NOVEL MULTI-LAYERED 3D BIOPRINTED CONSTRUCT AS ALTERNATIVE VASCULAR CONDUIT REPLACEMENT

Federica Potere (1), Giovanni Venturelli (1), Beatrice Belgio (1), Giuseppe Guagliano (2), Francesco Briatico Vangosa (2), Federica Boschetti (1), Paola Petrini (2), and Sara Mantero (1)

1. Laboratory of Biological Structure Mechanics (LaBS)- Department of Chemistry, Material and Chemical Engineering 'Giulio Natta', Politecnico di Milano, Italy;

2. Department of Chemistry, Material and Chemical Engineering 'Giulio Natta', Politecnico di Milano, Italy

Introduction

Cardiovascular disease-related mortalities have risen from 12.1 million in 1990 to 18.6 million in 2019. In the last 5 decades, no viable alternative conduits have been found to alleviate the supply limitation of gold standards vascular graft.

Thanks to the explored 3D printing technology, the new challenge focuses on obtaining a 3D structure with several distinct layers to replicate the hierarchical organization of tissues. The ideal bioink would have mechanical, rheological, chemical, and biological characteristics according to the desired physicochemical properties, such as mechanical strength and robustness, and adjustable gelation and stabilization.

This work aims to reproduce blood vessel substitutes compliant with the shape, functionality, and integrity requirements of the original tissues, combining the advantages of the 3D bioprinting, decellularization process, and natural polymers and accounting for the presence of different cellular species simultaneously.

Methods

Enzymatic decellularization with of 6-month-pig aortas, was optimized. The decellularized powder was produced by cryomilling under N_2, the lyophilized product of the decellularization process and then solubilized with pepsin digestion. Decellularized ECM was included in gelatin/alginate bioinks. The composition (concentration of precursors and type of crosslinking) was optimized, and the printability was evaluated by rheological characterization. After a design of the 3 tunicae structures (SolidWorks), the G-Code was produced with CELLINK HeartWare software, and the construct was printed by Cellink Incredible+. The biocompatibility of the designed bioink was tested by encapsulating mouse fibroblast cells (L929) in the bioink, and the cell viability is assessed up to 28 days of culture.

Results

Decellularization. The optimized decellularization protocol produced a residual quantity of DNA lower than 50ng/mg of tissue, and the DNA fragment length lower than 200 bp (base pairs). Moreover, the DNA and RNA components were not visible in DAPI or hematoxylin and eosin staining.

3D Bioprinting. It was necessary to optimize the composition of a bioink able to withstand the printing of a segment of tubular construct up to 20 mm (40 layers) and to produce the hierarchical structure of different cell layers in the physiological aorta. Among the several compositions tested, the suspension resulting from 1.2% w/v gelatin, 6% w/v alginate, and 0.66% w/v dECM combined with a pre-printing crosslinking phase with internal gelation and a post-printing crosslinking with 1% $CaCl_2$, was able to produce tubular segments with a height up to 2 cm were produced. (*Figure 1*).

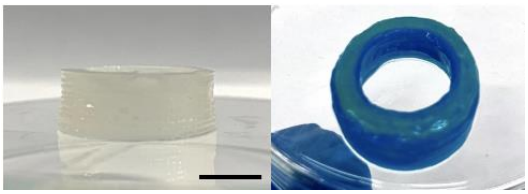

Figure 1. Structure of the multi-layered 3D bioprinted construct characterized by 40 layers of ECM-containing bioink. The dyed bioinks show the different tunicae: dark blue for intima and adventitia tunicae, and light blue for media tunica. Scale bar 1 cm.

Biocompatibility evaluation. Live&Dead staining showed cells retained their printed position on day 1 after printing. After 5 days of culture, the cells started to extend and connect. These results indicate that the printing step, the post-printing process, and the novel-derived bioink are biocompatible and cytocompatible. After day 14 of static culture, cells proliferated and infiltrated. These results demonstrate that high cell viability can be achieved in the printed tubular constructs after printing.

Discussion

The bioink described in this study proved to be suitable for printing multi-layered constructs, capable of maintaining the three tunicae and avoiding the overlap of the different inks through the thickness, despite the increase in the number of layers up to 20 mm-segments. As predicted by the rheological results, we achieved printability and shape fidelity, considering that the geometrical structures and the dimensions of the printed structures were very close to those established in the design phase. By tailoring the printing parameters and the amount of dECM the desired mechanical properties can be met. The next step includes the use of three different cell lines simultaneously to replicate the native three-tunicae structure of large blood vessels.