

## **BioNanomanufacturing of Carbide Aerogels**

**Devin Keck**  
Mechanical Engineering  
Clemson University  
dkeck@g.clemson.edu  
**Rodrigo Martinez-Duarte**

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### *Biography*

My name is Devin Keck and I am from Charleston, SC. I received my B.S. in Mechanical Engineering at Clemson University. I began my graduate education in the fall of 2015 and am currently seeking a PhD in Mechanical Engineering. My research interests include microfabrication, microfluidics, biomaterials, and the patterning/control of microorganisms with electrical field gradients. Outside of school my experience includes a one year cooperative education program with Robert Bosch Corporation where I worked on a variety of projects to reduce scrap, decrease cycle times and increase productivity for an electrical steering linkage assembly line.



### **Overview**

There are a number of existing microorganisms, both in the wild and engineered that are capable of synthesizing materials from the nutrients found in their environment. The goal of this research is to develop a technique to pattern and manipulate the cellulose synthesizing bacteria *Gluconoacetobacter xylinus* (*G. xylinus*) using electrical fields. If successful, the technology would enable the ability to structure cellulose fibrils with nanometer diameters into scalable matrices of designed porosity which could be used as structural materials, scaffolding for functional composites, or as a carbon precursor for carbon composites. The use of microorganisms as nanomanufacturing tools will offer a cheap, sustainable alternative to the current state of the art techniques.

### **Motivation**

The patterning and structuring of bacterial cellulose (BC) is an intriguing area of research. Bacterial cellulose can achieve up to 90% crystallinity and offers high mechanical strength and excellent biocompatibility. Compared to other polymers, BC sheets are found to offer a high young's modulus in the range of 15-35 GPa and high tensile strength between 200-300 MPa [1]. Additionally, as a material BC has a water holding capability of up to 99% and naturally produces stable nano-fibril structures with extremely large surface areas. These unique characteristics enable the combination of BC with nanoparticles and other polymers of low molecular weights.

### **State of the Art**

While the patterning of microorganisms using electrical fields is a widely studied area of research, the patterning of *G. xylinus* with electrical fields is largely understudied. To date, research groups have investigated the growth of BC while exposed to electric fields under 1V/cm. The results showed visible fibril alignment of the BC when the *G. xylinus* was exposed to electrical field gradients at or above 0.303 V/cm [2]. Additional results concluded that the crystalline structure of the BC is unaffected when exposed to electrical field gradients less than 1V/cm [3]. Furthermore, it was

determined that the growth rate of the bacterial cellulose decreases when exposed to electrical fields over 0.25 V/cm.

The patterning of *G. xylinus* without the use of electrical fields is the more traditional approach. Perhaps the easiest and most developed method of patterning cellulose is the production of cellulose sheets using static growth conditions. More complex patterning methods include the creation of honeycomb cellulose structures by growing BC on honeycombed patterned agarose and the magnetic patterning of bacterial cellulose through embedding the cellulose with metal oxide nanoparticles and exposing magnetic fields.

### Intellectual Merit

The focus of this research can be split into four specific goals. The first goal is to investigate the use of electric fields for the manipulation of *G. xylinus* to structure planar cellulose fibril patterns. It is hypothesized that successful manipulation of *G. xylinus* using light induced dielectrophoresis (LiDEP) will offer the means to fabricate reproducible fibril patterns on the nanoscale. The second goal is to understand the effect of the electrical fields on the properties and the throughput of the cellulose fibers. It is hypothesized that the electrical fields will have an effect on the fiber dimensions and properties as well as effect the throughput of the *G. xylinus* bacterium. The next goal is to develop a technique to fabricate 3-D cellulose structures using electric field particle manipulation. It is hypothesized that layer by layer fabrication can be utilized for the creation of 3-D cellulose structures. Lastly the final goal is to functionalize the cellulose structures with various metal oxide nanoparticles in order to create carbides. The hypothesis is that the cellulose can be functionalized with nanoparticles and heat treated to create many different types of carbides.

### Broader Impact

If successful, the results of this research could be expanded to a number of microorganisms with the ability to biosynthesize materials. This research will facilitate the use of microorganisms as nanomanufacturing tools and create precedence for future research and advancements in the use of synthetic biology for green manufacturing. Additionally, if successful, this research could facilitate the use of microorganisms for the creation of designed materials and composites with properties tailored on the nanoscale.

