Quantitative Polarized Light Imaging of Histologically Stained Rat Humerus Cartilage

Gustavo De Paiva
Washington University in St. Louis

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Independent Study:
Quantitative Polarized Light Imaging of Histologically Stained Rat Humerus Cartilage
Lake Lab
Fall 2021
Gustavo De Paiva
December 22, 2021
Dr. Spencer Lake’s lab uses rat elbows to study the effects of post traumatic joint contracture on the elbow. Quantitative polarized light imaging (QPLI) is a method used by the Lake lab in order to study the microstructural makeup of musculoskeletal soft tissues. I used quantitative polarized light imaging to quantify the collagen in rat elbows. To do this, I imaged the injured and healthy rat elbows that were previously mounted by my predecessors in the lab. Various stains enhance the visibility of particular tissues, allowing humans to see. In our case, picrosirius red is used to stain collagen. Our results from this QPLI study may be able to tell us how humerus collagen is affected within its various tissues such as calcified and articular cartilage after injury and over time. These factors: timepoint, disease state, and humerus tissue along with one other would end up becoming the focus of my research.

The objective of my independent study was to understand how the collagen strength and alignment look across healthy and injured elbow samples. Over the summer I was a fellow under the Washington University Summer Engineering Fellowship, and I worked under Mike, a post-grad doing his research in the Lake lab. I was tasked with creating a protocol for capturing images of the rat’s capsule and humerus cartilage. I was then responsible for processing these images through a masking program which allows me to section off the tissue of interest. I then run these masks through Matlab for QPLI where images of the stdAoP and avgDoLP are outputted. Through QPLI, we are able to see the orientation and strength of the collagen fiber alignment. Having this process streamlined allowed me to more quickly analyze images and deliver results. My objective for the fall was to continue this research but focus on a particular region of the tissue rather than the entire calcified cartilage region for example. Standardizing the region of interest will erase any discrepancies we may have had in capturing entire tissue regions. This is our fourth factor, region, of which we captured images of 3 regions. We
standardized the size of these rectangular regions, and tried to standardize their position on each humerus by placing each rectangle a certain distance from the edge of the humerus. An image of each region within the humerus is pictured below.

After capturing the data associated with the rat elbows across timepoint, disease state, humerus tissue, and region, my most pressing challenge became manipulating the data for data analysis. Because data analysis is so complex and specialized, I spent weeks conducting various statistical tests with the counsel of Mike. The challenge was deciding which statistical test best fit for my data comparisons. I conducted t-tests and one-way ANOVAs before realizing two-way ANOVAs would be the proper test to conduct if comparing data across two factors. In my case, I sought to compare data across two factors: timepoint(42 v. 84 days) and disease state(healthy v. injured). My results are included below.
We found statistically significant avgDoLP results in region 3 of injured calcified cartilage between 42 v. 84 days. Other statistically significant results include: stdAoP for injured calcified cartilage between 42 v. 84 days in region 2, stdAoP for healthy calcified cartilage between 42 v. 84 days in region 3, and stdAoP for healthy articular cartilage between 42 v. 84 days in region 3.

While the results from the statistical tests I conducted are of interest, the immensity and complexity of the data is the real area of focus. After presenting my research to the lab, many sought to provide input on what statistical tests could be conducted to extract the most comprehensive statistical differences. Dr. Lake and I have spoken about having a specialist review the data above and providing their input on what tests could be used to compare the data. We will continue with this plan during the following semester, and hope to have a more elaborate set of statistically significant results then.

Aside from consulting with a specialist, it may be valuable to conduct the same research with capsule tissue in the rat elbows and with smaller and numerous rectangular sections. It may also be valuable to look at the research with a different magnification for each image or to orient the elbows differently. Next semester, I will explore these options in tandem with my mentor.