kara Özenler[1,2], Mark van Voorst[1,2], Juuso Tuppurainen[3,4] , Janne Mäkelä[3,4], Mylène de Ruijter[1,2,5], and Jos Malda[1,2,5]

[1]Department of Orthopedics, University Medical Center Utrecht, Utrecht, The Netherlands
[2]Regenerative Medicine Center Utrecht, Utrecht, The Netherlands
[3]Department of Technical Physics, University of Eastern Finland, Kuopio, Finland
[4]Diagnostic Imaging Center, Kuopio University Hospital, Wellbeing services county of North Savo, Kuopio, Finland
[5]Department of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

UMC Utrecht

UNIVERSITY OF EASTERN FINLAND

INTRODUCTION

Articular cartilage (AC) provides frictionless joint movement while transferring loads to the subchondral bone [1]. Its ability to resist high stresses is based on the distinctive arcade-like organization of collagen type II fibers. Although many regenerative medicine strategies aim to replicate this hierarchical microarchitecture, none have successfully achieved this. In this study, we investigate how collagen type II organizes within engineered microtissues of predefined shapes, aiming to identify the mechanisms that govern fiber orientation during AC repair and regeneration.

MATERIALS & METHODS

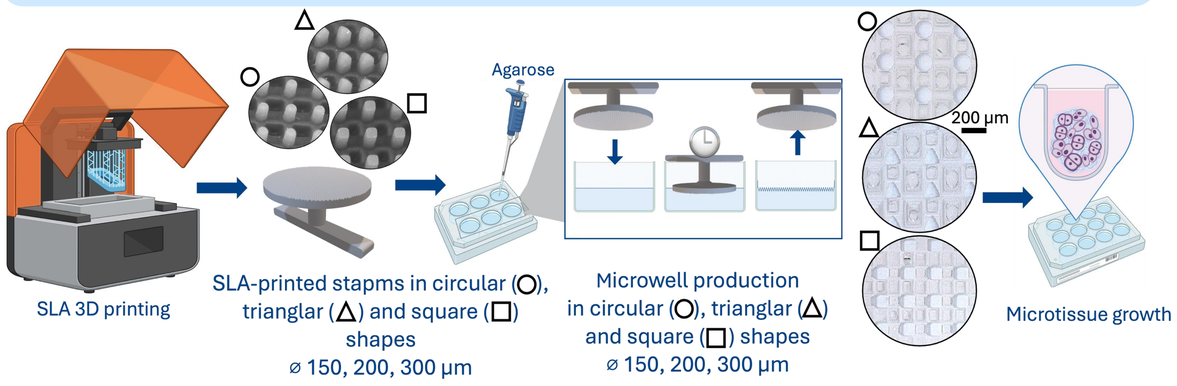


Figure 1. Schematic illustration of the study.

Negative molds of different geometrical shapes (square, triangular, circular, ∅ =150, 200, and 300 µm) were produced by stereolithography 3D printing technique (SLA, Formlabs). Microwells were prepared using these molds by patterning into the agarose. Articular cartilage progenitor cells (ACPCs) were seeded into the microwells. Cell behavior was examined using light microscopy (Olympus BX-43) and fluorescence imaging (Leica Thunder Live Cell Imager). Deposition of extracellular matrix (ECM) components was assessed by (immuno)histochemical staining and biochemical assays. Collagen fiber orientation was observed with polarized light microscopy (PLM). Moreover, collagen organization was modelled as fibril-reinforced poroviscoelastic swelling (FRPVES) model using Abaqus (v6.12-3, Dassault Systemes, USA) [2].

