Mimicking osteochondral architecture through integrated multi-scale 3D in vitro scaffolds

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1 Introduction

Degenerative joint diseases such as osteoarthritis (OA) affect nearly one quarter of Europe's population and remain a major therapeutic challenge



Cartilage and subchondral bone restoration requires precise tissue architecture and mechanical integrity. This study presents the development of a six-layer osteochondral model replicating the native cartilage-bone interface, aiming to provide an efficient tool for the *in vitro* modelling of cartilage regeneration.



2 Methodology

Fabrication of six-layer gradient pore osteochondral scaffolds

Hydroxyapatite incorporation into calcified cartilage and subchondral bone layers

Morphological and physicochemical characterization

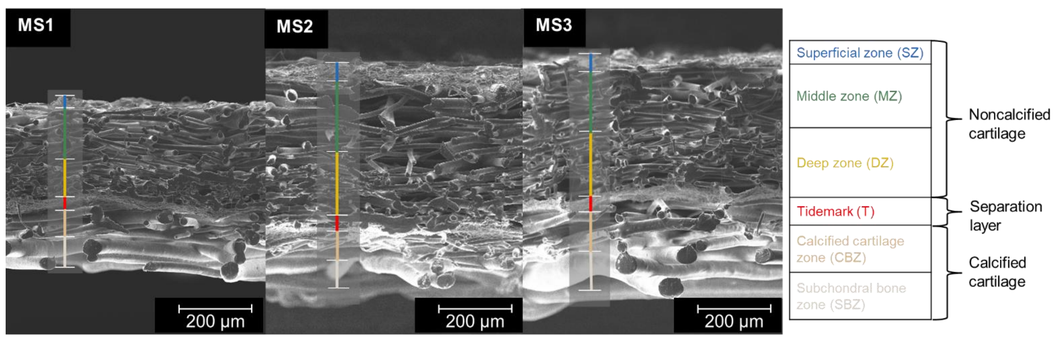
Fluorescence imaging and 3D evaluation of cell distribution across tidemark

Compartment specific seeding and fluorescent labeling of C28/I2 chondrocytes

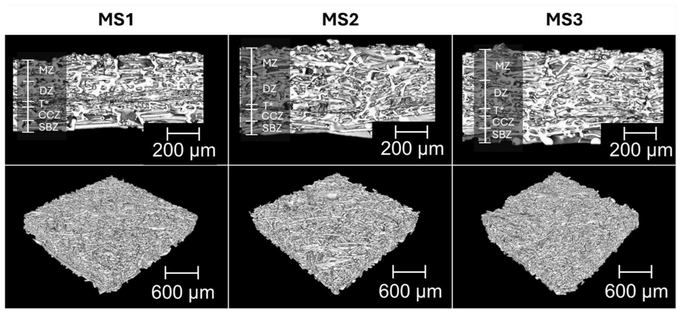
Mechanical, swelling and degradation assessment

3 Results

To replicate the unique structural organisation of articular cartilage, homogeneous macroporous MS1, MS2 and MS3 scaffolds comprising six interconnected zones namely the superficial zone (SZ), middle zone (MZ), deep zone (DZ), tidemark (T), calcified cartilage zone (CCZ), and subchondral bone zone (SBZ) were successfully fabricated.

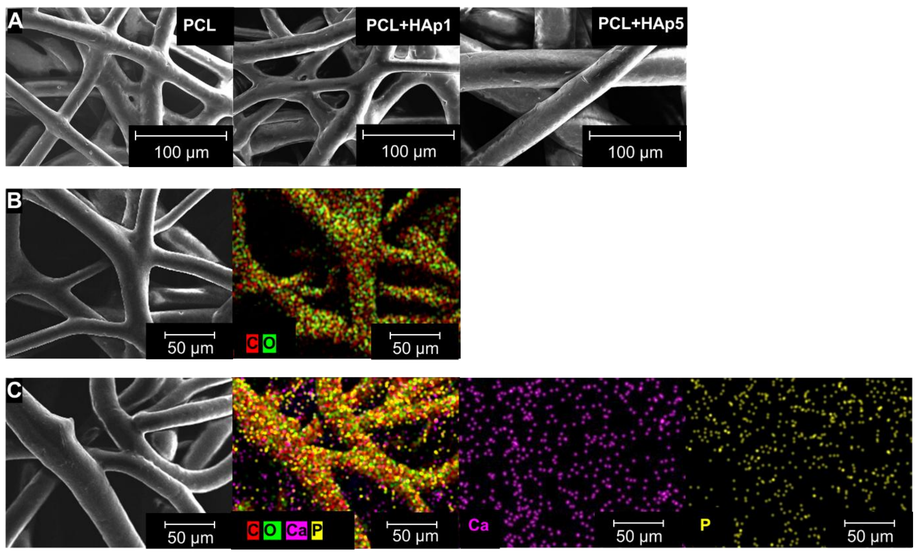


Micro-CT was used to characterize scaffold morphometrical properties.



