Automated Analysis of OA-Related Histological Changes Across the Rodent Tibial Plateau using Deep-Learning

Jacob L. Griffith[1], Justin Joseph[1], Kyle D. Allen[1,2]
[1]J.Crayton Pruitt Family Department of Biomedical Engineering, [2]Department of Orthopedic and Sports Medicine, University of Florida, Gainesville, FL
jacob.griffith@ufl.edu

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INTRODUCTION: Histological grading is commonly used to assess tissue degeneration and joint-level changes in rodent models of osteoarthritis (OA). Disease progression in these models results in changes to multiple tissues throughout the tibial plateau. Traditional grading schemes utilize ordinal ranks to describe these changes and identify differences between experimental groups. To build on the information provided by the ordinal rank systems, we have previously introduced quantitative approaches that summarize joint remodeling in the medial compartment using area calculations to analyze changes in cartilage, subchondral bone, and synovium [1-3]. Unfortunately, these systems are time intensive and tend to focus on specific regions and measurements that are assumed a priori. To overcome these limitations, the current study creates a high-throughput histological analysis pipeline focused on quantifying and visualizing joint remodeling in rodent knees across the tibial plateau. Here, deep-learning driven segmentation is used to automate identification of cartilage, subchondral bone, bone marrow, growth plate, and osteophytes (**Fig. 1**), allowing for rapid assessment of spatial and morphometric information from histological sections. Probabilistic heat maps are then created by aligning the regions of interest across all animals in the study. These heat maps allow us to visualize differences within and between groups without prior assumptions on the areas of remodeling/degeneration.

METHODS**:** Histological images used in this study were obtained from our lab's previous work; these include naïve animals, sham procedures, and OA-inducing surgeries across a breadth of OA severities. All histological images were collected from IACUC-approved studies at the University of Florida. Moreover, all images were produced from frontal sections (10 μm) representing the loading region on the medial tibial plateau; images from the anterior or posterior aspects of the joint were not considered.

To generate a high throughput histological analysis pipeline, deep-learning driven segmentation was first used to identify relevant regions of interest using the pre-existing U-Net architecture as described in [4]. Using Keras and the TensorFlow library in Python, we trained our U-Net using histological images (n=303) with regions of interest labeled by a skilled grader. To account for additional variability in image quality we implemented image augmentation using the Python Imaging Library – similar to the approach described in [4] for data augmentation with U-Nets for biomedical image. The resulting training set (n=1818) included 5 augmented images for each original input, with each image having a unique variation in saturation and rotation angle. After training, the U-Net was tested on 67 images and segmentation performance was assessed using a confusion matrix.

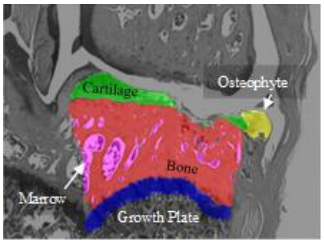

Figure 1. Representative regions of interest.

Following segmentation, the pipeline visualizes the histological data using plots of multiple quantitative metrics along with probabilistic heat maps. The quantitative metrics focused on morphometry of each region of interest including, but not limited to, area, thickness, and eccentricity. Additionally, location-dependent morphometric changes of a given tissue were analyzed across the medial compartment. This analysis allows us to spatially identify regions of the medial compartment most affected by remodeling. Statistical analysis for the morphometric data can be performed using linear mixed effects regression models. Finally, probabilistic heat maps of cartilage, growth plate, bone, bone marrow, and osteophyte growth were created by co-registering the binary images of the histological regions of interest, taking the sum of the stacked image, and then dividing by the number of images in the stack. This allowed us to visualize and qualitatively evaluate histological changes across the entire medial tibial plateau.

The overall pipeline was validated by fully analyzing histological images – not used in pipeline development – from 35 animals that underwent either a skin-incision sham surgery (n=17) or the medial collateral ligament transection plus medical meniscus transection (MMT+MCLT) model (n=18). Three histological sections were analyzed per animal and the average of each quantitative metric was used for statistical analyses. Probabilistic heat maps were created, as described above, to visualize the differences between the skin incision sham and MMT+MCLT models.

