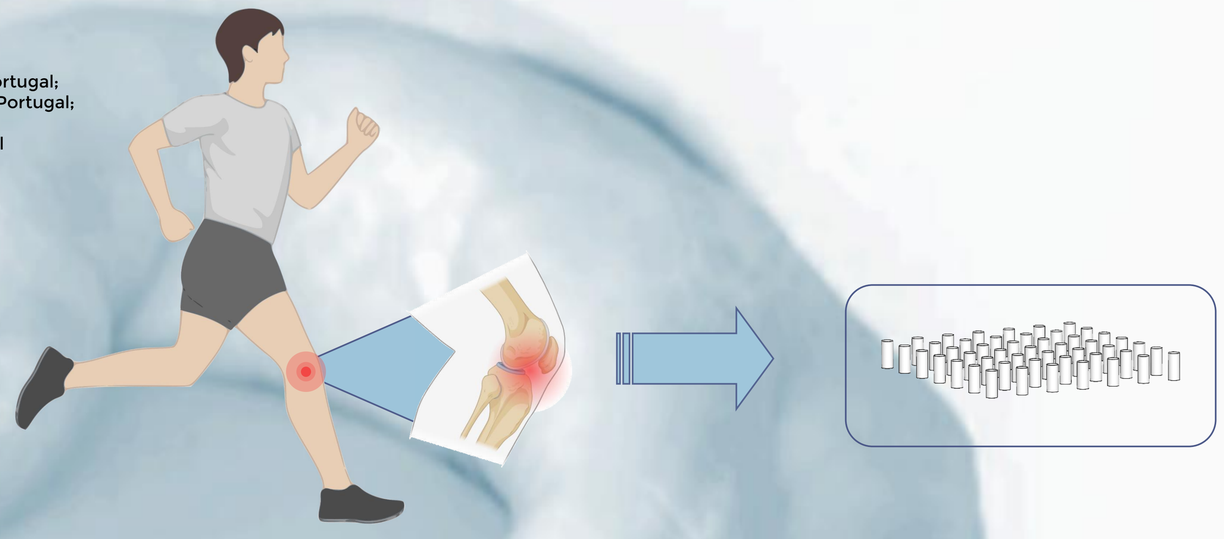
Columnar scaffold design development and multiaxial bioreactor validation for the deep articular cartilage zone

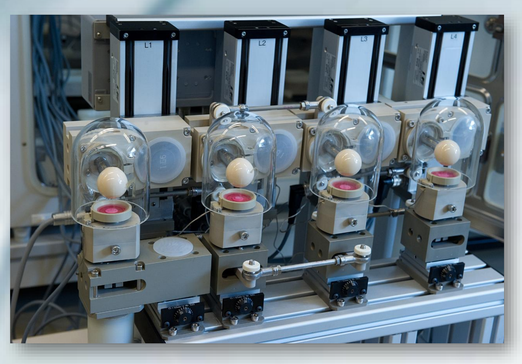
Rachel Cordeiro[1,2,3], Maja Schlittler[4], Laura Mecchi[4], Rui Alvites[1,5,6], Ana C. Maurício[1,5], Nuno Alves[2,7], Carla Moura[3,8], Martin J. Stoddart[4]

[1] Departamento de Clínicas Veterinárias, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS - UP), Porto - Portugal;
[2] Centre for Rapid and Sustainable Product Development (CDRSP-IPLeiria), Marinha Grande, Portugal;
[3] Polytechnic University of Coimbra, Coimbra - Portugal;
[4] AO Research Institute Davos, Davos Platz – Switzerland;
[5] Laboratório Associado para a Química Verde (LAQV),Rede de Química e Tecnologia (REQUIMTE), Universidade do Porto, Porto - Portugal;
[6] Instituto Universitário de Ciências da Saúde (IUCS), Cooperativa de Ensino Superior Politécnico e Universitário (CESPU), Paredes – Portugal;
[7] Associate Laboratory for Advanced Production and Intelligent Systems (ARISE), Porto - Portugal;
[8] Research Centre for Natural Resources Environment and Society (CERNAS), Polytechnic University of Coimbra, Coimbra - Portugal



Purpose

The deep zone (DZ) is the most challenging to mimic among the four zones comprising the articular cartilage due to its distinct architecture and mechanical properties. Adapted to sustain compressive loads, it unveils a fibrillar alignment perpendicular to the surface. This study investigates whether mimicking the DZ's native structure and mechanical stimulation create a microenvironment that promotes cartilage differentiation/proliferation.

