Thesis submitted to fulfill the requirements for the degree of 'Doctor in Health Sciences'

Promotor

Prof. dr. Heidi Declercq
Ghent University, Belgium
KU Leuven, Belgium

Guidance committee

Prof. dr. Anne Vral
Ghent University, Belgium

Prof. dr. Peter Dubruel
Ghent University, Belgium

Examination committee

Prof. dr. Jolanda van Hengel (chair)
Ghent University, Belgium

Prof. dr. apr. Olivier De Wever
Ghent University, Belgium

Prof. dr. Ward De Spiegelaere
Ghent University, Belgium

dr. Jasper Van Hoorick
Ghent University, Belgium

Prof. dr. Veerle Bloemen
KU Leuven, Belgium

Prof. dr. ir. Jeroen Rouwkema
University of Twente, Netherlands

Contact

Department of Human Structure and Repair
Tissue Engineering and Biomaterials Group

Lise.DeMoor@ugent.be
T +32 9 332 34 47
https://www.ugent.be/ge/hsr

The manuscript can be requested by email

FACULTY OF MEDICINE AND HEALTH SCIENCES

Biofabrication

of soft and vascularized tissues by bioprinting

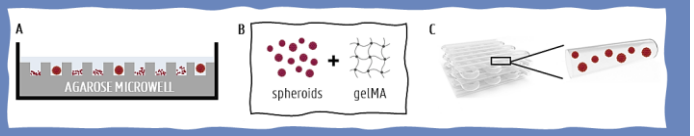
Lise De Moor
May 20th, 2021

GHENT UNIVERSITY

Summary

Tissue engineering (TE), is an interdisciplinary research field, combining the principles of engineering and life sciences towards the development of bioartificial tissue substitutes to restore, replace, or improve the function of damaged biological tissues or whole organs. The rise of biofabrication techniques, such as three-dimensional (3D) bioprinting, has a great impact on the field because it enables tissue engineers to mimic the 3D configuration of the native tissue by the precise layer-by-layer placement of several cell types and biomaterials along multiple axes with high spatial control.

In this dissertation, we tackle the TE of two extremes, **vascularized tissue** versus **avascular cartilage tissue,** by pursuing the same biofabrication strategy and workflow: hybrid bioprinting. First, cellular spheroids are formed (figure A). These are very small 3D tissues that are used as biological building blocks. To form a bioink, the spheroids are combined with a hydrogel, a methacrylamide-modified gelatin (gelMA, figure B), which functions as a supporting material, entrapping the spheroids while printing. This bioink can then be processed by extrusion bioprinting (figure C) to create a 3D tissue construct, mimicking the properties of the tissue of origin.



Cartilages within the knee joint are prone to injury because of the high mechanical stress the knee joint withstands on a daily basis. Therefore, TE strategies are pursued in order to develop new regenerative therapies. To generate fibro- or hyaline cartilage, porcine fibrochondrocyte (FC) and articular chondrocyte (AC) spheroids were formed using agarose microwells, and redifferentiation was further induced by culture in chondrogenic medium and a low oxygen tension environment. Only AC redifferentiated into 3D cartilage spheroids displaying an aggrecan and collagen type II-rich extracellular matrix, suggesting that AC are a suitable cell type for articular cartilage engineering. When the spheroids are combined with gelMA, hydrogel intrinsic cues can deeply affect cell fate and to date the influence of hydrogel encapsulation on the viability and the phenotype of spheroids is hardly described.

To investigate the influence of gelMA encapsulation on cartilage spheroids, different maturation stages of AC spheroids (3-, 7- or 14-day-old) were combined with various concentrations of gelMA (10, 15 and 20 w/v%) with Irgacure 2959 as a photo-initiator (PI). Overall, increasing the gelMA concentration, and thus increasing hydrogel stiffness, is associated with a decrease in collagen type II and an increase in collagen type I. Departing from the same AC cell source, different cartilage phenotypes could be induced by varying these parameters. To enhance clinical translation, this workflow was also tested with human bone marrow-derived mesenchymal stem cells.

The key challenge in the biofabrication of biologically functional products remains the integration of a vascular tree. Especially the creation of **microvasculature** of the small micron range (< 10 µm) remains challenging due to the limited resolution of most bioprinting techniques. To address this shortcoming in a biology-driven way, we developed prevascularized spheroids that can be incorporated throughout the bioprinted construct as capillary-containing building blocks. Human umbilical vein endothelial cells (HUVEC) were cocultured with human foreskin fibroblasts (HFF) and adipose tissue-derived mesenchymal stem cells (ADSC) as supporting cells. By balancing several parameters such as the cell number per spheroid, different cell type combinations and the applied cell ratio, HUVEC/HFF/ADSC small diameter spheroids wherein HUVEC spontaneously self-organized in a capillary-like network were generated. When encapsulated in 10 w/v% gelMA, HUVEC were able to sprout throughout the hydrogel. Post extrusion printing, the photocrosslinking conditions affected the angiogenic potential of the spheroids.

In conclusion, for spheroid generation, the agarose microwell system proved to be suitable for the formation of both cartilage and prevascularized spheroids with small diameters compatible for deposition by the print needle. GelMA is a promising material to function as the biomaterial component of the spheroid-laden bioink as it exhibits favorable properties in terms of printability and it supports spheroid viability, the chondrogenic phenotype of the cartilage spheroids and the vascular network in the prevascularized spheroids. The maturation stage of the spheroids before encapsulation proved to be important to achieve the desired phenotype, especially for the cartilage spheroids. Finally, the spheroid-laden bioinks proved to be printable and the gelMA concentration, type of PI, and post printing photo-crosslinking conditions can affect the spheroid functionality, and the spheroid phenotype.

Curriculum Vitae

Education	Ghent University
2015 – 2021	PhD in Health Sciences Tissue Engineering & Biomaterials Group
2013 – 2015	Master of Science in Biomedical Sciences
2009 - 2013	Bachelor of Science in Biomedical Sciences
Experience	Ghent University
2015 – 2021	PhD candidate *Biofabrication of cartilage & vascular tissues*
	Teaching assistant *Basic and Systematic Histology, Cell culture*

Publications

De Moor L, Merovci I, Baetens S, Verstraeten J, Kowalska P, Krysko D V, De Vos W H, Declercq H. High-throughput fabrication of vascularized spheroids for bioprinting. Biofabrication 2018; 10, 035009.

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