Hydrogel cryostructuring to pattern pore orientation in cartilage-mimetic scaffolds

Luciano Riso [1] , *Cosimo Loffreda* [2] , *Andrea Barbero* [3] , *Florian Thieringer* [2, 4], *Fabiana Arduini* [5] , *Valentina Basoli* [2] , *Javier Ramon* [1, 6]

[1]*Institute for Bioengineering of Catalonia, Barcelona, Spain;* [2]*Medical Additive Manufacturing Research Group (Swiss MAM), Department of Biomedical Engineering, University of Basel, Allschwill, Switzerland;* [3]*Cartilage Engineering Group, Department of Biomedicine, University Hospital of Basel, University of Basel, Basel, Switzerland;* [4]Clinic of Oral and Cranio-Maxillofacial Surgery, University Hospital Basel, Basel, Switzerland; [5]Department of Chemical Science and Technologies, University of Rome "Tor Vergata", Roma, Italy; [6]Catalan Institution for Research and Advanced Studies (ICREA), Barcelona, Spain

Articular Cartilage

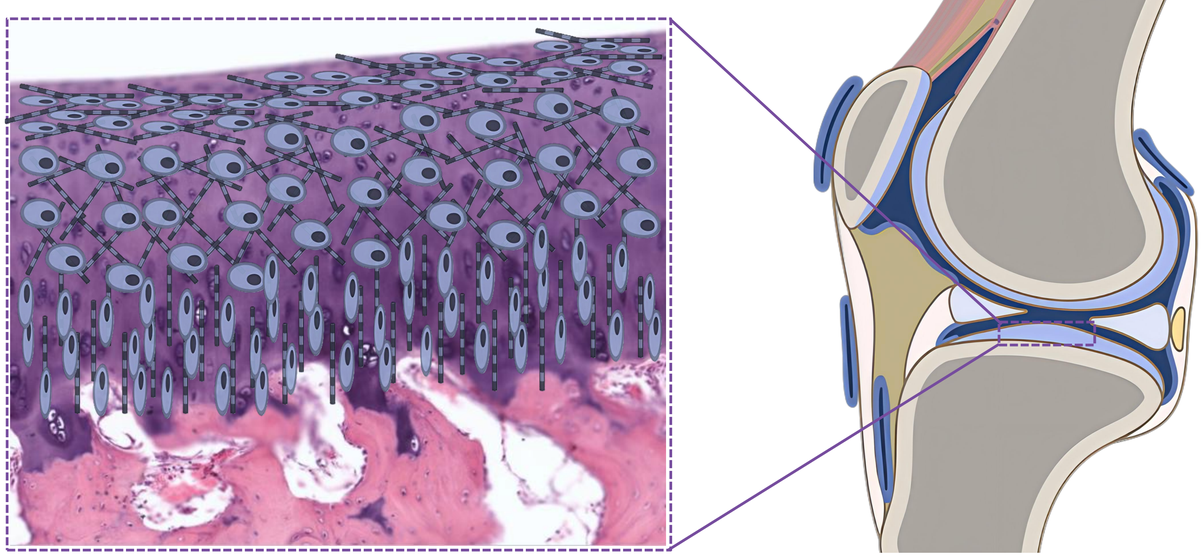


Figure 1. *Structural organization of articular cartilage. (Left) Schematic representation of knee joint. (Right) Schematic representation of cell morphology and collagen fibers arrangement within different cartilage zones.*

Composition & fabrication protocol

Hydrogels were fabricated using a 2% w/v gelatin and 2% w/v carboxymethyl cellulose (CMC) solution and adipic acid dihydrazide (ADH), crosslinked using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) / N-Hydroxysuccinimide (NHS) chemistry. After initial crosslinking, gels were subjected to isotropic or anisotropic freezing at -20°C.