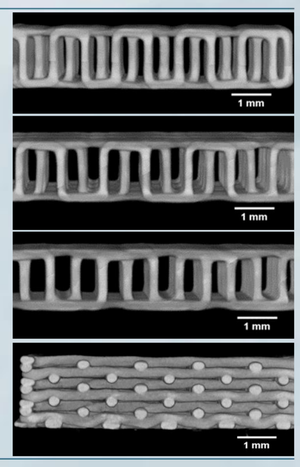


Methodology

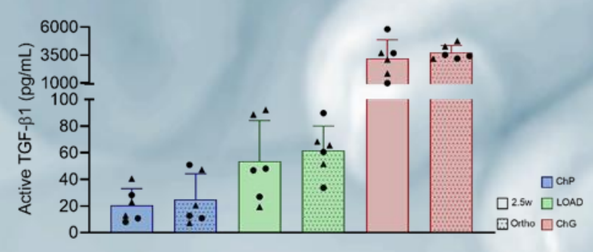
Three different columnar scaffolds with intercolumnar distances of 1.5, 2.0, and 2.5 mm (1.5w, 2.0w, and 2.5w, respectively), along with an orthogonal design as control, were manufactured using thermoplastic polyurethane (TPU). Scaffolds were characterized morphologically, followed by mechanical analysis in a multiaxial bioreactor containing cell culture medium supplemented with 50 ng/mL latent TGF-β1. The latent TGF-β1 activation was quantified after 6 hours of loading. After selecting the columnar scaffold with the best performance, a 21-day multiaxial mechanical study was performed using human Mesenchymal Stromal Cells (hMSCs). Three experimental groups were established : (i) scaffolds exposed to multiaxial mechanical stimulation with chondropermissive medium (LOAD); (ii) scaffolds maintained under static conditions with chondropermissive medium (ChP) as a negative control; and (iii) scaffolds maintained under static conditions with chondrogenic medium (ChG) as a positive control for chondrogenic differentiation. Chondropermissive medium consisted of Dulbecco's modified Eagle medium 4.5 g/L glucose, 0.11 g/L sodium pyruvate, 3.7 g/L sodium bicarbonate, 1% (v/v) Penicillin–Streptomycin, 50 μg/mL L-ascorbic acid 2-phosphate sequimagnesium salt hydrate, 1% (v/v) ITS+ premix universal culture supplement, 100 nM dexamethasone, 1% non-essential amino acids, and 5 μM 6-aminocaproic acid to prevent fibrin degradation. Chondrogenic medium was prepared by adding 10 ng/mL of TFG-β1 to the chondropermissive medium. Scaffolds and medium were biochemically evaluated.

Results

The columnar scaffolds were evaluated morphologically and mechanically, and their ability to activate latent TGF-β1 during mechanical stimulation was assessed to identify the most suitable design for future testing.

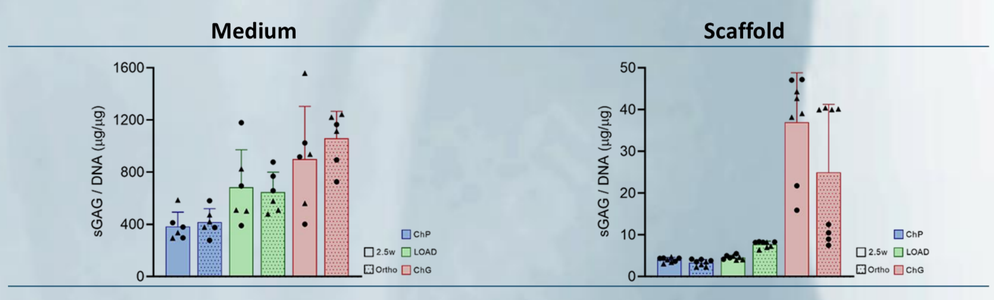
Scaffold	Porosity (%)	μCT image
1.5w	83.50 ± 1.16	
2.0w	88.32 ± 0.43	
2.5w	88.86 ± 1.27	
Ortho	73.91 ± 0.96	

Morphologically, columnar scaffolds demonstrated porosities similar to native cartilage (between 83.50% and 88.86%) while the orthogonal scaffolds showed a lower porosity (73.91 ± 0.96%).

