Cell sheet as a bioink for 3D bioprinting

E Bakirci[1,2], B Toprakhisar[1,2], M. C. Zeybek[2], G. O. Ince[2], B Koc[1,3]

[1]*3D Bioprinting Lab* [2]*Material Sciences and Nanoengineering* [3]*Manufacturing and Industrial Engineering, Sabanci University, Istanbul, Turkey*

INTRODUCTION: Cell sheet technology is a growing area in tissue engineering. It enables a sheet of interconnected cells which is enriched with cell-extracellular matrix (ECM) and cell-cell interactions. Poly (N-isopropylacrylamide) (PNIPAm) coating based thermoresponsive culture dishes are used as one of the advanced cell sheet technology methods [1]. It allows the surface to demonstrate temperature responsive wettability changes in aqueous environments. Different methods can be used to fabricate PNIPAm surfaces such as initiated chemical vapor deposition (iCVD) which offers a control of the polymer thickness [2]. In this research, we showed that thermoresponsive surfaces can create cell sheet which can be used as a bioink in 3D direct cell bioprinting [3]. The aim of this work is to show that cell sheets can be used to increase mechanical strength of bioink.

METHODS: 35 mm polystyrene culture dishes were coated by using initiated chemical vapor deposition (iCVD). The thickness of PNIPAm films were 30nm measured by using ellipsometry. Mouse embryonic fibroblast-like cells (NIH 3T3) seeded on PNIPAm coated polystyrene culture dishes with 100 cells/cm^2. Figure 1 shows how bioink is prepared during the proposed methdology.

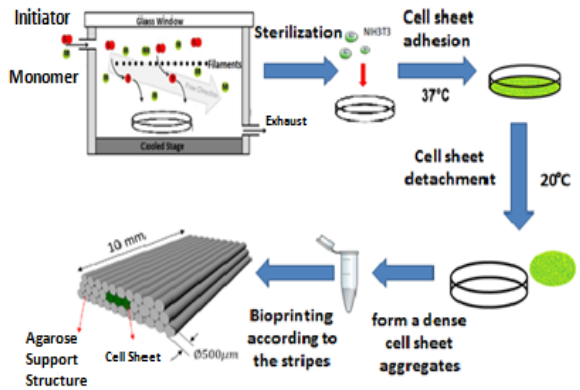

Fig. 1: The preparation of bioink

The prepared cell-sheet aggregates were 3D bioprinted using Novogen MMX Bioprinter according to the developed codes (Figure 2). The bioprinted constructs were incubated for 7 days so that the printed bioinks fuse together to form a tissue network. During the maturation period, the same culture medium was used. In order to visualize the fusion of cell sheet aggregates, they were stained with green or red membrane-intercalating dyes before printing.

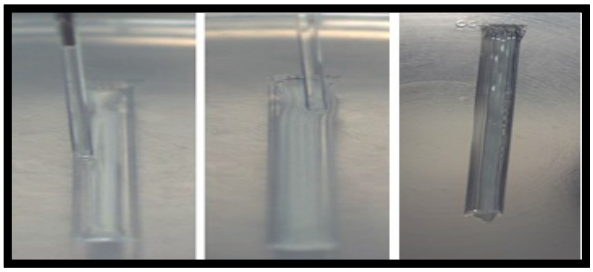

Fig. 2: The bioprinting process

RESULTS: After the printing process, the cell sheet aggregates fused within 0-7 days. The fusion of the printed cell sheet aggregates was examined at the first, third and seventh day after printing using a Zeiss LSM710 confocal microscopy. The fusion of alternate sequences of green and red cylinders is shown in Figure 3 and reveals fusion between the printed cell aggregates.

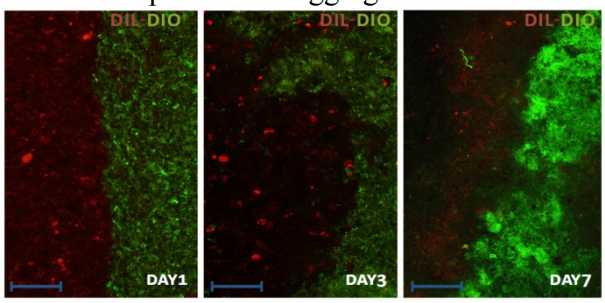

Fig. 3: Fusion evaluation of the printed cell sheets pattern (scale bar = 250 μm)

DISCUSSION & CONCLUSIONS: A novel cell-sheet based bioink developed for bioprinting. The developed bioink were used for direct cell printing. The results show that cell-sheet based aggregates can be bioprinted and fuse together. The results also showed that printed 3D structures have a better cell-cell and cell-ECM interactions, which is important for complex communication network of tissue constructs.

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