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Looking into the Eye with REAP

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Commentary

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—Ryann Boudreau

My academic and professional interests took root long before I came to the University of New Hampshire (UNH) to study bioengineering. Encouraged by my parents to explore, ask questions, and experiment with my own creative designs as a child, I developed a device named the Micro Sling Shot, built a cell phone charger, and engineered what I referred to as the Smiley Faced Pull Toy, all from household items. My intellectual curiosity included all things science, and I was fascinated with how and why things work as they do.

Academically, I also gravitated toward the sciences. I was the only girl in my class building model rockets at Summer Science Camp during middle school. During high school I discovered bioengineering. I spent four summer weeks studying in the biotechnology/nanotechnology module of the University of New Hampshire's Project SMART Program. There, I researched and presented a poster on pharmacogenomics, which is the study of how pharmaceuticals can be chosen for people based on their specific DNA. This experience strengthened my passion for laboratory science and in particular, biological engineering.

The author in the lab at UNH. *(Photo by Jeremy Gasowski.)*

Combining my love for math and science to help people was my dream. Whether through disease prevention, the creation of organs, or even the formation of genetically modified organisms, I wanted to be part of it. The summer after my first year as a bioengineering major at UNH, I participated in the Research Experience and Apprenticeship Program (REAP). Eager to dive right in with REAP, I set out with Dr. Kyung Jae Jeong, my faculty research mentor, to create an artificial cornea to help people who have corneal blindness.

Keratoprosthesis and the Need for Innovation

Keratoprosthesis is a surgically implanted artificial cornea that replaces a diseased cornea. The cornea is the clear, outer layer of the eyeball that bends and contorts light entering the eye to help the retina create a visual image. Keratoprosthesis is usually the last resort after a failed attempt to replace the scarred cornea with a donor cornea (keratoplasty). With keratoplasty, as with any donor procedure, the host's body sometimes rejects the donor tissue. In the case of keratoplasty, the rejection causes the cornea to turn white and opaque. Keratoprosthesis offers a unique solution that bypasses the possible host rejection issues associated with keratoplasty.

Currently, a device called Boston KPro, developed by a research team at Massachusetts Eye and Ear Infirmary, fills the need for an artificial cornea (Saeed, Shanbhag, and Chodosh 2017). This device consists of four pieces (see Figure 1): (1) a front part that is made of a clear transparent plastic called polymethylmethacrylate (PMMA); (2) a corneal graft (a piece of donor cornea); (3) a porous back plate that allows for nutrient transport throughout the device; and (4) a titanium locking ring that holds the device together. With the implementation of the clear front part, the patient will still be able to see even if his or her body rejects the donor cornea. The plastic, unlike a rejected donor cornea, will not turn opaque. All Boston KPro procedures do require a donor cornea, because it helps connect the device to the patient's eye.

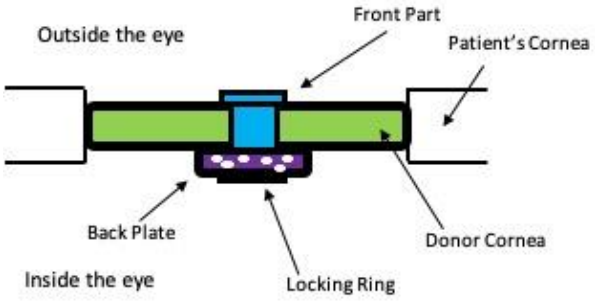

Figure 1. Boston KPro's design, featuring the four-piece configuration.

Boston KPro's design, however, has inherent flaws. The clear, plastic front part and the donor cornea are screwed together with the locking ring. This binding isn't airtight, resulting in a very small space between the pieces. This space permits bacteria and other harmful pathogens to travel into the eye, which can cause inflammation, or endophthalmitis (Behlau et al. 2014). Endophthalmitis can eventually damage the retina, and once the retina is permanently damaged, all devices, including the Boston KPro, become ineffective in curing vision loss. Subjects using the Boston KPro must take lifelong prophylactic antibiotics to avoid infection. Taking lifelong antibiotics is not ideal. Stronger pathogens that are immune to the antibiotic can develop, and the antibiotic can cause an imbalance, such as yeast infections, in the body. In addition, Boston KPro's design is dependent on fresh donor corneas, which are in short supply.

With the poor integration of the Boston KPro segments and the shortage of donor corneas, the fabrication of a new design for keratoprosthesis is in high demand. I, along with the rest of Dr. Jeong's team, set out to create a new device that addresses issues with the Boston KPro device. For my REAP, I focused primarily on the development of a hydrogel that will act as an essential part of the new keratoprosthesis design.

Silk and Gelatin as Components of the Hydrogel

We developed a hydrogel made from silk and gelatin for the new keratoprosthesis design for several reasons. First, these components make a hydrogel with properties similar to natural human corneas. Gelatin is made from collagen, which makes up the natural cornea. Gelatin has many desired qualities, including working well with the living cells in the eye (biocompatibility), creating a comfortable, habitable environment for them; interacting and responding the way we want it to in the eye (bioactivity); and breaking down into safe substances that won't harm the eye (biodegradability).