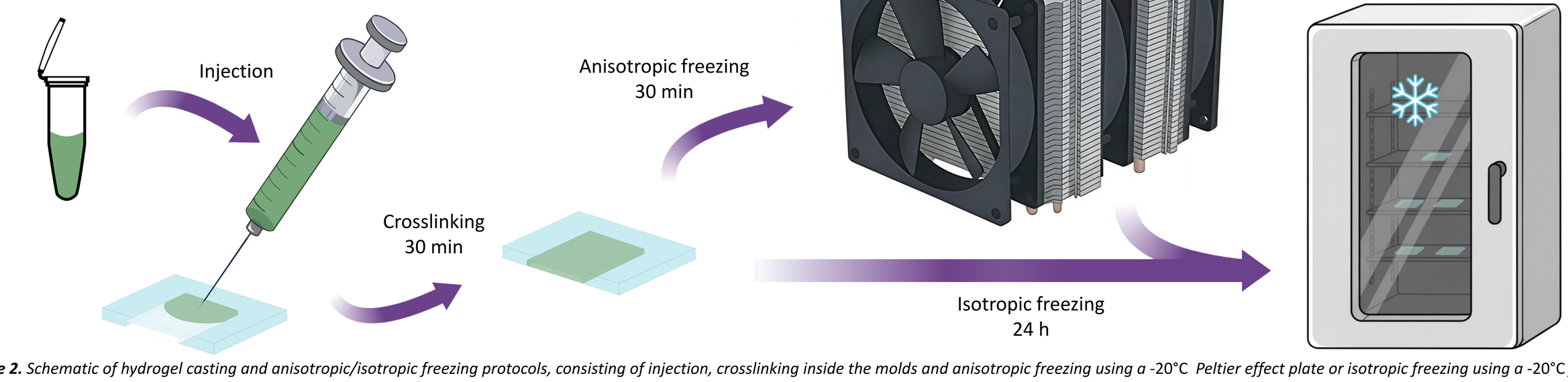


Figure 2. *Schematic of hydrogel casting and anisotropic/isotropic freezing protocols, consisting of injection, crosslinking inside the molds and anisotropic freezing using a -20°C Peltier effect plate or isotropic freezing using a -20°C freezer.*

Results I

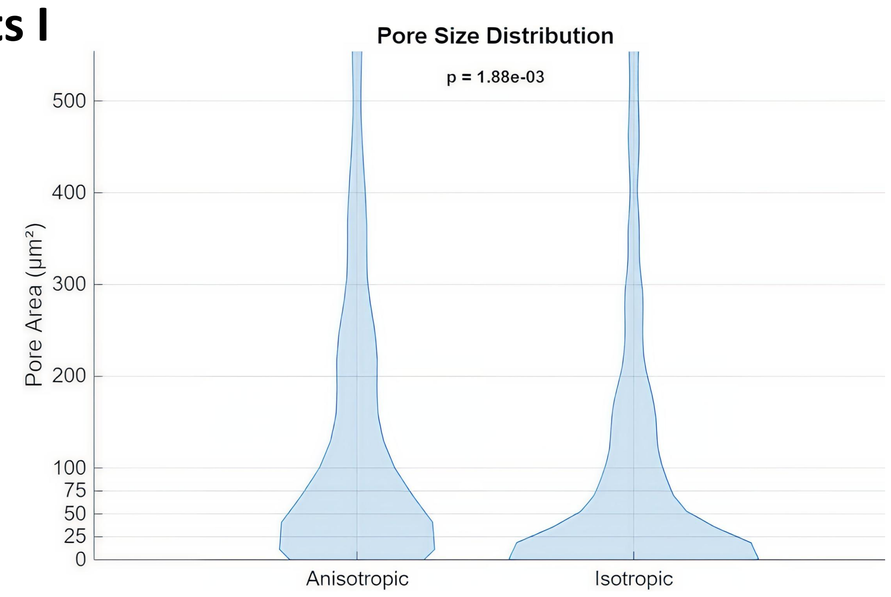


Figure 3. *Violin plot showing pore area distribution from anisotropic (left) and isotropic (right) hydrogels. (Kolmogorov-Smirnov test: distribution p = 1.00 // Mann-Whitney U test: median p = 1.88 × 10⁻³).*