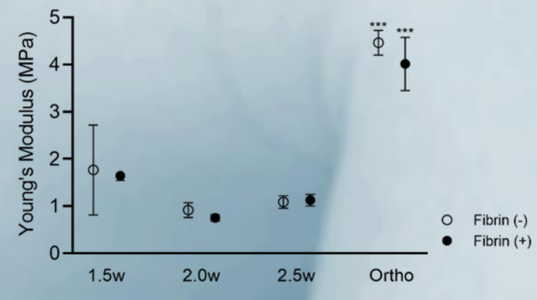


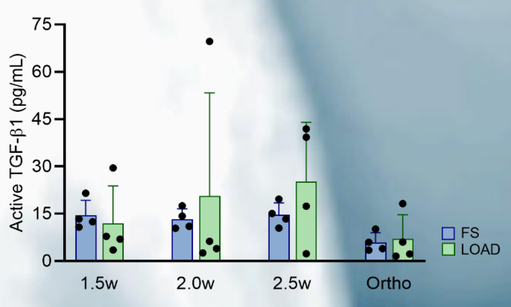
Levels of active TGF-β1 approximately doubled with loading (2.5w: 53.66 ± 30.59 pg/mL vs. 20.56 ± 12.48 pg/mL, LOAD vs ChP; and Ortho: 61.55 ± 18.62 pg/mL vs. 24.92 ± 19.28 pg/mL, LOAD vs ChP).

Accumulated sGAG/DNA in the medium was higher in the LOAD samples than in the ChP group, with a slightly higher value in the columnar scaffolds (LOAD: 683.76 ± 288.29 μg/μg vs 645.99 ± 154.92 μg/μg, 2.5w vs Ortho). Loading also increased the amount of sGAGs retained within the scaffold, with a greater effect in Ortho scaffolds (LOAD: 7.74 ± 0.75 μg/μg vs 4.58 ± 0.54 μg/μg, Ortho vs 2.5w).



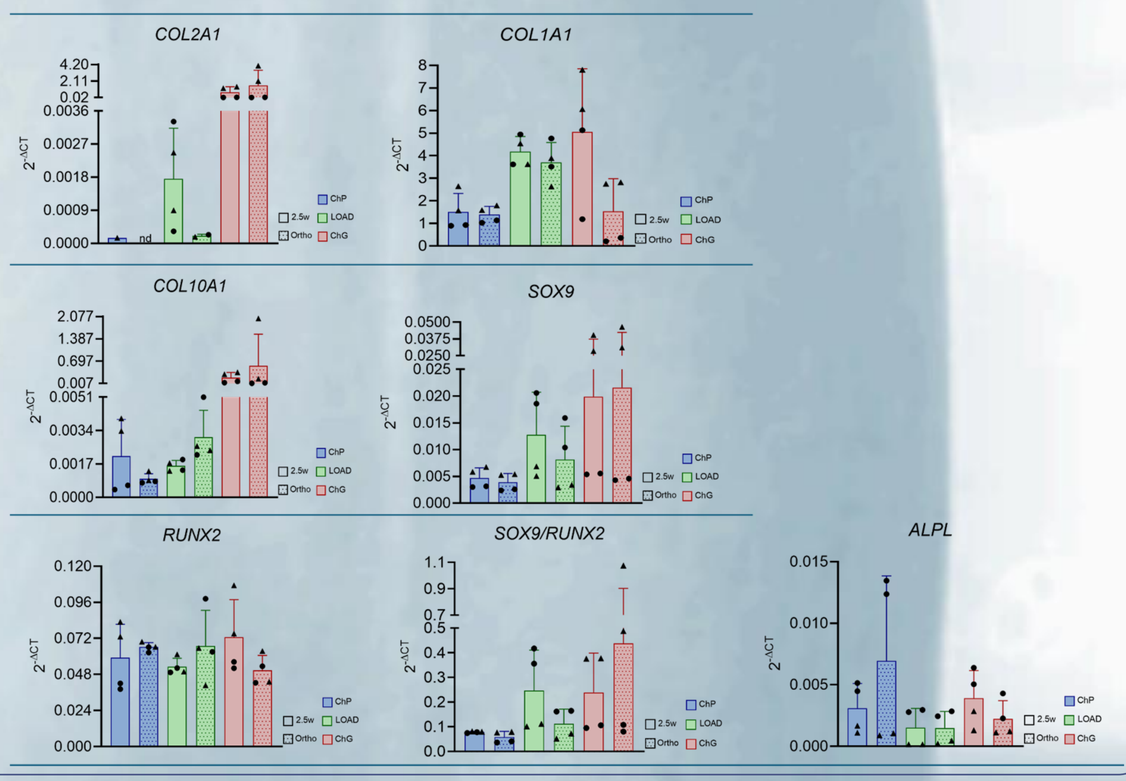
The dynamic mechanical test demonstrated that the Young's modulus of the empty scaffolds (Fibrin (-)) was highest for the Ortho (4.46 ± 0.26 MPa), followed by the 1.5w (1.77 ± 0.95 MPa), the 2.5w (1.09 ± 0.13 MPa) and the 2.0w scaffold (0.92 ± 0.16 MPa). Filling the scaffolds with hydrogel (fibrin (+)) did not result in a statistically significant effect on Young's modulus.