Gelatin also can be cross-linked chemically (or covalently) and physically, which connects the different strands of gelatin and creates a firmer gel. Covalently cross-linked gelatin creates a useful hydrogel for some needs, such as soft tissue regeneration, but is not stiff enough to survive the medical procedure for keratoprosthesis or a day in the life of a normal person. We needed to find a substance to incorporate with the gelatin that would stiffen the hydrogel. Previous research on the project led to the investigation of silk for this purpose.

Silk is a protein known for its stiffness and rigidity. Like gelatin, silk can be cross-linked to form a stronger substance. Silk causes the gelatin hydrogel to become tougher and possibly equivalent in strength to a natural human cornea. By cross-linking both the gelatin and the silk, we create an interpenetrating network. The silk and gelatin are woven together to compose the silk/gelatin artificial cornea.

Learning Experiences

During the spring semester of 2018, just before my REAP, I began creating different combinations of silk/gelatin hydrogels. This was a long process, because the combination of gelatin and silk had to be just right for the hydrogels to come out similar to the natural cornea. Over the summer, we perfected the combination of components for the hydrogels and conducted many tests on them.

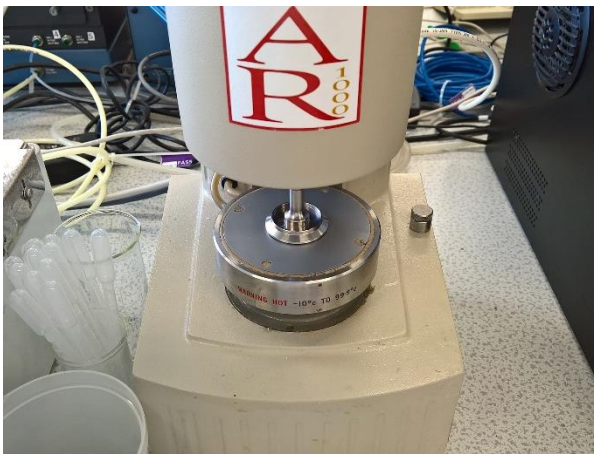

Figure 2. A rheometer shows the metal plate fully lowered down on top of the stage. The hydrogel was placed between the metal plate and stage to test stiffness. *(Photo copyright 2016 Nick Birse, Wikimedia Commons, "TA Instruments AR1000 Rheometer.")*

To test the hydrogel, I learned how to use a rheometer and a scanning electron microscope. A rheometer measures the movement of a substance when a rotational force is applied, and we used it to test the stiffness of the hydrogel (see Figure 2). In the rheometer, a metal plate pushes on the hydrogel in a rotational motion and measures the shear force pushing back on it. The stiffness properties of our first hydrogel were not those of a natural cornea. Our hydrogels were weaker. This was rough news for me, but I have learned that in research you're going to have many

failed attempts before you succeed. That's just part of the process. With this information, we brainstormed ideas on how to strengthen the hydrogels, one of which involved changing the combinations of components.

We also used a scanning electron microscope to evaluate the number of pores in our silk/gelatin hydrogels (see Figure 3). A porous hydrogel is very important for a keratoprosthesis application, because the living cells in the patient's eye need to flow through the hydrogel for integration and possibly the creation of new cornea cells. Before my REAP experience, I had minimal practical experience with the scanning electron microscope and had not fully appreciated the significance of every part of the microscope until I saw it in the functional context of the lab. I was excited to observe and participate in the whole process of using this delicate instrument, including the preparation of the hydrogels, the protocols of working the machine, and taking pictures of the product.

The preparation of the hydrogels consisted of lyophilization, which dehydrated the samples, and then coating them with a layer of metals. The layer of metals allows for the electrons emitted by the microscope to bounce off a sample, creating an image of the sample's micro to nano surface topography. In the case of my research, this allowed us to see the pores in our hydrogels.

The scanning electron microscope showed that our silk/gelatin hydrogels contained many pores. The picture looked like a flat surface with many grooves and bumps, similar to pictures of the surface of the moon (see Figure 4). These findings were promising and exciting to observe. The presence of pores meant that the hydrogel could possibly allow cells to spread through it and grow.

