NDnano Undergraduate Research Fellowship (NURF) 2014 Project Summary

1. Student name: Katherine Iliff

2. Faculty mentor name: Ryan K. Roeder and Diane R. Wagner

3. Project title: Nanoparticle contrast agents for detecting damaged cartilage or Cationic Gold Nanoparticle Contrast Agents for Detecting Damaged Cartilage and Tendon

4. Briefly describe any new skills you acquired during your summer research:
   During my project I learned several methods of characterization including ultraviolet-visible spectroscopy and dynamic light scattering to measure the size and zeta potential of the nanoparticles. I also learned how to image my tissue samples with micro-computed tomography.

5. Briefly share a practical application/end use of your research:
   Gold nanoparticle contrast agents can improve imaging of soft tissues, which would allow early diagnosis of joint diseases such as osteoarthritis. Early diagnosis could slow the disease progression to prevent debilitating injuries and the need for joint replacement.

Begin two-paragraph project summary here (~ one type-written page) to describe problem and project goal and your activities / results:

   Joint diseases such as osteoarthritis are often diagnosed in late stages because cartilage and tendon are difficult to noninvasively image. Current diagnosis is based on symptoms and imaging that indicates that the cartilage has already worn down significantly. Imaging early stages of damage is difficult because soft tissue has low X-ray attenuation, so there is low contrast between damaged and undamaged regions. Gold nanoparticles (AuNPs) are attractive as X-ray contrast agents because they are relatively biocompatible, have high X-ray attenuation, and can be easily functionalized to target biomarkers such as glycosaminoglycans (GAGs). GAGs are a negatively charged component of cartilage and tendon that are exposed when tissue is damaged. When the AuNPs are functionalized with positively charged poly-L-lysine (PLL) or poly(ethyleneimine) (PEI), they should target the negatively charged GAGs and, therefore, highlight regions of damage for micro-computed tomography.

   AuNPs were synthesized and functionalized with poly-L-lysine (PLL) or poly(ethyleneimine) (PEI) molecules. Bovine patellar cartilage and Achilles tendon samples were prepared. Using a drop tower cartilage samples were impacted to create articular surface fissures and damage throughout the depth. Other cartilage and tendon samples were manually damaged on the surface using a scalpel incision. Samples were either dyed with India ink or soaked in functionalized AuNP solution overnight and imaged. As-synthesized PLL-AuNPs and PEI-AuNPs exhibited near neutral pH and high positive zeta potentials. Functionalized AuNPs were stable as-synthesized but less stable in the presence of tissue samples, especially when concentrated for X-ray imaging. In cartilage samples where functionalized AuNPs remained stable, the AuNPs appeared to target the undamaged articular surface but not the damage site. In
tendon samples, the AuNPs targeted the surface damage (Fig. 1). This difference may be due to a difference in the GAG release or exposure mechanisms of each tissue.

Figure 1: Photographs of (a) the top surface of articular cartilage (6 mm diameter) damaged by scalpel and labeled by PLL-AuNPs and (b) the cross-section of tendon (9 mm wide) damaged by scalpel and labeled by PEI-AuNPs
Publications (Poster):
Below is the poster I presented at the 2014 Summer Undergraduate Research Symposium.

Cationic Gold Nanoparticle Contrast Agents for Detecting Damaged Cartilage and Tendon
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Introduction
The ability to distinguish between healthy and damaged regions in soft tissue can be problematic due to subtle differences in the key absorption characteristics which result in low contrast. Cationic gold nanoparticles (AuNPs) are a highly negatively charged component of both cartilage and tendons. After damage, cartilage content in cartilage decreases and cation content is found to increase. AuNP contrast agents have been shown to diffuse through tissue better than anionic contrast agents because of the high negative charge density from cation. AuNPs functionalized with p-amino-phenol (PA) and phenylboronic acid (PA) have been shown to display increased hydrodynamic diameter and zero potential and remain stable under a wide range of conditions. Therefore, the objective of this study was to investigate PA and PA functionalized AuNPs as targeted cation contrast agents for damaged cartilage and tendon.

AuNP Synthesis and Functionalization

PLL- and PEI-AuNP Characterization

Labeling Damaged Cartilage and Tendon

Figure 1: Schematic diagram of AuNP surface labeled with PA (a) or PA (b).

Tissue Damage Models
- 0.5 mm diameter osteochondral plugs were drilled into the femoral condyle.
- Tendon samples were cut from bony edge of the patellar ligament.
- For the osteochondral plugs, a drill bore with a calibrated impeller was used to induce a traumatic traumatic injury creating surface fissures and damage throughout the entire depth with a maximum impact load of 300 N.
- A surgical needle approximately 1 mm deep was used to expose fissures in both cartilage and tendon.

Figure 2: Diagram of femoral head loaded with intercondylar ligament.

Figure 3: Photographs of intact cartilage, showing color of (a) and (c) AuNPs and (b) RhoDyl. Ultrastructural images (a) of PLL AuNPs with a peak absorption at 417 nm and (b) PEI AuNPs with a peak absorption at 630 nm. Dynamic light scattering (DLS) of AuNPs (c) and AuNPs with a peak absorption at 417 nm. Transmission electron microscopy (TEM) images of AuNPs (d) and AuNPs with a peak absorption at 417 nm.

Figure 4: Photographs of the articular surface of cartilage damaged with the deep tissue and held with a sterile articular surface of cartilage damaged with a deep tissue and held with a sterile saline solution. A group treated with a saline solution and a group treated with a saline solution and a group treated with a saline solution and a group treated with a saline solution.

Future Work
Future work will include efforts to improve stability and concentration of AuNPs for computer tomography imaging and to better understand the mechanisms behind the release of markers of damage after damage in both cartilage and tendon.

Acknowledgements
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References