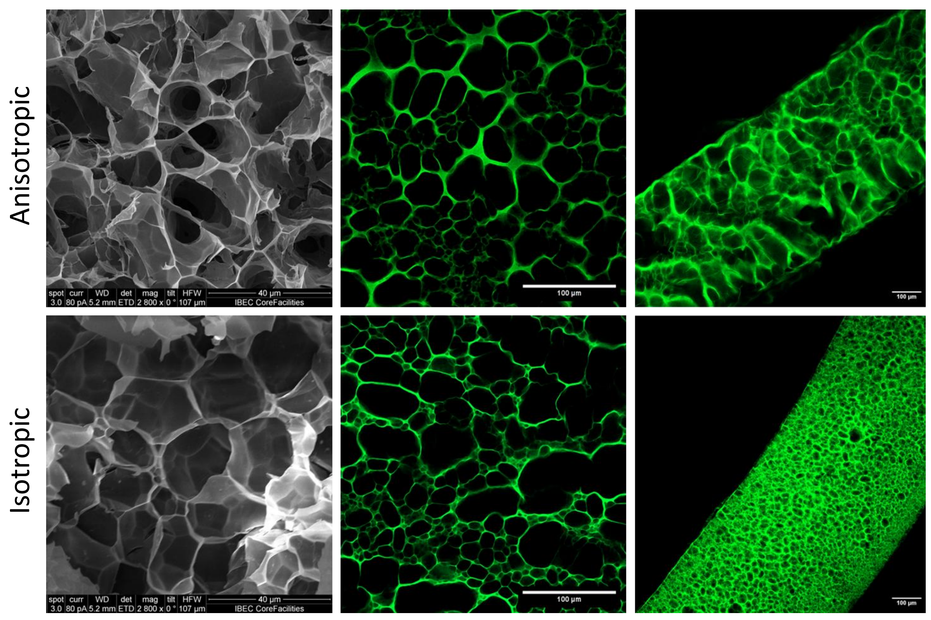


Figure 4. *Structural characterization of hydrogels. Upper view SEM images (left) upper view confocal images with fluoresceinamine (green) staining (center) and side view confocal images with fluoresceinamine (green) staining (right)of anisotropic (top) and isotropic (bottom) cryogels.*