RESULTS - Microtissue Growth in Microwells

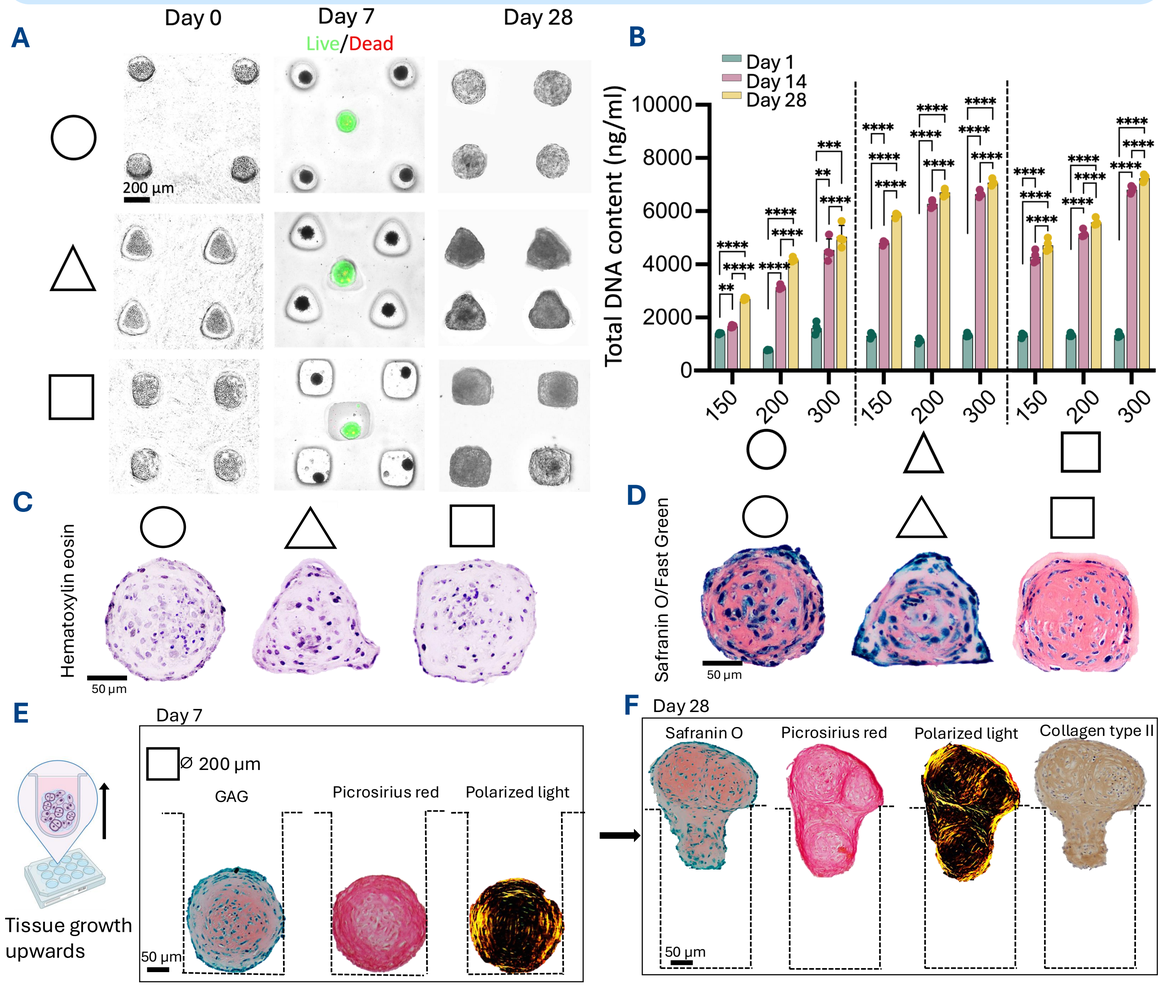


Figure 2. ACPC microtissue growth for 28 days culture period.

Light microscope images of ACPCs growing with high viability (live cells in green) in the circular, triangle, square microwells and reflecting predefined shapes (Fig 2A). Total DNA amount increased during the culture period that indicates cells proliferated within the microwells (Fig 2B). Data are shown as mean ± SD, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ indicate statistically significant difference between groups. General morphology of the microtissues stained with hematoxylin eosin (Fig 2C) and cartilaginous ECM deposition with GAG deposition on day 28 (Fig 2D). Microtissue growth in the vertical direction upwards from microwells showing GAG and collagen deposition within square shaped microtissues on day 7 (Fig 2E) and day 28 (Fig 2F).