Figure 3. Shown is a scanning electron microscope. Lyophilized hydrogels were placed into the machine, and a computer was used to find the perfect angle at which to take the picture. *(Photo by Phenom-World copyright 2016, Wikimedia Commons, "144243 PhenomWorld with Screenshots Tungsten.")*

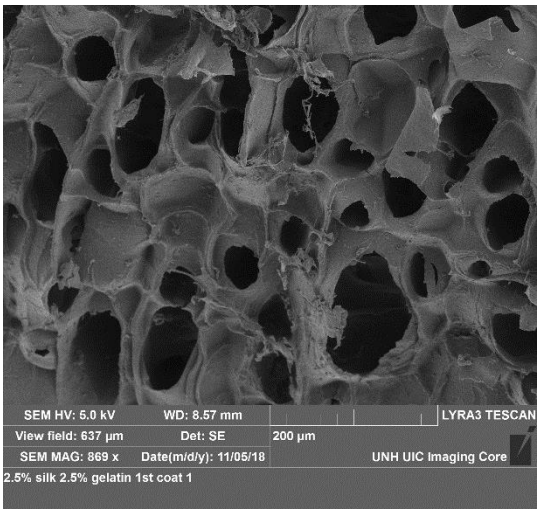

Figure 4. A scanning electron microscope image of the silk-gelatin composite hydrogel shows many pores.

With the new findings of a weak but porous hydrogel, we decided to experiment with the process of making the hydrogels. We hypothesized that if we changed certain intervals of time in the production process, the hydrogels would become stiffer. Our results supported our hypothesis (Park et al. 2019).

Through the hydrogel development process, I learned other techniques and procedures that would prepare me for future research, including multiplex assays; creating and changing cell medias; sterilization techniques; culturing, counting, passaging, and storing various types of cells; and creating silk fibroin from silkworm cocoons.

The process of creating silk fibroin was fascinating. We bought silkworm cocoons from a beauty supply shop (they can be used for skin care) and combined them with certain chemicals to create a concentrated liquid form of silk fibroin. It was a long, tedious process consisting of days of adding chemicals to the silk, letting the silk dry out, and allowing it to mix in a beaker filled with water (dialysis) to create the perfect concentration of silk needed. It was well worth it in the end, for we were able to create an essential component of the hydrogel for a much cheaper price.

By the end of the summer we had made strong hydrogels, and in the following fall and spring semesters further research involved creating a more porous hydrogel. By the end of the 2019 spring semester, I was working in the lab to make a hydrogel that is more mechanically strong, porous, and biocompatible.

Looking Toward the Future

Participating in REAP was an amazing opportunity for me, and one of the best experiences I have had as a student. It made me appreciate the level of dedication and persistence that is necessary when conducting research. During the academic semester, other obligations take away from the focus and time required to see results in the lab. REAP allowed me to spend most of my day, five days a week for ten weeks, immersed in what I am really interested in: researching and developing innovative technologies that can impact the quality of life for people around the globe. I was given the opportunity to advance my knowledge of laboratory practices and the opportunity to identify specific areas of interest, such as genetic and tissue engineering, that I eventually may pursue as a PhD student in biological engineering. I look forward to developing and reporting on my own research in the future.

I would like to thank some very important people who made this amazing, unforgettable experience possible. First of all, thank you to my mentor, Dr. Kyung Jae Jeong, and his postdoctoral trainee, Dr. Shiwha Park, who provided support and guidance without which I would not have been able to participate in such a fulfilling experience. I would also like to thank the other researchers on the team, who helped make my time over the summer educational and enjoyable. Special thanks to Mr. Dana Hamel and Dr. George Wildman for donations toward the research done during the ten-week REAP program, as well as Dr. John McClain and Dr. Karsten Pohl for recommending me. Thank you to the University of New Hampshire and especially to the Hamel Center for Undergraduate Research for the opportunity as an undergraduate to be a part of such a fundamental aspect of my career. Finally, thank you to my family and friends for always believing in me and encouraging me to do my best work.

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Author and Mentor Bios

Ryann Boudreau, from Londonderry, New Hampshire, came to the University of New Hampshire (UNH) to major in bioengineering and minor in genetics. She is in the University Honors Program and plans to complete the Honors in Major track as well. She will graduate in May 2021 with a bachelor of science degree. Interested in researching tissue engineering concepts, Ryann met with Dr. Jeong, who was studying biomaterials, and worked in his lab on an artificial cornea design through the Student Research Experience course during the spring of 2018. She continued this research as a participant in the Research Experience and Apprenticeship Program (REAP) during the summer of 2018. Ryann heard about *Inquiry* through one of the editors and was happy to share how her research provided her with valuable knowledge about the research process and experience with equipment involved in biological engineering. Ryann is passionate about her career path and looks forward to researching and developing treatments that will improve the health and quality of life for people around the world.

Kyung Jae Jeong is an assistant professor in the chemical engineering department at the University of New Hampshire (UNH), where he began teaching in 2013. He specializes in biomaterials and their applications in medicine. Before teaching at UNH, Dr. Jeong worked with ophthalmologists at Massachusetts Eye and Ear Infirmary during his postdoc training, and his collaborative work with them continues. He has mentored several undergraduates conducting research, but Ryann Boudreau is the first to contribute to *Inquiry*. He finds it rewarding to train undergraduate students, and he was particularly pleased that Ryann was a coauthor of an article about their work published in a major biomaterials journal in summer 2018.