### Research Approach

The primary concept to be exploited for this research is the use of dielectrophoresis (DEP) to manipulate the *G. xylinus* bacterium. DEP can be used as a method of noncontact cell manipulation by using an electric field gradient to induce a dipole within the cell membrane. The type of DEP to be used in this research is light induced DEP (LiDEP) otherwise known as optoelectronic tweezers. LiDEP is a more capable tool for cell patterning due to the fact that a photoconductive layer is used to create the electric field gradient, which allows for the electric field gradient to be dynamic and reconfigurable. Figure 1 features a common LiDEP setup in which cells are manipulated by sandwiching the cell culture between two conductive chips while an electrical

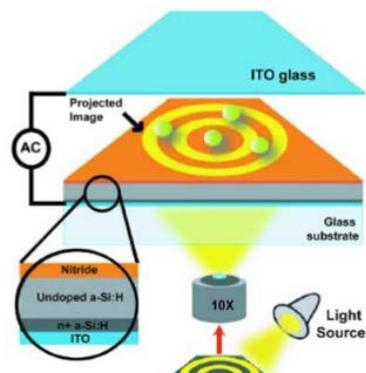


Figure 1: LiDEP setup to be used for the manipulation of *G. xylinus* [4]

field gradient is introduced by shining a nanoscale sized movie onto the photoconductive layer using a commercial DLP projector and microscope lens [4].

The primary force that allows for the manipulation of a microorganism using an electric field is termed the DEP force. The resulting DEP force acting on a particle with volume  $v$  can be determined using equation 1:

$$(1) \quad F_{DEP} = \frac{1}{4} v Re[\alpha] \nabla [E]^2$$

where  $\alpha$  is the effective polarizability of the particle and  $E$  is the applied electric field [5].

The experimental plan will begin by constructing an LiDEP setup similar to the one shown in figure 1. The capabilities of the device will be tested through the manipulation of popularly researched cells whose DEP parameters are well established. While studying these cells improvements will be made to the LiDEP device moving towards the goal of manipulating a single cell. Experiments using Carbon DEP will be conducted on the *G. xylinus* with the goal of determining the optimal amplitude, frequency, and media conductivity for DEP manipulation. These results will be expanded to the LiDEP setup. A culture of cellulose will continuously be synthesized and used to study the growth/throughput of different media environments, methods of functionalization and heat treatment into carbides, and the mechanical properties of the cellulose grown under different culture conditions.

### **Findings to Date / Conclusions**

To date the construction of the LiDEP device is complete and its functionality has been tested through the successful manipulation of yeast cells. The potential for *G. xylinus* to be manipulated using DEP force has been illustrated using carbon electrodes. Thus far the cells show the strongest DEP force when suspended in a media with a conductivity of  $1500 \mu S/cm$  using an electric field with an amplitude of  $10 V_{pp}$  and a frequency between 15-20 MHz. Lastly, bacterial cellulose has been functionalized with ammonium meta-tungstate and heat treated to successfully create tungsten carbide. The optimal heat treatment process took place in a vacuum environment with a temperature of  $1300^\circ C$ , a dwell time of 3hrs, and a heating rate of  $5^\circ C/min$ .

### **References**

- [1] Y. Huang, C. Zhu, J. Yang, Y. Nie, C. Chen, and D. Sun, 2014, "Recent advances in bacterial cellulose," Cellulose, vol. 21, no. 1, pp. 1-30, 2014
- [2] M. B. Sano, A. D. Rojas, P. Gatenholm, and R. V. Davalos, 2010, "Electromagnetically controlled biological assembly of aligned bacterial cellulose nanofibers," Ann. Biomed. Eng., vol. 38, no. 8, pp. 2475-2484
- [3] X. Zheng, C. Zhong, M. Liu, A. Guo, Y. Li, and S. Jia, 2014, "The Cells of Gluconoacetobacter xylinus Response to Exposure," in Proceedings of the 2012 International Conference on Applied Biotechnology (ICAB2012), Lecture Notes in Electrical Engineering, T.-C. Zhang, P. Ouyang, S. Kaplan, and B. Skarnes, Eds., pp. 1749-1757.
- [4] P. Y. Chiou, A. T. Ohta, and M. C. Wu, 2005, "Massively parallel manipulation of single cells and microparticles using optical images," Nature, vol. 436, no. 7049, pp. 370-372.
- [5] M. Castellarnau, A. Errachid, C. Madrid, A. Juarez, and J. Samitier, 2006, "Dielectrophoresis as a tool to characterize and differentiate isogenic mutants of Escherichia coli," Biophys. J., vol. 91, no. 10, pp. 3937-3945.