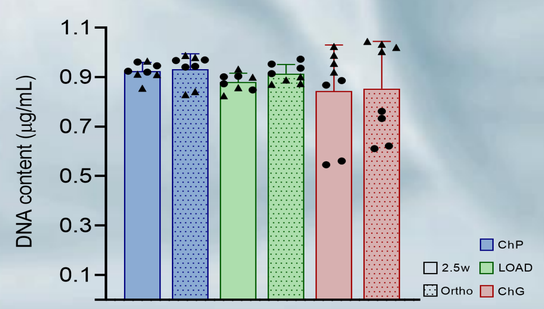


Regarding latent TGF-β1 activation, the 2.5w design appeared to activate the greatest amount of TGF-β1 (25.23 ± 18.81 pg/mL), followed by the 2.0w (20.63 ± 32.72 pg/mL), the 1.5w (11.98 ± 11.87 pg/mL), and the Ortho design (7.02 ± 7.70 pg/mL).

In the LOAD group, *COL2A1* expression increased in 2.5w scaffolds and remained low in Ortho scaffolds (2.5w: 0.00175 ± 0.00010; Ortho: 0.00020 ± 0.00004). On the contrary, *COL1A1* expression was lower in the ChP group (2.5w: 1.51300 ± 0.84729; Ortho: 1.38895 ± 0.43146) than in the LOAD group (2.5w: 4.18123 ± 0.14114; Ortho: 3.70152 ± 0.61921). *COL10A1* was detected in all samples, with the highest expression observed in the Ortho design for ChG and LOAD group (ChG: 0.54897 ± 0.75590; LOAD: 0.00306 ± 0.00079). The *SOX9/RUNX2* ratio was upregulated under LOAD conditions, but only in 2.5w scaffolds (0.24645 ± 0.19795). *ALPL* expression was upregulated in ChP Ortho scaffolds (0.00694 ± 0.00844) compared to all the other conditions.



Based on the comparable mechanical properties of the three columnar scaffolds and the highest TGF-β activation observed, we selected the 2.5w design as the most promising. The Ortho scaffold was used as a control for subsequent cell-based studies.



DNA was present in all scaffolds after 21 days, suggesting no toxic effect of the setup, with ChP showing slightly higher values (2.5w: 0.92 ± 0.02 μg/mL; Ortho: 0.93 ± 0.03 μg/mL) than LOAD (2.5w: 0.88 ± 0.001 μg/mL; Ortho: 0.91 ± 0.04 μg/mL).

Conclusion and Future Prospects

The results demonstrate the columnar scaffolds' chondrogenic differentiation potential, making them a promising design to guide cartilage repair. In the future, and considering that this is a proof-of-concept design trial, a biodegradable material should be considered for clinical applications.

Acknowledgements: This work was supported by the AO Foundation and the Horizon 2020 programme of the European Union (Marie-Curie grant agreement No. 101034412), and by EMBO Scientific Exchange Grant Nº10990. Was also supported by FCT - Fundação para a Ciência e Tecnologia, I.P. through Doctoral Studentships reference 2022.09638.BD (DOI: 10.54499/2022.09638.BD). FCT also supported this project through the following projects: CDRSP Funding (DOI: 10.54499/UID/04044/2025), ARISE (DOI: 10.54499/LA/P/0112/2020), InnovaBIOMAS (DOI: 10.54499/2022.10564.PTDC), Laboratório Associado para a Química Verde - Tecnologias e Processos Limpos (DOI 10.54499/UID/50006/2025), and institutional scientific employment program-contract (CEECINST/00077/2021/CP2798/CT0004).