3D bioprinting of chondrocytes with bovine collagen type I bioinks

Y. Sbirkov[1,2], M. Redzheb[3], V. Dzharov[2], E. Tanova[1,2], T.Todorova[1,2], P. Voinov[4] , R. Hatar[4,5], and V. Sarafian[1,2]

[1] *Department of Medical Biology, Medical University of Plovdiv, Plovdiv, Bulgaria*

[2] *Research Institute at Medical University of Plovdiv, Plovdiv, Bulgaria*

[3] *Matrichem, Sofia, Bulgaria*

[4] *Unihospital, Panagyurishte, Bulgaria*

[5] *Department of propedeutics of surgical diseases, Medical University of Plovdiv, Plovdiv, Bulgaria*

Key words: Collagen, chondrocytes, cartilage, 3D bioprinting

INTRODUCTION

The development of highly reproducible *in vitro* models of cartilage through 3D bioprinting holds great potential for the better understanding and treatment of diseases like osteoarthritis and for regenerative medicine where damaged cartilage may be replaced by *in vitro* engineered tissues. Even if the cellular component of this type of connective tissue is well-defined, providing the extracellular scaffolds that will allow for the desired growth, spatial organisation and functionality of chondrocytes presents a challenge that is yet to be tackled. Therefore, novel biomaterials and 3D bioprinting strategies need to be explored.

The extracellular components of cartilage are mainly made of different types of collagen fibres.This, together with the good bioavailability and low immunogenicity make collagen the primary biomaterial of choice for many research projects. In this pilot study, we focussed on bovine-derived thermally-responsive collagen I bionks. Importantly, bovine collagen has already been shown to be biocompatible, biodegradable, and has earned approval by the FDA and EMA (e.g. as a scaffold carrier for BMP-2 and 7) [1,2]. Furthermore, relatively high concentrations of collagen (10-20mg/ml) have been demonstrated to provide excellent printability and accuracy of the desired 3D structures [3]. Therefore, the goal of this pilot study was to evaluate the printability and cytocompatibility of bovine collagen type I bioinks with chondrocytes in the context of disease modelling and regenerative medicine.

MATERIALS AND METHODS

CHON-001 chondrocyte cell line was used for originally-developed extrusion-based 3D bioprinting protocols with a BioX bioprinter (Cellink, Sweden). Bioinks with two different concentrations of collagen, 1% and 2%, were evaluated initially for cell viability. Calcein AM/PI staining was used to determine percentage of live cells between 24 hours and 3 weeks after bioprinting. Live cell imaging and morphological assessment were implemented to assess CHON-001 cells within the bioprints.

RESULTS AND DISCUSSION

Fluorescent analysis of live/dead cells through calcein AM/PI staining, brightfiled live cell imaging and morphological analyses revealed that bovine-derived collagen I bioink formulation and the bioprinting protocol used allow for significant cell adhesion and spreading within the bioprints within 24 hours post printing. They also provided intercellular communication, migration through the hydrogel and proliferation. Furthermore, upon histological analysis of the 3D constructs, we could also observe lacunae-like structures around the chondrocytes, which are typical of hyaline and elastic cartilage. Current experiments are aiming to characterise further the properties of the cells and the composition of the extracellular matrix around them. The validation of this *in vitro* model could establish it as a physiologically relevant platform for disease studying and as a potential basis for novel approaches in regenerative medicine.

CONCLUSIONS

Extrusion-based bioprinting with bovine collagen type I bioinks is a promising approach for the mid- to long-term 3D culturing of chondrocyte *in vitro* and thus holds great potential for further tissue engineering studies.

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ACKNOWLEDGEMENTS

This work was funded by the Bulgarian National Innovation Fund (13IF-02-9/07.12.2022), by Medical University of Plovdiv (NO-04/2021), and NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project № BG-RRP-2.004-0007-C01.