ND*nano* 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.

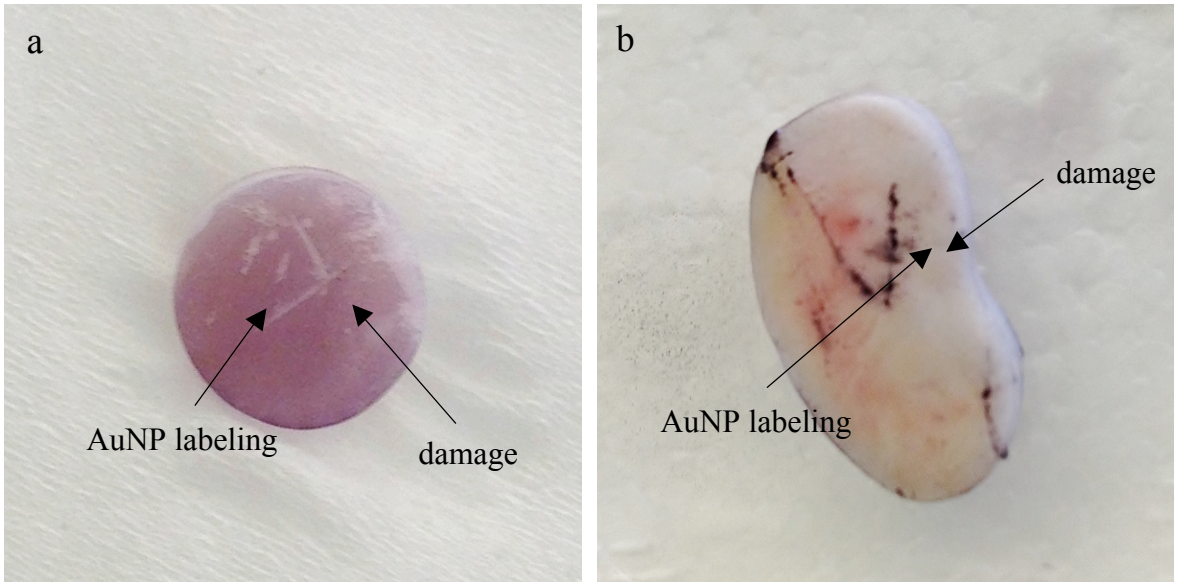

Figure 1: Photographs of (a) the top surface of articular cartilage (6 mm diameter) damaged by scalpel and labeled by PLL-AuNPs and (b) the cross-section of tendon (9 mm wide) damaged by scalpel and labeled by PEI-AuNPs

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 X-ray attenuation which results in low contrast.

Glycosaminoglycans (GAGs) are a highly negatively charged component of both cartilage and tendon. After damage, GAG content in cartilage decreases and GAG content in tendon increases.[1]

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 GAGs.[2]

Cationic contrast agents have been shown to diffuse through tissue better than anionic contrast agents because of the high negative charge density from GAGs.[3]

AuNPs functionalized with poly-L-lysine (PLL) and poly(ethyleneimine) (PEI) have been shown to display desirable hydrodynamic diameters and zeta potentials and remain stable under physiological conditions.[4]

Therefore, the objective of this study was to investigate PLL and PEI functionalized AuNPs as targeted X-ray contrast agents for damaged cartilage and tendon.

AuNP Synthesis and Functionalization

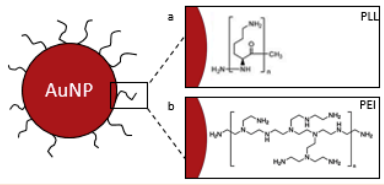

Figure 1: Schematic diagram of a AuNP surface functionalized with (a) PLL or (b) PEI.

Tissue Damage Models

- 9.5 mm diameter osteochondral plugs were drilled from bovine patellae.
- Tendon samples were cut from bovine Achilles tendon.
- For the osteochondral plugs, a drop tower with a rounded impactor was used to model a compressive traumatic injury creating surface fissures and damage throughout the entire depth[5] with a maximum impact load of 1000 N.
- A scalpel incision approximately 1 mm deep was used to expose GAGs in both cartilage and tendon.

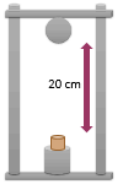

Figure 2: Schematic diagram of drop tower loaded with osteochondral plug.

PLL- and PEI-AuNP Characterization

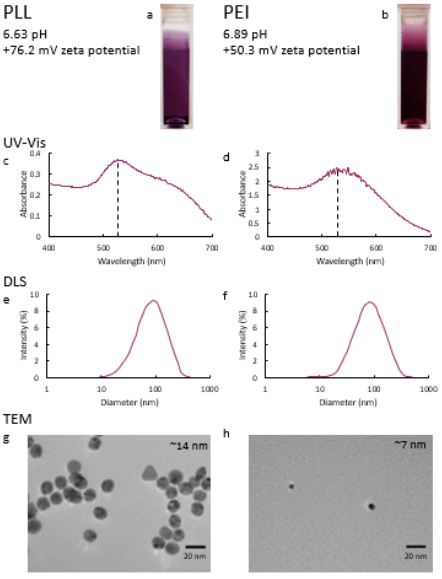

Figure 3: Photographs of plastic cuvettes displaying color of (a) PLL-AuNPs and (b) PEI-AuNPs. Ultraviolet-visible spectroscopy (UV-Vis) of (c) PLL-AuNPs with a peak absorbance at 527 nm and (d) PEI-AuNPs with a peak absorbance at 527 nm. Dynamic light scattering (DLS) of (e) PLL-AuNPs with a mean hydrodynamic diameter of 98.8 nm and (f) PEI-AuNPs with a mean hydrodynamic diameter of 67.6 nm. Transmission electron microscopy (TEM) images of (g) PLL-AuNPs and (h) PEI-AuNPs with a physical particle diameter of 14 nm and 7 nm, respectively.

Labeling Damaged Cartilage and Tendon

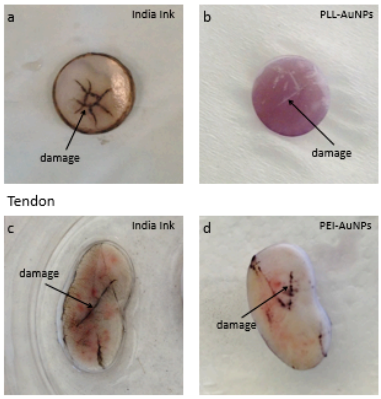

Figure 4: Photographs of the (a) articular surface of cartilage damaged with the drop tower and dyed with India ink; (b) articular surface of cartilage damaged with a scalpel and labeled by PLL-AuNPs; (c) cross-section of tendon damaged with a scalpel and dyed with India ink; (d) cross section of tendon damaged with a scalpel and labeled by PEI-AuNPs.

Future Work

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

Acknowledgements

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References

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