A Microfluidic Fibrosis-On-a-Chip Model to Study Biomaterial-Immune Interactions

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Statement of Purpose: The foreign body response leads to fibrosis, which can cause multiple negative effects such as hypertrophic scarring, excessive inflammation, and a reduction in the efficacy of cancer therapeutics. During the fibrosis formation process, crosstalk between macrophages and fibroblasts leads to isolation of biomaterials from the native tissue. Traditionally, these interactions are evaluated by in vivo placement in mouse or rat models. These models provide important data but cannot provide a complete understanding of the interactions that take place in human tissues. The goal of this work was to develop a 3D microfluidic model ("Fibrosis Chip") of biomaterial-immune and cancer-immune interactions. After comparing in vitro and in vivo mouse fibrosis, we aim to use human cells within the Fibrosis Chip to better understand fibrosis in humans.

Methods: Microfluidic molds were prepared using standard photolithography techniques and devices were fabricated with polydimethylsiloxane (PDMS). L929 mouse fibroblasts and RAW264.7 mouse macrophages were fluorescently labelled with red and green CellTracker dyes respectively. Extensive studies were conducted to optimize cell seeding density, culture times, and biomaterial implantation. Cells were co-cultured in 2D or within microfluidic devices, after which cancer spheroids or alginate-based biomaterials were implanted within the devices. Immunostaining, fluorescence microscopy, Griess assays, and histological analysis were used to evaluate immune cell activation and fibrosis formation in response to the spheroids and implants.

Results: We have developed a 3-channel microfluidic device for use in the co-culture of fibroblasts and macrophages with 3D cancer spheroids or biomaterials (Fig. 1A). The center channel contains fibroblast-embedded 3D Matrigel into which biomaterial implants or 3D spheroids can be placed (Fig. 1B). The outer channels serve to introduce macrophages and chemokines, respectively. In preliminary 2D experiments, we found that L929 fibroblasts alone did not activate RAW264.7 macrophages (Fig. 1C) and confirmed the effects of lipopolysaccharide (LPS) stimulation on macrophage morphology and activation; we are now working to extend these measurements to our 3D microfluidic system. We also determined the effects of cell count and incubation time on B16-F10 melanoma spheroid formation and used this to select optimal parameters to form spheroids for use in the microfluidic device. Device characterization results and data from ongoing microfluidic co-cultures of fibroblasts and macrophages with melanoma spheroids and alginate-based biomaterials will be presented.

Overall, we have developed an in vitro model that combines physiologically relevant 3D matrices, spheroids, and fluidic flow to a) provide a platform to study the basic biology behind the foreign body response, and b) be used as a platform for screening biomaterials, antifibrotic drugs, and cancer therapeutics. After optimization of experimental protocols and validation in mouse systems, the use of human cells within the device will enable extrapolation of expected human in vivo immune responses and eventual utilization of the chip for personalized and predictive medicine.

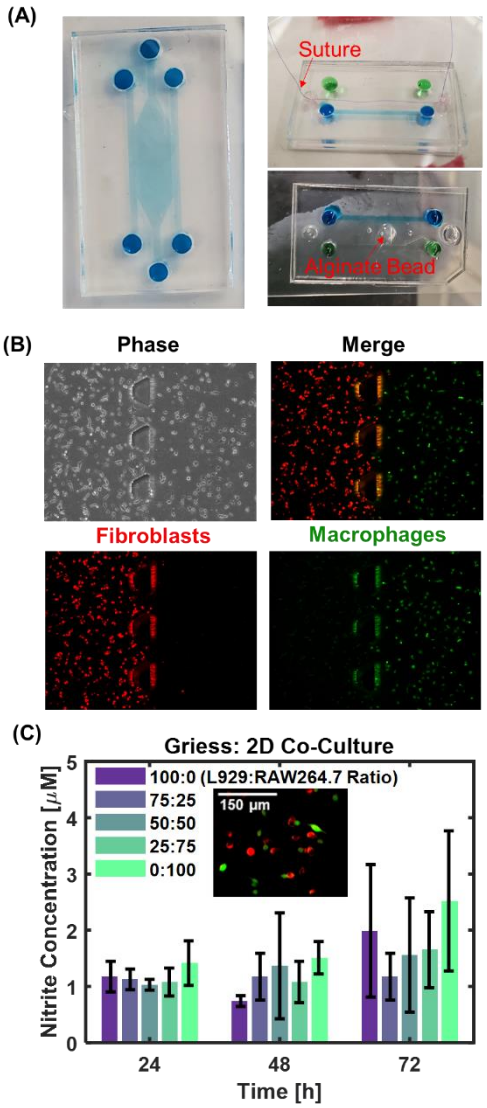

Figure 1. (A) Fabricated fibrosis chip and biomaterial implantation. **(B)** Fibroblast-macrophage co-culture in Fibrosis Chip. **(C)** Effect of fibroblast-macrophage co-culture on macrophage activation.