RESULTS – Collagen Assessment

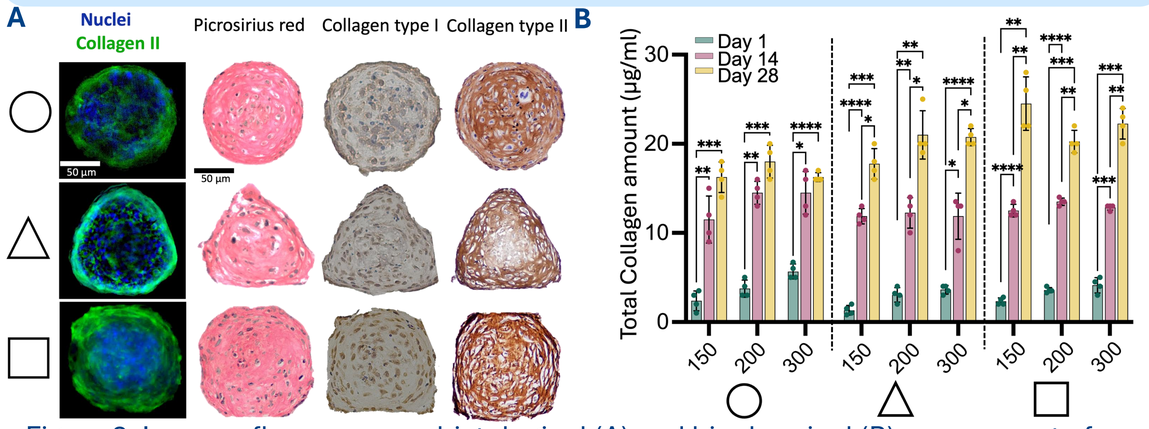


Figure 3. Immunofluorescence, histological (A) and biochemical (B) assessment of collagen.

Fluorescence images show collagen type II deposition (green) predominantly on the outer region of the microtissues (Fig 3A). Histology staining shows total collagen (picrosirius red), collagen type I and type II deposition (Fig 3A). During maturation, the collagen alignment was concentrated specifically at the outer edges of the predefined shape. Total collagen amount increased during the culture period (Fig 3B). Data are shown as mean±SD, $^{*}p<0.05$, $^{***}p<0.001$, $^{****}p < 0.0001$ indicate statistically significant difference between groups.

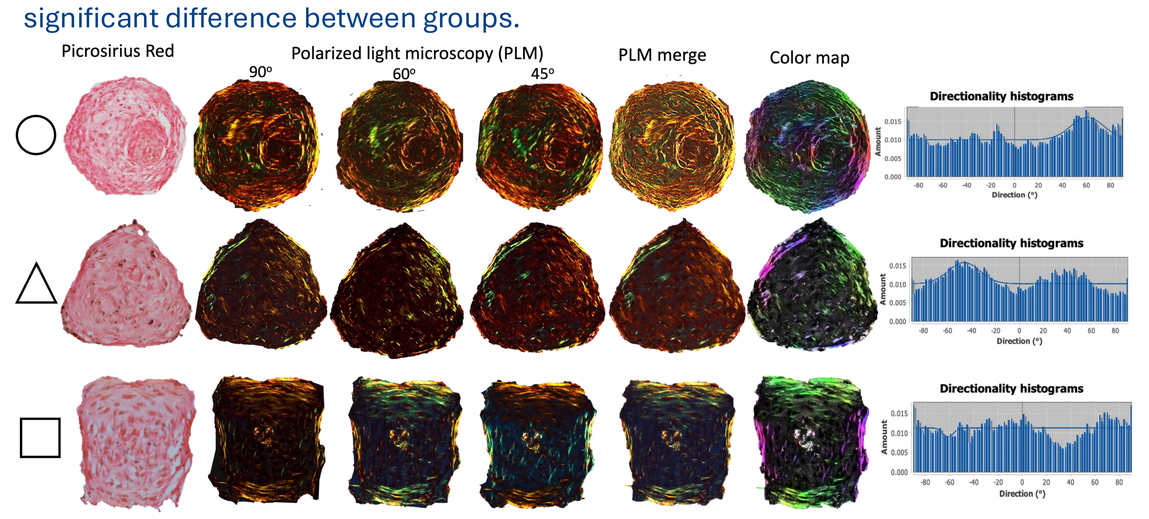


Figure 4. Assessment of collagen fibers within different shapes of microtissues.

