Proceedings of the ASME 2009 Summer Bioengineering Conference (SBC2009)
June 17-21, Resort at Squaw Creek, Lake Tahoe, CA, USA

SBC2009-206787

DIELECTROPHORETIC MICROWEAVING: BIOFABRICATION OF ALIGNED BACTERIAL NANOCELLULOSE FOR REGENERATIVE MEDICINE

Michael B. Sano (1), Rafael V. Davalos (2), Paul Gatenholm (2,3)

(1) Engineering Science and Mechanics
Virginia Polytechnic and State University,
Blacksburg, VA

(2) School of Biomedical Engineering and Sciences (SBES)
Virginia Tech - Wake Forrest University
Blacksburg, VA

(3) Material Science and Engineering
Virginia Polytechnic and State University
Blacksburg, VA

INTRODUCTION

The use of natural and synthetic polymers as scaffolding material for regenerative medicine is far from clinical translation for most tissue applications. This is due primarily to lack of manufacturing control over mechanical properties and 3D architecture which promote cell attachment and proliferation. Cellulose, a natural polymer produced by the majority of plants, can be assembled into nanofibrils by bacteria. The advantage of bacterial cellulose is that it has unique biocompatibility, mechanical integrity, hydroexpansivity, and is stable under a wide range of conditions [1]. It is thus ideal as a scaffolding material on which to seed cells for regenerative medicine applications. The bacteria Acetobacter Xylinum produces nanoscale cellulose ribbons at an average rate of 2μm/min [2].

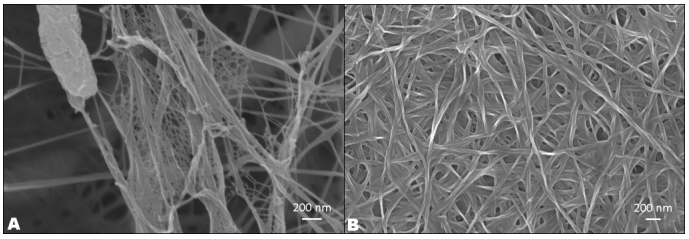

Figure 1: a) A single A. xylinum cell is entangled in b) the dense random cellulose network it helped produce.

In order for the cellulose networks to be viable solutions for biomedical applications, such as implants and tissue scaffolds, their orientation must be controlled and customizable. Existing scaffold fabrication techniques suffer from fundamental manufacturing limitations that have, to date, prevented their clinical translation. These limitations result from the distinct lack of processes capable of reproducibly creating structures on the nano-, micro-, and millimeter scales that adequately promote cell growth and function. We have made steps towards overcoming this limitation by manipulating the shape and nanoscale morphology of bacterial cellulose material through a new process which we call Dielectrophoretic Microweaving.

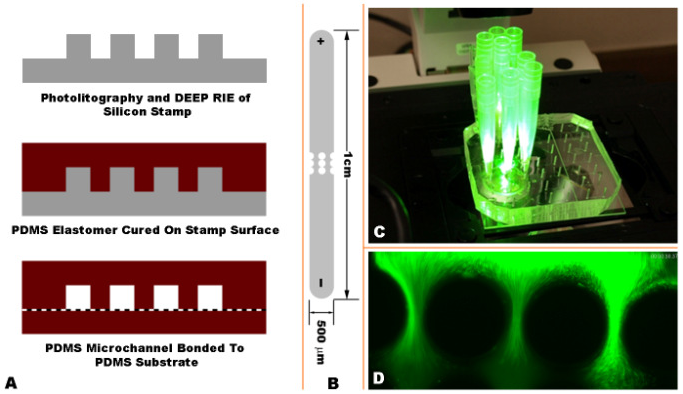

Figure 2: a) Fabrication process for creating microchannels. b) Schematic showing the top view of the channels with insulating pillars. c) Experimental setup for interfacing with microfluidic channels. d) Fluorescently labeled A. xylinum cells being controlled through a microchannel dielectrophoretic forces.

Dielectrophoresis (DEP) is the motion of a particle due to its polarization induced by the presence of a non-uniform electric field

[3]. The velocity of the bacteria under an electric field is calculated by:

$$\overrightarrow{V_p} = \mu_{ek}\vec{E} + \mu_{DEP}\,\nabla(\vec{E}\circ\vec{E}) \quad (1)$$

where μ_{ek} and μ_{DEP} are intrinsic parameters of the bacteria in their culture media. DEP has been shown is an effective means to manipulate and differentiate cells based on their size, shape, internal structure, and intrinsic properties such as conductivity and polarizability [4]. Insulator-based dielectrophoresis (iDEP) uses insulating obstacles, instead of electrodes, within the device to produce spatial nonuniformities in the electric field [5, 6].

