3D BIOPRINTING OF HUMAN STEM CELLS TO CONSTRUCT BLOOD VESSEL STRUCTURES

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THE 43rd FEBS CONGRESS PRAGUE 7-12 JULY 2018
FEBS YSF 2018 PRAGUE
11/ P.05-006

INTRODUCTION Three dimensional (3D) bioprinting combines cells, growth factors, and biomaterials to reconstruct living tissue and organs preferably using the patient's own cells. The aim of this study was to create blood vessel-like structures using human amniotic fluid stem cells (AFSC) and BioInk® hydrogel.

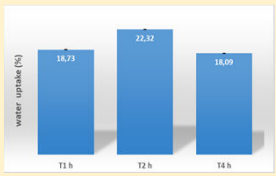
BIOMATERIALS

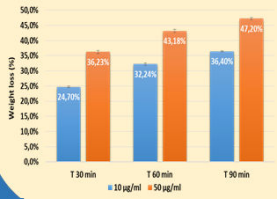
CELL INK

BioInk® (RegenHU) is a semi-synthetic hydrogel based on collagen, hyaluronic acid and polyethylene glycol. It is sterile and photopolymerizable.

$$\text{Wateruptake} = \frac{Ww - Wd}{Wd(g/g)}$$

The weight of the hydrogel increased by 22% at 2 hours after immersion in PBS.





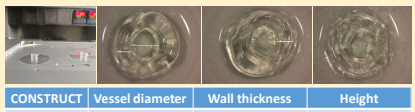
Enzymatic degradation showed that in the presence of collagenase (50 µg/ml), the construct decreased by 47.2%. The hydrogel used has the ability to take up water and is biodegradable.

3D BIOPRINTING

Printer parameters

Diameter	Number of layers	Pressure	UV	Speed rate	Temperature	Needle
2.5 mm	10	1.1 bar	5 sec/layer	1mm/sec	18ºC	0.2 mm

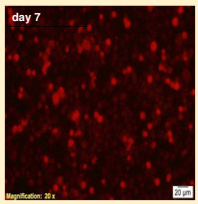
Printing was done using a 0.2 mm diameter needle, under 1.1 bar pressure, 1 mm/sec speed rate, diameter of vascular tubes 2.5 mm, thickness and height of each layer 0.3 mm, followed by 5 seconds polymerization of each layer. Bioprinted blood vessel constructs have a mean diameter of 2.46 ±0.41 mm, with a mean wall thickness of 1.43 ±0.10 mm and a mean height of 2.73 ± 0.05 after 10 printed layers.

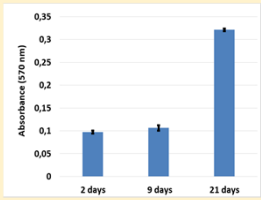


CONSTRUCT	Vessel diameter	Wall thickness	Height
Construct 1	2.95 mm	1.43 mm	2.73 mm
Construct 2	2.24 mm	1.49 mm	2.82 mm
Construct 3	2.21 mm	1.28 mm	2.84 mm

STEM CELLS

Amniotic fluid stem cells (2 million AFSC) were mixed with 1 ml BioInk hydrogels and printed in vessel structure model with defined line spacing. AFSC were stained with cell tracker Red CMTPX and analyzed by fluorescent cells. microscopy. MTT test was used to evaluate the viability of the amniotic fluid stem cells.

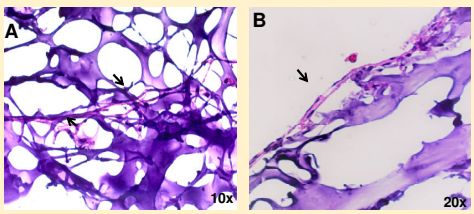




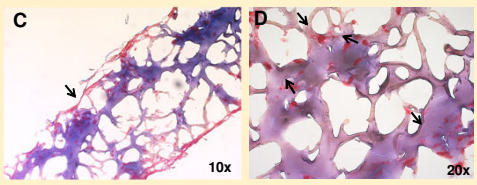
The fluorescent microscopy images confirm the MTT biochemical test, showing that the viability is maintained after 21 days of cultured in BioInk® hydrogel.

AFSC have a great potential in cardiovascular regeneration demonstrated by their ability to form capillary-like networks when cultivated in hydrogels. Our results showed that AFSC express mesenchymal markers such as CD73 (92%), CD90 (98%), CD105 (67.5%). After 21 days of culture in endothelial media (EBM2) the immunophenotype did not show changes in surface markers. Cryosections (5 µm) of the vessel constructs showed that the BioInk hydrogel has a porous structure with large lacunae that allow attachment and growth of AFSC.

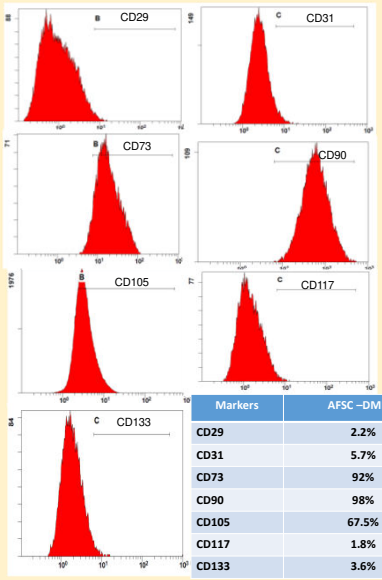
Hematoxylin and Eosin staining (A and **B)** showed that cells are able to connect with each other, the dimension of pores provided a favorable scaffold for cells to growth and proliferate.



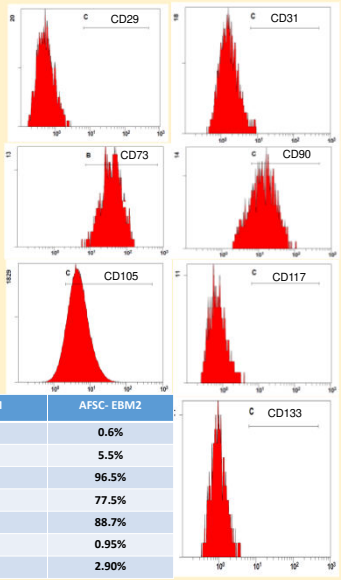
Trichromic Masson staining (C and **D)** revealed that AFSC are able to infiltrate and adhere to BioInk hydrogel, being anchored to the hydrogel network. The collagen is colored in blue and AFSC in red.



AFSC-DMEM



AFSC-EBM2



Markers	AFSC –DMEM	AFSC- EBM2
CD29	2.2%	0.6%
CD31	5.7%	5.5%
CD73	92%	96.5%
CD90	98%	77.5%
CD105	67.5%	88.7%
CD117	1.8%	0.95%
CD133	3.6%	2.90%

In conclusion BioInk hydrogel enables growth of AFSC being a good printable biomaterial for vascular tissue engineering. For understanding the blood vessel development, functional analyzes of these constructs (contractibility and elasticity by myograph techniques) and experiments using animal models will be performed.

Acknowledgements. This work was supported from the Project PN-III-P1-1.1-PD-2016-1660, BIOPRINT. E-mail: floriniordache84@yahoo.com