Picrosirius red staining and polarized light imaging at different angles reveal that collagen fiber orientation in microtissues is aligned following the shapes.

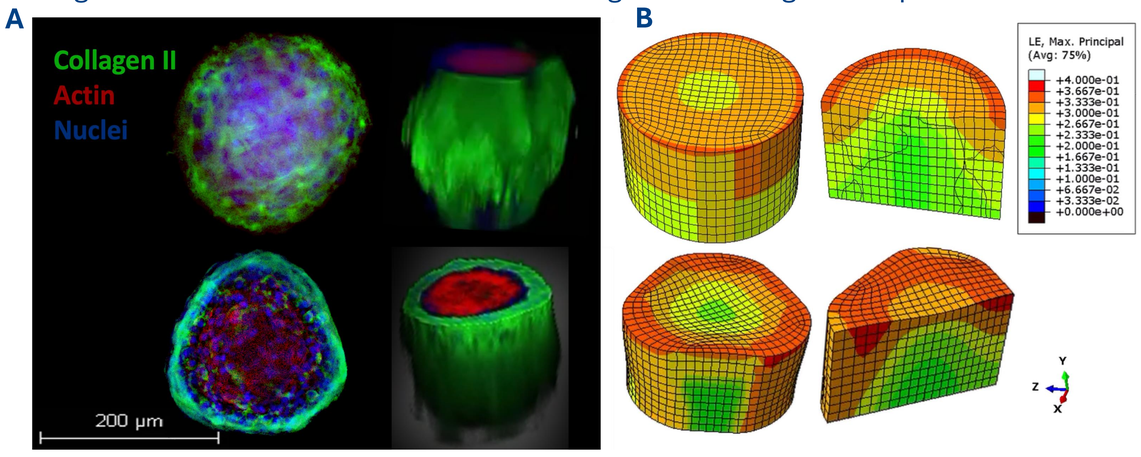


Figure 5. Assessment of the collagen organization with fluorescence microscopy (A) and computational modelling (B).

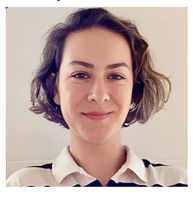
Fluorescence images (Fig 5A) showed collagen type II predominantly accumulated on the outer part of the microtissue. Computational modeling (Fig 5B) of microtissue growth shows mechanical stresses and strains in the outer regions of the microtissues, consistent with the fluorescence data. These mechanical stresses and strains caused by tissue swelling can also guide collagen fiber organization.

CONCLUSIONS

This study provides a proof-of-principle for guided collagen type II deposition through the differentiation of ACPCs in predefined shapes. The results demonstrate that spatial confinement influences extracellular matrix deposition and organisation. Furthermore, the organization of the collagen network is affected by the shape of confinement, predominantly through mechanical stresses within the microwells.

This study offers insights into how shape-guided tissue formation can be leveraged, for example in biofabrication strategies to generate functional and durable AC grafts.

References
[1] Benninghoff, A. Form und Bau der Gelenkknorpel in ihren Beziehungen zur Funktion. Z.Zellforsch 2, 783–862, 1925.
[2] Wilson, W et al., Journal of Biomechanics, 38, 1195-1204, 2005.



UMC Utrecht
Aylin Kara Özenler
a.karaozenler@umcutrecht.nl

TERMIS EU-Chapter 2026 PALMA DE MALLORCA 21-24 APRIL / SPAIN
LS·CARE micro2MACRO materials-driven regeneration
DRIVE-RM
Funded by the European Union
erc European Research Council Established by the European Commission