SINGLE OR BI-TISSUE MULTIMODAL BIOREACTOR FABRICATION IN POLYDIMETHYLSILOXANE USING SACRIFICIAL 3D PRINTED MOLDS

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1 ABSCTRACT

Bioreactors are essential for obtaining viable in vitro cell cultures mimicking realistic in vivo conditions in a tridimensional microenvironment. Since target culture cells are sensitive and respond actively to microenvironment modulation, applying precise stimulation that impacts the microenvironment through electromagnetic and mechanical stimuli has been considered to promote specific cellular responses like proliferation, growth, and differentiation. Grouping the need for exploratory research that requires bioreactors with flexible designs to accommodate tissue samples, scaffold structures, and sensors, while maintaining their ability to deliver precise stimulation conditions, we took advantage of our previously devised strategy [1], called JANUS, to integrate finite element model prediction data into the design phase respecting established bioreactor goals. Here, those goals are: the design must comply with the delivery of precise electromagnetic and mechanical stimulation ranges sustaining a continuous perfusion operation, with anti-bubble formation traps; the design must be capable of supporting a single culture in one chamber or a co-culture in a dual chamber; the design or its construction structures have to be 3D printable, maintaining a highly reproducible fabrication, after which its operation could be numerically predicted and experimentally validated.

In this work, we improve upon the design and construction of our previously released open-source bioreactor, replacing the construction material C8 (a proprietary composite thermoplastic) with polydimethylsiloxane (PDMS), an autoclavable silicone, by this way upgrading our previous design with leakproof and sterilization capabilities. Here we took advantage of butene-diol vinyl alcohol copolymer (BVOH) degradation in warm water to produce a sacrificial 3D printed mold with perfusion channels. All mold parts were entirely made in a commercial 3D printer (Creality Ender 3 S1-Pro, China), according to the materials manufacturer-recommended instructions. Complementary parts that fit together were designed to implement either the fluid flow perfusion and mechanical stimulation system, and the capacitive coupled electric field stimulation system was composed of indium tin oxide (ITO) electrodes connected to electrical wires with conductive silver epoxy glue. After sacrificial mold production, PDMS (Sylgard 184 Silicone Elastomer Kit, applied in a 10:1 (w/w) ratio of base to curing agent) was degassed and poured into the mold, left to cure for over 48 hours at room temperature. The resultant structure was carefully de-molded, after which BVOH was removed in a hot bath (40 ºC) for 72 hours. Both parts were assembled, and the overall operation was validated in a perfusion closed circuit.

Finite element method numerical models of the developed single-well design predict at a domain point probe (x=-0.057, y=0.009, z=0.025) placed on a scaffoldless chamber, that maximum peristaltic flow of 50 mL/min (0.3281 m/s) can deliver a range of fluid flow shear stress up to 0.62 mPa (shear rate of 0.89 s^{-1}). In addition, predictions show that a broad range of capacitively-coupled electric field magnitudes can be applied, mostly depending on the signal generator capabilities. This single-well design is scalable in number and dimension. It will allow the realization of high throughput experiments with an array of identical bioreactors, supporting scaffolds from any custom diameter size ranging from a few to tens of millimeters. Taking advantage of the same fabrication technique, this work also conceptualizes a bi-tissue co-culture, allowing the separate use of cell culture medium in addition to different external stimulation protocols (mechanical and electromagnetic). The bi-tissue culture concept supports a multilayered scaffold, stacked into a frame support, as proposed by Baba *et al.* [2]. To effectively isolate the contents of each chamber fluidic contents, the scaffold stacked layer solution must contain an isolation membrane, if not, a mixture of both chamber's contents will occur. In conclusion, this work updates our previous JANUS strategy [1] by improving the fabrication technique and overall properties of the bioreactors, while maintaining the flexibility of 3D printing design processes, giving a more viable experimental bioreactor fabrication for each design hypothesis supported by numerically predicted data.

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