RESULTS: Prediction accuracy for our U-Net segmentation model was 92.1% for subchondral bone, 91.5% for cartilage, 87.6% for growth plate, 85.2% for bone marrow and 49.7% for osteophytes. **Fig. 2A-D** illustrates examples morphometric data generated using the independent images from 35 animals to demonstrate full function of the processing pipeline. This morphometric data is plotted as mean ± 95% confidence interval and show 2A) a significant decrease in cartilage area, 2B) location dependent decreases in cartilage thicknesses, 2C) a significant increase in subchondral bone area, and 2D) location dependent increases in subchondral bone thickness. For additional visualization of spatial information, **Fig. 3** compares the presence of **3A)** cartilage and **3B)** subchondral bone in the skin-incision sham (n=17) and MMT+MCLT (n=18) groups using probabilistic heat maps.

DISCUSSION: Histological analysis is an important tool for understanding the progression of OA. In this study, we introduce a pipeline to identify, quantify, and visualize regions of interest in the medial compartment of the tibia in rat OA models. Incorporation of an automated, deep-learning guided segmentation tool allows for quicker analysis of histological images. This system can segment over 100 images and be ready for statistical analyses in less time than it previously took us to manually label regions of interest for a single image. Future work will refine region of interest identification to include other joint areas, including improved osteophyte detection across multiple sections, synovial lining features, and remodeling of the femoral condyle. Inclusion of histological images from other labs will also allow our approach to be applied to multiple species, boost overall prediction accuracy, and include histological features not represented in our training set.

SIGNIFICANCE/CLINICAL RELEVANCE: This pipeline provides a framework for an efficient and unbiased approach to histological grading. Comprehensive analysis and visualizations can be produced quickly, preserve spatial information, and be modified to include additional regions of interest.

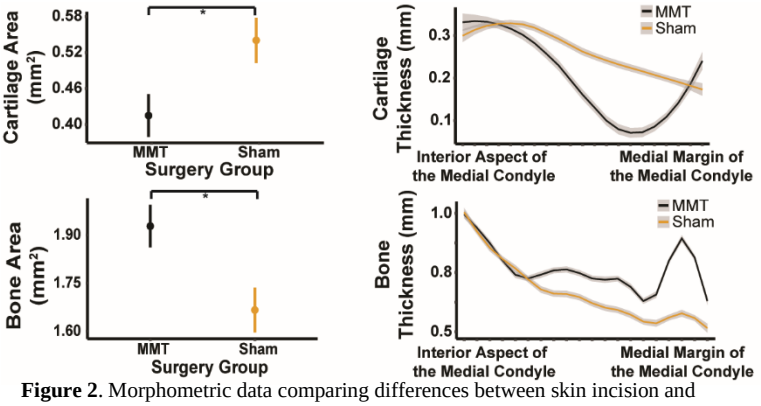

Figure 2. Morphometric data comparing differences between skin incision and MMT+MCLT in **A)** cartilage area, **B)** location dependent cartilage loss, **C)** subchondral bone area, and **D)** location dependent increases in subchondral bone.

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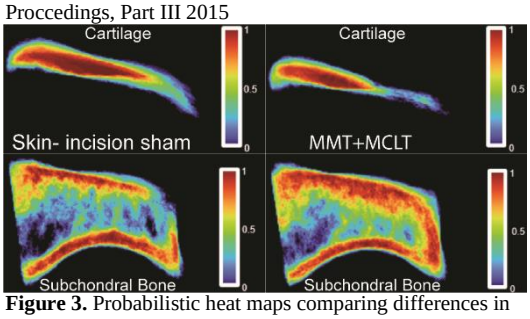

Figure 3. Probabilistic heat maps comparing differences in cartilage and bone between the skin incision sham and MMT+MCLT groups.