Free Session

Additive Manufacturing/3D Printing

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Upscaling Modular Tissue Engineering

Gregor Weisgrab[*1,2], Olivier Guillaume[1,2], Aleksandr Ovsianikov[1,2]
[1]TU Wien, 3D Printing & Biofabrication, [2]Austrian Cluster for Tissue Engineering, Vienna, Austria

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Introduction: The field of tissue engineering currently relies on two distinct approaches, either scaffold-based or scaffold-free cell culture. The first approach offers protected cell growth within a temporary structure at the expense of inhomogeneous cell seeding and an overall low initial cell number. The latter approach allows for initial higher cell densities and their homogeneous distribution at the cost of less controllable mechanical properties to favor rapid tissue formation.

Recently, a third strategy was proposed combining both methods to provide tissue constructs with high cells density and biomimetic environment all the while shielding the bulk with a highly porous cage [1]. This approach promotes the fusion of cell spheroids into larger tissue constructs while also protecting the cells from mechanical damage. To generate a construct of a relevant size, a large amount of single building blocks is required. Therefore, we propose a microfluidic handling system to automate the separation, seeding and culture of single building blocks in an approach to reproducibly fabricate cell-laden scaffolds with a specific number of cells.

Experimental methods: Scaffold fabrication:

High-resolution 3D scaffolds were 3D-printed from a range of biomaterials, including polycaprolactone (PCL), poly-d, l-lactic Acid (PDLLA), poly(trimethylene carbonate) (PTMC) and zirconium-hybrid (ZrHb). M2CMK was used as a photoinitiator at 0.5 wt%. The scaffolds were fabricated with 2-photon polymerization (2PP) using a femtosecond-pulsed laser at 800 nm and a 10x objective. After polymerization, the structures were developed in THF.

Microfluidic sorting:

A multi-part mold of the microfluidic sorting chip was designed in SolidWorks (Dassault Systèmes, France) and 3D-printed using SLA. The molds were then casted from Polydimethylsiloxane (PDMS) and plasma bonded in between 2 glass slides. A flow regulator (OB1 Mk3, Elveflow, France) is used to introduce the scaffold-laden liquid into the sorting chip and to actuate the valves of the sorting mechanism pneumatically (figure 1B).

Image:

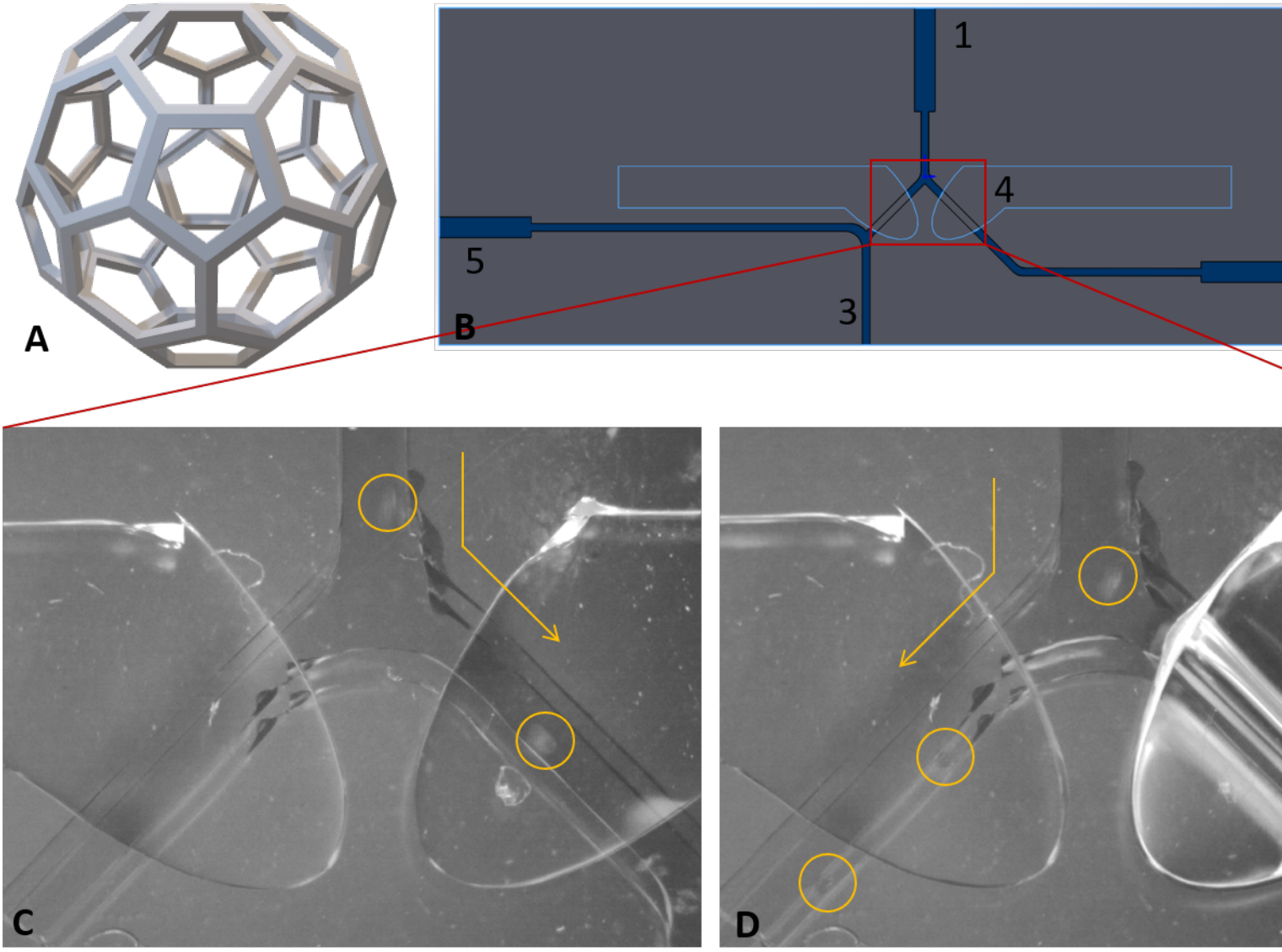

Table: Figure 1 Automated tissue sorting system (A) 3D model of a scaffold for spheroid culture (B) CAD model of microfluidic sorting chip. Scaffolds (yellow) are either delivered to the waste channel (C) or the ejection channel (D).

Results and discussions: Scaffolds were produced in a shape resembling a Buckminster fullerene with a diameter of 300 µm and an overall porosity of 96% to promote rapid cell invasion (Figure 1 A).

From the proposed biocompatible materials, ZrHb was chosen for the initial test run as it proved easiest to separate the bulk into single units. Scaffolds made from PCL, PDLLA and PTMC formed clumps in the carrier solvent (1-Propanol) and clogged the microfluidic chip.

The microfluidic chip was designed to dispense single scaffolds from the bulk solvent stream in as little volume as possible. A scaffold-laden liquid enters the chip (1) and is either directed to the waste channel (2) or the dispensing channel (3) based on the status of the valve system (4). The ejection from channel 3 is done via pressurized air (5) to minimize the amount of carrier liquid in the culture plate.

Conclusions: We propose a microfluidic system to sort a large number of scaffolds into single cell culture wells for the reproducible fabrication of spheroid-laden scaffolds.

This approach yields single scaffolds that can then be seeded with a precise number and type of cells. The separation of soft core and hard shell allows the tunability of the porous shell in terms of shape and material. Further research into

surface modifications and other carrier solvents is required to process scaffolds from the other proposed biomaterials with this method.

References/Acknowledgements: [1] Ovsianikov A. et al., Trends in Biotechnology, April 2018, Vol. 36, No. 4

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