METHODS

We have used the strain *Acetobacter xylinum* subspecies sucrofermentas BPR2001, trade number 700178™, from the American Type Culture Collection. They were cultured in a modified fructose media with an addition of corn steep liquid. For precultivation, 6 cellulose-forming colonies were cultured for 2 days at 30ºC in a 300ml rough flask. The bacteria will be liberated by vigorous shaking and inoculating in the desired amount into the culture media.

To study the response of *A. xylinum* to such electric fields, we created microfluidic devices in PDMS using a silicon master stamp fabricated using standard photolithography and deep reactive ion etching. A typical schematic of one of our devices is presented in Figure 2b. *A. xylinum* cells in culture media were injected into the microfluidic channels and pressure was allowed to equalize. Platinum electrodes were then used to apply small electric fields across the channels inducing electrokinetic and dielectrophoretic forces that guided the bacterial cells as they produced cellulose nanofibers.

RESULTS AND DISCUSSION

Figure 2d shows the progression of the bacteria labeled with BacLight™ (Invitrogen, Carlsbad, CA) through the channel. Under an applied field, the bacteria are electrokinetically driven through the channel. The dielectrophoretic forces become more dominant in directing the bacteria as the field is increased. When the applied field reaches a critical value on the order of 400V/cm the DEP forces overcome all other forces and hold the bacteria in place. The results in figure 2d show that the applied electric field can be varied to control bacteria motion.

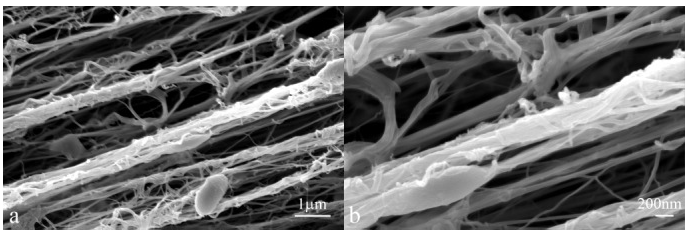

Figure 3: FESEM of the cellulose network produced by A. xylinum under an applied electric field.

A broad range of experiments have been conducted varying a number of parameters including the strength of the applied field and microfluidic chamber dimensions. In small geometries, the cellulose produced quickly clogs the channels and electrokinetic movement of the bacteria is halted. In large geometries such as cuvettes and test tubes, the dielectrophoretic forces no longer dominate. If the applied electric field is too low, the dielectric forces fail to navigate the bacteria through the microfluidic environment. In these scenarios a random cellulose network is produced. Conversely, if the applied field is too high, the bacteria are moved too quickly and cellulose production is switched off.

We have found that we can control the morphology of the cellulose scaffolds when the bacteria are subjected to electric fields between 0.25V/cm and 1.0V/cm, are in a 19 mm long, 500 micron deep PDMS encased microfluidic chamber, and allowed to produce cellulose for 48 hours while being guided through the chamber by electrokinetic and dielectrophoretic forces. At these field strengths, the bacterial cells are being controlled with velocities on the order of 1 micron/s.

A FESEM image of the cellulose produced under 0.45V/cm in which interwoven strands of nanocellulose fibrils are aligned in the direction of the applied electrical fields is shown in Figure 3. Variations in the strength of the applied field change the morphology of the cellulose structure produced. Figure 3 is in clear contrast with Figure 1 which shows a randomly distributed network of nanocellulose. The ellipsoid shaped particles on top of the strands in Figure 3 are the bacteria which have been fixed to the cellulose fibers during the freezing process. The results in the Figure 3 clearly show that the orientation of cellulose fibers and the architecture of the network can be predictably controlled using electric fields.

This process can be adapted to mimic the complex fiber orientations found in physiological tissues. The ability to control the direction of fiber orientation could be readily expanded to weave structures of multiple fiber layers, with each layer grown in a prescribed direction, by simply changing the orientation of the applied electric field. These structures could be tailored to have the desired mechanical properties. The creation of scaffolds for tissue engineering will require adapting this technique on much larger scales and to this effect; we have created continuous scaffold structures with controlled fiber orientation with thicknesses of 500 microns or greater and surface areas of 80 mm^2.

ACKNOWLEDGEMENTS

We acknowledge the Institute for Critical Technologies and Applied Science (ICTAS) at Virginia Tech for financial support and the NCFL at ICTAS for support with imaging. We thank Dr. Aase Bodin for introducing Mike Sano to the bacterial cellulose field as well as Jaclyn Brennan and Hadi Shafiee for experimental assistance.

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