Development of High Throughput Printer for Combinatorial Screening of Biomaterials for Tissue Engineering Applications

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Statement of Purpose: Engineering biomaterials for modulation of cell responses is very attractive in tissue engineering. Cell responses to biomaterials depend on cell-cell and cell-ECM interactions, which in turn depend on composition and concentration of ECM, and growth factors [1]. Current macro-scale combinatorial screening approach is cost-prohibitive, labor intensive and limited by prohibitively large experimental space of matrix proteins and growth factors. In this project, we have developed a high throughput platform for generating combinatorial libraries of biomaterials from base scaffold, matrix proteins and soluble factors. The platform has throughput of generating and screening large libraries to uncover the complex interactions and engineer optimal matrix combination for suitable cell responses. Our high throughput system comprises of fluid handling elements that create combinatorial libraries by mixing individual components at desired concentrations and compositions, and dispensing them in spatially addressable micro-well.

Methods: To develop the dispensing system, syringe pump drivers (BASi), stepper motor controller (Pololu) and controller boards (Arduino) were assembled and calibrated at CATS. The mixing system consists of 12 and 24 element static mixer (Nordson). The microwell plates are set on XYZ stage. FITC – BSA (Invitrogen) doped collagen (MP Biomedicals) solution was mixed in different combinations with media in a static mixer and dispensed to form hydrogels. Gels were fixed immediately and sectioned using a microtome and imaged on Olympus IX81 microscope. Schwann cells were mixed with collagen and Matrigel solution in the static mixer and viability after mixing process was ascertained using Calcein AM – ethidium homodimer viability kit (Invitrogen).

Results: Fig 1 shows a schematic of the high throughput automated platform we have assembled to print combinatorial libraries of ECM proteins to form 3D hydrogels. Syringe pumps were programmed to dispense combinations of biomaterials. Mixing is a very critical process in this setup as the biomaterial components need to be uniformly distributed throughout the material. A static mixer was selected as a mixing device. To test the effectiveness of the static mixer, FITC BSA doped collagen was mixed in a 24 element static mixer with media in a 1:3 and 1:1 combination and dispensed into a microwell to form hydrogel. Fluorescent micrographs of the gels indicate uniform mixing is achieved using static mixer and it is comparable to the hand mixed control (Fig 2A & B). The 24 element mixer had better mixing compared to the Y and T mixer tested which showed signs of unmixed regions (Fig 2A & C). To ensure the printing process does not alter cellular viability, a viability test was performed on printed cells. Schwann cell viability was determined to be 90% in both control and printed gels (Fig 2D).

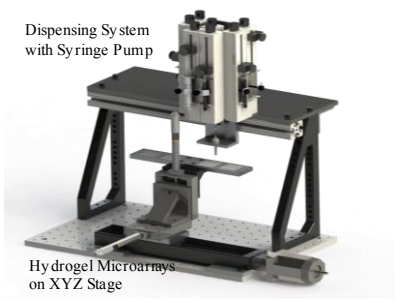

Fig 1: Schematic representation of high throughput printer setup

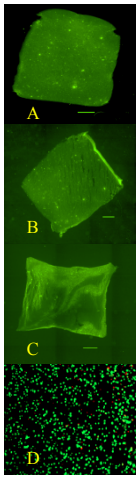

Fig 2: Microscopy images of collagen gel sections. A) Uniformly mixed solution with static mixer. B) Fully mixed control. C) Non uniformly mixed control D) Live Dead Stain of printed gel (Scale bar-500 μm)

Conclusions: High throughput screening technologies have been widely used in drug discovery processes but very few platforms exist for screening biomaterials for tissue engineering applications [2, 3]. We are reporting a platform for combinatorial screening of biomaterials by employing syringe pumps to dispense various combinations of ECM proteins and uniformly mixing these components in a static mixer and dispensing into a microwell to generate a 3D collagen hydrogel. We have shown the static mixer with 24 spiral elements can uniformly mix viscous hydrogel material like collagen and matrigel when compared to conventional T & Y mixers. The mixing process with static mixers does not affect the viability of printed Schwann cells. Current work is focused on generating collagen matrigel libraries by combinatorial variation of collagen concentration and matrigel composition to determine cell response like morphology, migration of Schwann cells on collagen matrigel biomaterials for neural tissue engineering applications. The advantages of our platform include high throughput for screening large libraries of biomaterials, low utilization of resources and ease of operation.

References:

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