OSTEOCHONDRAL BIOREACTOR FOR DRUG SCREENING AND TOXICITY ASSESSMENTS

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Introduction

Osteoarthritis represents one of the most prevalent and debilitating chronic diseases worldwide. The modest efficacy of available treatments combined with the the significant percentage of failed pre-clinical trials demonstrate an urgent need for realistic tools for *in vitro* drug screening, able to reproduce osteo-chondral interactions in health and disease condition. To generate *in vitro* model of adjacent tissues, we developed and validated an innovative millifluidic bioreactor suitable to accommodate biphasic cell constructs and guarantee real-time optical monitoring.

Materials and methods

Models of the flow path in one-inlet and dual-inlet bioreactors (Fig. 1) were created using SolidWorks and tested using ANSYS Fluid Flow (CFX), with an inlet volume flow rate of 1 ml/day and outlet open to the environment [1].

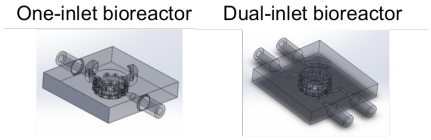

Figure 1: Bioreactors design.

The bioreactors were 3D printed by stereolithography. At day 7 biological tests were performed on the one-inlet bioreactor hosting hMSCs in 8% gelMA construct in the central chamber: Alamar Blue® assay was performed adding the reagent to the inlet medium and analysing the output medium using a plate reader. A Dynamic Live Assay was performed adding Calcein-AM to the inlet medium and collecting bioreactor central chamber z-stacks images. The biphasic bioreactor design fluidic was tested by flowing two different colour media and quantifying the degree of mixing in the outlets using a plate reader. The differentiation of hMSCs towards a chondrogenic and osteogenic phenotype in the two sides of the bioreactor was tested at day 21 via fluorescence test of the Col2 transfected sentinel cells and Col2 PCR analysis.

Results

From the CFD results, one-inlet and dual-inlet bioreactors showed comparable results. Mean velocity of 1.08E-6 μm/s and extremely low shear stress values confirmed simultaneously no cellular damage and suitable nutritional supply in both the devices. Both Alamar Blue® and Live/dead results in one-inlet bioreactor showed no differences in terms of cell metabolic activity if compared to the static culture. Comparing the absorbance intensity of input and output solutions, we validated the absence of fluid mixing inside the chamber. The differentiation of hMSCs towards a chondrogenic and osteogenic phenotype in the two sides of the bioreactor construct was optically validated by fluorescent tests and confirmed by quantitative RT-PCR analysis (Fig 2).

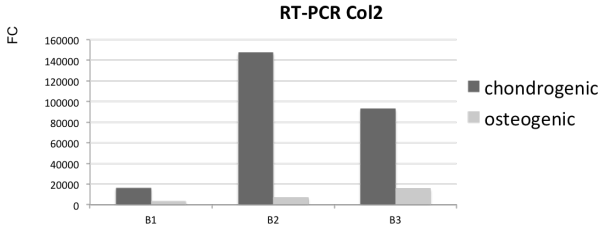

Figure 2: Col2 RT-PCR results for three dual-inlet bioreactors.

Discussion

We validated the millifluidic biphasic bioreactor capability in guaranteeing cell viability, continuous optical monitoring, no mixing of fluids in the culture chamber hosting each tissue phase. Moreover, we validated the designed device in inducing two specific differentiation pathways in the same chamber.

In light of this, the new-designed biphasic bioreactor represents a suitable device for high throughput micro-physiological systems for drug screening and toxicity assessment.

References

1. Nichols, D. A. et al., Biomed. Microdevices 20, 1–8 (2018).

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