Electrowetting-based Multi-microfluidics Array Printing of High Resolution Tissue Construct with Embedded Cells and Growth Factors

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Statement of Purpose: Tissue engineering (TE) is evolving as a potential solution for repair and reconstruction of diseased or damaged tissues (Langer 1993). By combining the advantages of SFF method, electrowetting-based microstructure printing, and biomaterials into one innovative tissue manufacturing system, all limitations can be overcome. Electro Wetting on Di-Electric multiple microfluidic array printing can create droplets of size less than 10 µm, work with a variety of hydrogels, and include cells and growth factors during scaffold fabrication. Electrowetting on di-electric (EWOD) has never been used in TE. EWOD microarrays within a printing mechanism would enable control of droplet size as well as precise deposition of droplets and create a unique combination of high precision and flexibility of materials compared to existing micro-droplet technology.

Methods: The system will integrate a new EWOD array design with a computer-controlled motion system to position the EWOD chip for chitosan hydrogel, tri-polyphosphate crosslinker, growth factor suspended in de-ionized water and cells such as cardiomyoblasts or endothelial cells suspended in complete culture medium based on CAD models. EWOD uses the electrocapillary principle: surface tension (ST) is a function of electric potential across an interface, and the change in liquid-solid ST changes the contact angle at the liquid-solid-gas interfaces. When voltage is applied between the liquid and electrode, surface energy is balanced by electrical energy and the change in liquid-solid ST is lowered, as expressed in Lippmann's Equation. Young's equation relates contact angle and ST, and Lippman-Young's equation relates contact angle to voltage (Cho 2002). Charge accumulates at the solid-liquid interface and the surface wettability is modified from hydrophobic to hydrophilic by applying a voltage between the liquid and an electrode under the dielectric layer. By applying a sequence of voltage to electrodes patterned under the dielectric layer, four fundamental droplet manipulation mechanisms can be established: creating, cutting, joining, and transporting from a reservoir and in the fluid path. The anticipated built rate will be around 1cm/min for a nanoliter droplet and for picoliter-volume droplets, the diameter will be on the order of 10µm. The continuously varying material composition produces gradation in material properties, often known as functionally gradient materials (FGM) (Cheng 2005). The data processing system processes the scaffold models from a computer design, CT or MRI image and converts it into a layered process tool-path. The 3D model of the imaged parts will be reconstructed from these high-resolution multi-planar images through biomimetic model software.

Results: The capability of EWOD for handling hydrogels and cells has been demonstrated. Various amounts ranging from 0.2% to 2% (w/v) of low molecular weight (LMW) and high molecular weight (HMW) chitosan were mixed with glacial acetic acid, 1% and 2%. The final solutions were tested with a rheometer to acquire their corresponding viscosities (Figure 1).

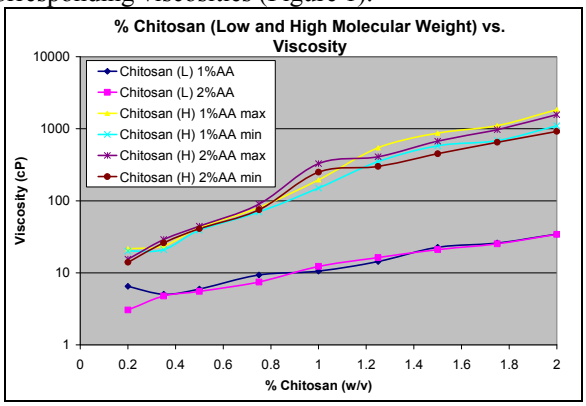

Figure 1 - % Chitosan vs. Viscosity

The largest viscosity the EWOD could handle without the material becoming fixed in place was 250cP. Too high of a viscosity will hinder EWOD movement. HMW chitosan behaves as a non-Newtonian fluid while LMW chitosan behaves as a Newtonian fluid. The relationship between the shear rate and shear stress for a LMW chitosan is linear while not linear for a HMW chitosan. All experiments were performed at room temperature. Dispensing, transport, combining, and splitting were demonstrated using the chitosan solutions and an algenate solution. Initial tests were conducted on the EWOD chip with human fetal osteoblast cell line. After actuation, the EWOD chips were observed under a fluorescent microscope to quantify live and dead cells. The fraction of live cells was about 94% compared to the solution before loading into the chip. Even after 2h with 60V applied to the switching electrodes, there was no noticeable change in the ratio of live-to-dead cells in the immersed droplet.

Conclusions: EWOD chips have been developed to dispense droplets of size less than 100µm. A complete EWOD-based multi-microarray printing system and CAD program has been designed studied. Rheological testing and fundamental droplet operations were completed and studied on chitosan hydrogels and crosslinker solutions. On-chip crosslinking and cell manipulation on a EWOD chip were demonstrated, which shows the system is capable to make micro array printing for tissue construct.

References:

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