Application of rapid prototyping to high throughput screening of 3D dynamic environments

Pedro F. Costa[1], Cedryck Vaquette[2], Christina Theodoropoulos[2], Manuela E. Gomes[1], Rui L. Reis[1], Dietmar W. Hutmacher[2]
[1]3B's Research Group, University of Minho, Portugal
[2]Institute of Health and Biomedical Innovation, Queensland University of Technology, Australia

Statement of Purpose: The regeneration of tissues greatly results from the interaction of cells with their highly complex surrounding environments[1]. High throughput screening can be used to perform wide arrays of tests combining multiple variable factors such as cells, materials and physical/chemical stimuli[2]. We have developed a method employing solid freeform fabrication to easily, quickly and economically build high throughput screening devices which are capable of simulating the interaction of cells/tissues with multiple environmental factors in a controlled manner. As a proof of concept we CAD-designed and built, in a single step, enclosed multi-culture devices containing scaffolds which were successfully seeded and cultured with osteoblasts under fluid flow-generated shear stimuli.

Methods: Computational fluid flow modeling was utilized to optimize the device's design primarily taking into attention the architecture of the scaffolds to be used. The selected scaffold design consisted of an 8mm cube composed of 0,27mm thick layers containing struts horizontally spaced apart by 1,5mm and with 0 and 90 degree orientation. Chambers were designed to tightly and fluid-efficiently enclose the model scaffold while allowing as well for its easy removal. Chambers were as well provided with individual upper medium reservoirs and inlets/outlets for gas renewal/exchange. On the bottom part of the device was also designed a mini-fluidic network connecting every individual chamber to one common medium inlet/outlet. The optimized three-dimensional design was then used for rapid prototyping the device. A dual extrusion fused deposition modeling-based equipment was used to simultaneously prototype the porous scaffolds (made of poly(lactic) acid (PLA)) enclosed into the device (made of acrylonitrile butadiene styrene (ABS)). Given the low hydrophilicity of PLA, scaffolds contained into the devices were surface treated by filling the device's chambers with 2M sodium hydroxide solution for 30 minutes. The scaffold-containing devices were then analyzed by micro-CT and SEM in order to verify their morphology, integrity and watertightness. After sterilization, device's air inlets/outlets were capped with syringe filters while a silicone tube was used to attach the device's common medium inlet/outlet to a 1ml syringe mounted onto a syringe pump. This was used for injecting primary human osteoblast suspensions ($1{,}25 \times 10^5$ cells per chamber) into the device's chambers. By bidirectionally circulating the cell suspension, and later culture medium, through the scaffolds, cells were seeded and perfusion-cultured into the scaffolds. The perfusion culture was maintained for 6 weeks and scaffolds collected every 2 weeks for DNA quantification, MTT and FDA/PI viability staining and SEM imaging.

Results: A multi-chamber device (Fig. 1a and 1c) was designed and built resorting to computational fluid flow simulations and rapid prototyping (Fig 1a-g). Its walls defined perfusion chambers around scaffolds (Fig. 1d). Scaffolds were positioned in homogenous perfusion fluid flow regions by means of porous supports. Structural analysis was performed via micro-CT and SEM imaging. DNA quantification analysis (Fig. 1e) performed along the 6 week culture with osteoblasts showed that the seeding of cells into scaffolds had been effective and that the amount of cells had increased during that period while FDA/PI viability staining (Fig. 1f) showed that cells remained viable. MTT staining and SEM imaging (Fig. 1g) showed that cells were well distributed throughout both inner and outer regions of the scaffold while keeping a typical osteoblast morphology.

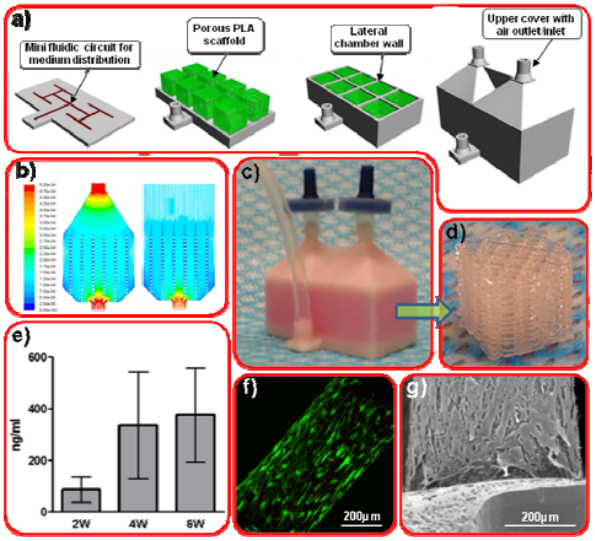

***Fig. 1** – a) design of culture device, b) Fluid flow models, c) perfusion culture, d) scaffold removed e) scaffold's DNA content over 6 weeks culture, f) FDA/PI staining showing cell viability and g) SEM of cells on scaffold.*

Conclusions: We have developed and validated a rapid prototyping-based single-step method for fabricating multiple 3D freeform scaffolds readily contained into case-specific culture devices. These perfusion-based devices provide completely enclosed and controlled environments for seeding and culturing cells into scaffolds under personalized conditions and stimuli. This method is able to simplify the scale-up of devices to full multi-sample and multi-variable combinatorial platforms necessary for high throughput screening.

References: (1) Bancroft GN, et al. Proc Natl Acad Sci (2002) 99(20): 12600-12605.
(2) Figallo E, et al. *Lab Chip* (2007) **7**, 710-719.

Acknowledgements: We thank the Australian Research Council for funding and the Portuguese Science Foundation for Pedro Costa's PhD grant (SFRH/BD/62452/2009).