Open-Source 3D Printable Bioreactors for High Throughput Bone Organ Culture

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INTRODUCTION: A polycarbonate bioreactor (PCB) system has been previously employed for *ex vivo* bone studies [1]. While allowing for controllability over culture conditions, PCBs are difficult and expensive to fabricate. As an alternative, we are establishing an open-source 3D printable bioreactor using the photopolymer, MED610. MED610 is listed as biocompatible for permanent skin contact but is limited to 24 hours for mucosal membrane contact [2]. While some studies agree that MED610 is not suitable for culture environments [3, 4], others suggest that its biocompatibility is dependent on the sterilization method used [5, 6]. Here, we report on long-term toxicity testing of MED610 using the human osteosarcoma Saos-2 cell line.

METHODS: Using the Stratasys Objet30 Prime 3D printer, MED610 rectangular constructs were printed and sterilized using four different methods: manufacturer's protocol (MP) [2], sonication protocol (SP) [5], and MP or SP with autoclaving (MP+A, SP+A). Post-sterilization, constructs were placed in sterile dishes containing culture media with serum at 37°C and 5% CO_2. Conditioned medium was applied to cells cultured in 96-well plates (15,000 cells/well, n=6/group) every two days. Cell viability was assessed via CellTiter-Blue on days 2 and 7, and compared to positive (autoclaved PCB) and negative (sterile unconditioned media) controls using a one-way ANOVA and Tukey's multiple comparison test ($\alpha = 0.05$). Similarly, a static bioreactor test was performed with two MED610 bioreactors, one sterilized using MP and one using SP+A, with cell viability assessed on day 7.

To assess MED610 in a perfusion system, a printed bioreactor sterilized using the SP+A method and an autoclaved PCB (positive control) were assembled with sterile tubing and a peristaltic pump. Media was circulated (6.6 mL/hr [1]) through the chamber for 21 days; every two days, the system medium was changed, and conditioned medium was applied to cells in a 96-well plate. Cell viability was assessed on days 7 and 21.

RESULTS: No significant differences were observed for MP and SP+A compared to the controls for the static constructs. For the static bioreactor study, MP and SP+A viabilities were significantly different from the controls, though SP+A was 9.67% and 15.8% higher than the negative and PCB controls, respectively. In the perfusion study, cell viability using the SP+A conditioned medium was not significantly different compared to the PCB but was 13.0% higher than the negative control (Figure 1).

DISCUSSION: This study demonstrated that a combination of sonication and autoclaving did not adversely affect the biocompatibility of MED610 and that 3D printed MED610 bioreactors can be successfully sterilized using this method. While further studies are warranted to assess the role of MED610 in improving cell viability, these findings bring us closer to establishing an open-source printed bioreactor for bone culture.

REFERENCES:
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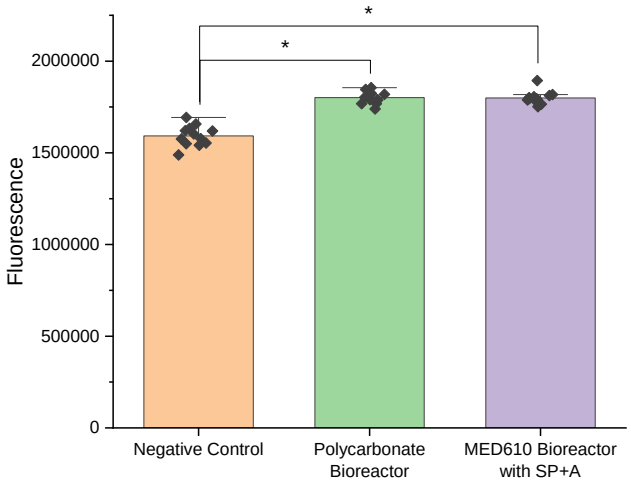

Figure 1: Fluorescence data from CellTiter-Blue assay indicating the cell viability of the negative control (sterile culture medium), positive control (autoclaved PCB), and the SP+A sterilization method on Day 21 for the perfusion bioreactor study.