APPLICATION OF DIFFERENT CELL POPULATIONS IN HYDROGEL BIOINKS FOR ZONAL CARTILAGE BIOFABRICATION

Riccardo Levato, Department of Orthopaedics University Medical Center Utrecht, The Netherlands
Iris Otto, Department of Orthopaedics, University Medical Center Utrecht, The Netherlands.
i.a.otto@umcutrecht.nl
Richard Webb, Center for nanohealth, Swansea University, UK
René van Weeren, Department of Equine Sciences, Faculty of Veterinary Sciences, Utrecht University, The Netherlands.
Ilyas Khan, Center for nanohealth, Swansea University, UK
Jos Malda, Department of Orthopaedics, University Medical Center Utrecht, The Netherlands. Department of Equine Sciences, Faculty of Veterinary Sciences, Utrecht University, The Netherlands.

Key Words: Bioprinting, bioink, stem cells, articular cartilage

Functional regeneration of articular cartilage is still a major challenge in human. Bioprinting permits to mimic the complex architecture of articular cartilage, by coordinating the deposition of multiple cell types and materials, termed bioinks. For this purpose, cells with high potential for zonal differentiation need to be encapsulated in bioinks that provide an instructive niche for extracellular matrix (ECM) synthesis. The recent identification of multipotent articular cartilage chondroprogenitor cells (ACPCs) represents a new opportunity to generate bioinks with defined zonal affinity.

The aim of this work was to print zonal constructs using hydrogel bioinks encapsulating ACPCs, alone or in combination with other cell types, obtained from equine donors. Gelatin methacryloyl (gelMA)-based inks were used to culture ACPCs, bone marrow mesenchymal stromal cells (MSCs) and chondrocytes (CHs) in casted gels. The expression of zonal markers and ECM molecules by each cell type was studied. Constructs composed of two adjacent regions, each containing a single cell type were also fabricated, as models for zonal co-culture of the possible MSCs, CHs, and ACPCs pairings. Finally, zonal constructs were printed using ACPC-laden gelMA as superficial zone-competent bioink, and a MSC-laden ink for the deeper zones, via bioink extrusion in a sacrificial poloxamer frame. The effect of printing on long-term cell performance was evaluated during 56 days of culture. GAG/DNA quantification, histological and qPCR analysis revealed that all cell types underwent chondrogenic differentiation in gelMA bioinks. Additionally, a differential expression of zonal markers was detected between MSCs and ACPCs, the latter significantly upregulating the superficial zone marker PRG4. Conversely, MSCs had higher expression of collagen type X, a marker for the calcified zone. Differential distribution of ECM molecules was preserved also in zonal co-cultures. These results pave the way to the biofabrication of multicellular, functional constructs with zone-mimicking composition to be used for cartilage regeneration or as *in vitro* tissue models.