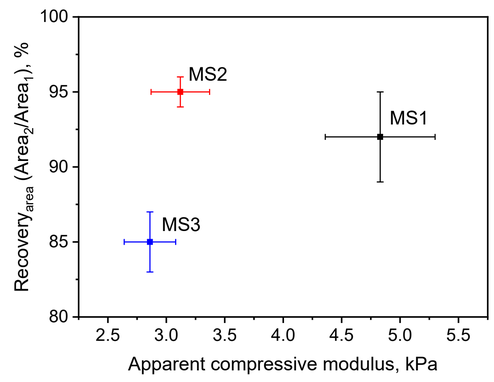
Scaffold type	Volume fraction, %	Porosity, %	Specific scaffold surface area (1/mm)
MS1	12.3	87.8	165
MS2	10.1	89.9	168
MS3	13.9	86.1	156

To create a more favourable microenvironment for cells in the CCZ and SBZ layers, hydroxyapatite (HAp) was incorporated into the fibers. Elemental mapping showed uniform distribution of Ca and P across the fiber surfaces, indicating the absence of micron-scale HAp agglomerates and confirming homogeneous HAp incorporation within the fibers.

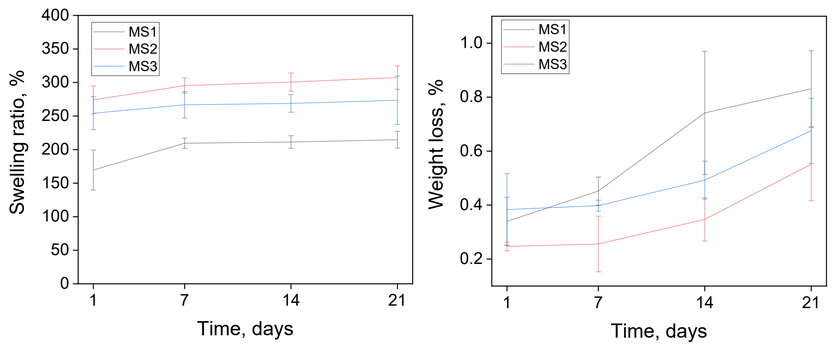


The MS1 scaffold exhibited the highest apparent compressive modulus (4.83 ± 0.47 kPa), while lower values were obtained for MS2 (3.12 ± 0.25 kPa) and MS3 (2.86 ± 0.22 kPa). The present results indicate that decreasing pore size and increased fiber density enhance resistance to compressive deformation.

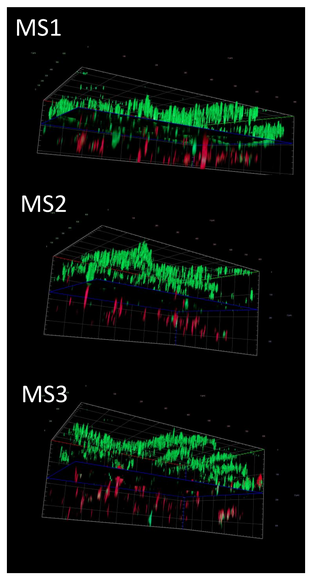
All scaffolds exhibited high shape recovery (~85-95%) after the compression test

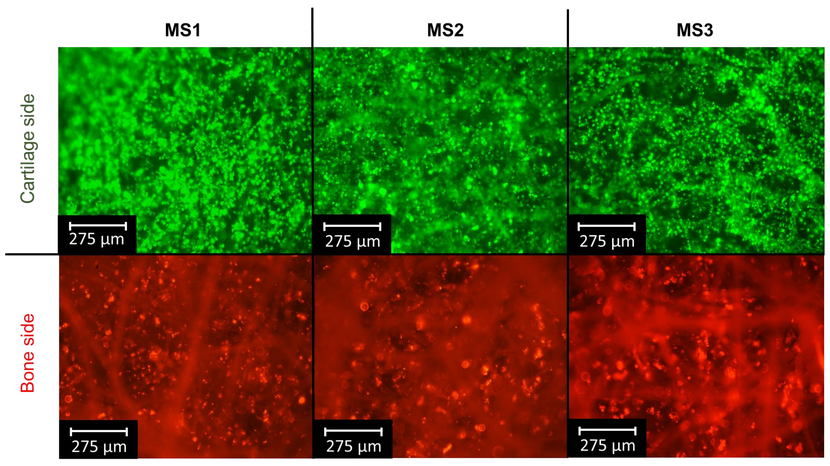


All scaffolds reached swelling equilibrium after 7 days. MS2 showed the highest swelling ratio. No significant weight loss was detected over 21 days, indicating minimal degradation and good structural stability of PCL multizonal scaffolds.



C28/I2 chondrocytes seeded on opposite sides of the osteochondral 3D polymer scaffold were labeled with different fluorescent dyes: Luminicell Tracker 670 on the bone side (red) and BioTracker 490 on the cartilage side (green). 3D projections (MS1-MS3) showed that the cells remained mainly in their respective regions of the scaffold. No clear overlap of red and green signals was observed across the tidemark layer, indicating limited cell migration between the bone and cartilage compartments.





Representative fluorescence images confirmed successful cell attachment on both sides of the osteochondral scaffold. Green-labeled cells were observed on the cartilage side, while red-labeled cells were detected on the bone side, indicating side-specific cell seeding and zone dependent cell distribution.

4 Conclusions

- A continuous six-layer multizonal PCL scaffold was successfully fabricated, reproducing the main structural features of the native osteochondral tissue.
- Hydroxyapatite incorporation in the CCZ and SBZ layers created a mineralized bone mimicking microenvironment with homogeneous Ca and P distribution.
- The scaffolds showed good *in vitro* stability, reaching swelling equilibrium by day 7 with no significant degradation over 21 days.
- MS2 showed the highest recovery, while MS1 had the highest compressive modulus.
- The tidemark layer acted as an effective boundary and maintained separation between the two scaffold zones.
- Cells were successfully attached on both sides of the osteochondral scaffold and showed compartment-specific distribution.

5 References

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