Results II

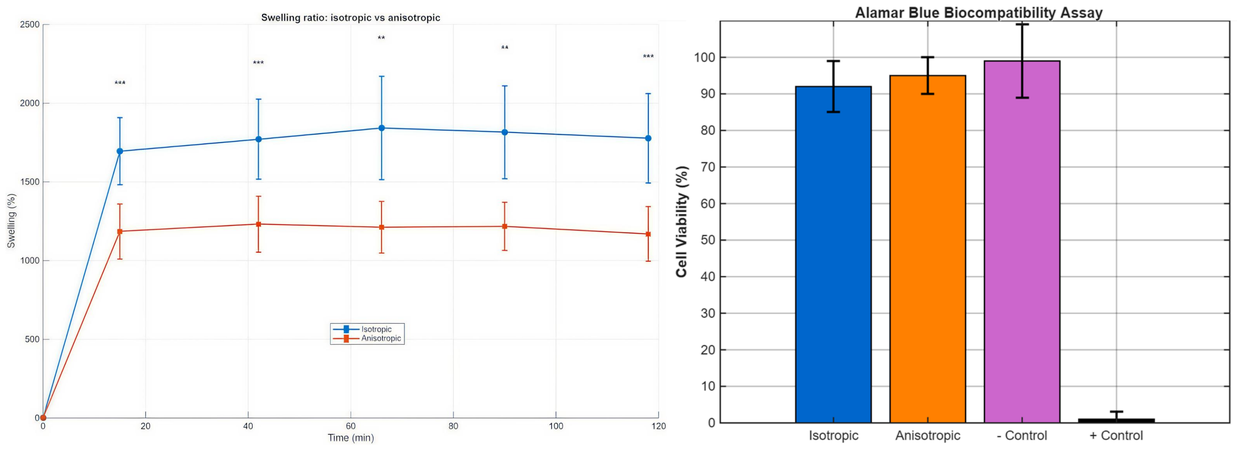


Figure 5. Swelling ratio and biocompatibility assay. (Left*)* Swelling ratio (%) of isotropic (blue) versus anisotropic (orange) CMC-gelatin-ADH hydrogels over time (0–120 min) ***p<0.001. *(Right)* Alamar Blue quantitative cell viability assay of *primary chondrocytes on isotropic (blue) hydrogels, anisotropic (orange) hydrogels and negative (purple) / positive (black) control.*

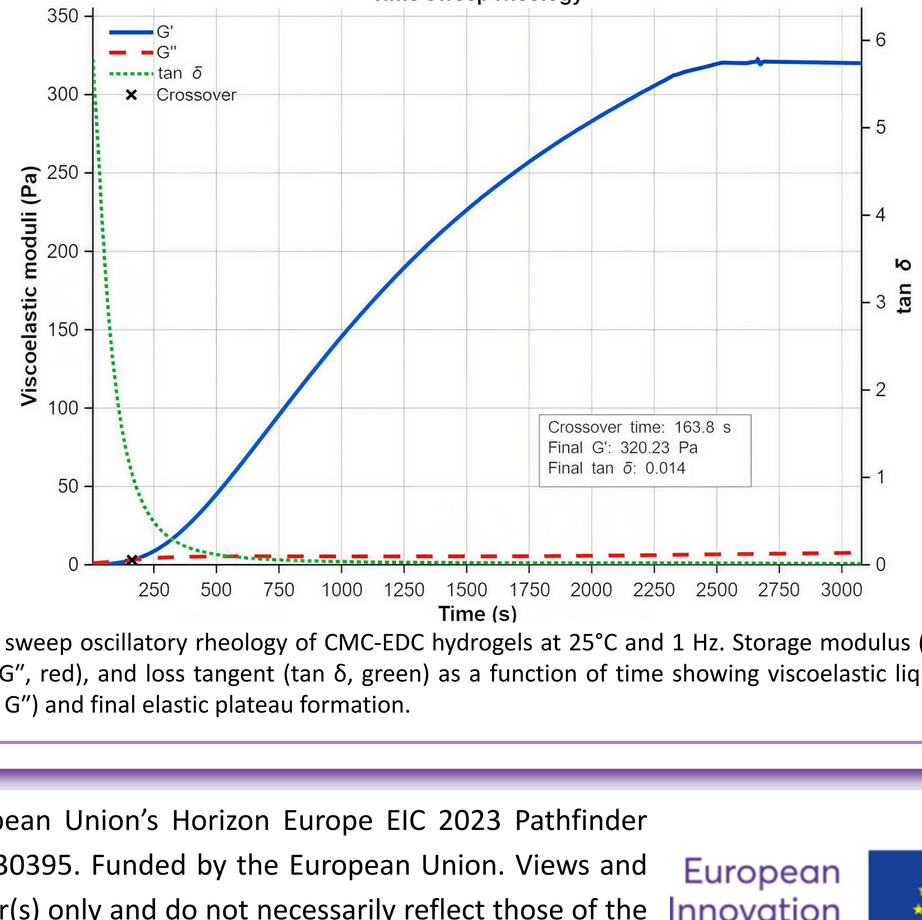


Figure 6. Time sweep oscillatory rheology of CMC-EDC hydrogels at 25°C and 1 Hz. Storage modulus (G', blue), loss modulus (G", red), and loss tangent (tan δ, green) as a function of time showing viscoelastic liquid-to-gel transition (G' > G") and final elastic plateau formation.

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