

Histologic Effects of the Submental Muscles After Radiation Treatment

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Introduction

Radiation based treatments are known to compromise swallowing function in patients with head and neck squamous cell carcinoma. Radiation doses to the submental muscles (digastric, mylohyoid, and geniohyoid) that form the floor of the mouth can increase the risk for radiation-induced dysphagia, resulting in aberrances in the oral and pharyngeal stages of swallowing. This can lead to altered diet consistencies, reduced nutritional intake, and reduced quality of life. One potential cause is that radiation injuries provoke fibrotic changes in the swallowing muscles effecting their mobility. Our objective was to examine the histological changes in the swallowing muscles post radiation and correlate them with changes in swallowing function in rat model. We hypothesized that collagen content would increase after 64Gy of fractionated radiation to the submental muscles.

Methods

Radiation Procedure: Sprague Dawley adult male rats (N=7) received 64Gy of fractionated radiation to the submental muscles with the mylohyoid at the depth of maximum dose. Clinac iX linear accelerator with 6 MeV electrons was used to administer 8 fractions of 8Gy. The target field was limited to 18x18mm using lead shielding. Superflab material was applied to the dermis to control for the depth of radiation. Control animals (N=7) received anesthesia only treatment.

Collagen Content: Picrosirius red staining was used to detect collagen fibers within the digastric and geniohyoid muscles following treatment. Sectioned frozen tissue was fixed in 4% PFA and incubated in Direct Red 80 dissolved in aqueous saturated picric acid, rinsed in acetic acid solution (0.5%), dehydrated, and mounted. Collagen was stained red and non-collagen components were stained orange or yellow. Images were taken at 20x and analyzed using the ImageJ color threshold option to quantify the area and percent area of red staining.

Swallowing Function: Videofluoroscopy studies were performed to evaluate changes in swallowing function 2-months post-radiation. Rats were given 25% iohexol added to vanilla-flavored sugar water ad libitum. Animals were acclimated to test chamber and test solutions prior to study. A low energy fluoroscope system was used at rate of 60 frames per second. Videos were analyzed frame-by-frame using prototype JawTrack™ software from University of Missouri. The following swallowing parameters were calculated as previously described¹: pharyngeal transit time (PTT), lick rate, lick-swallow ratio, inter-lick-interval, swallow rate, inter-swallow interval.

Viewing Cellular Structure: H&E stains were conducted.

Collagen Percentage and Swallowing Function

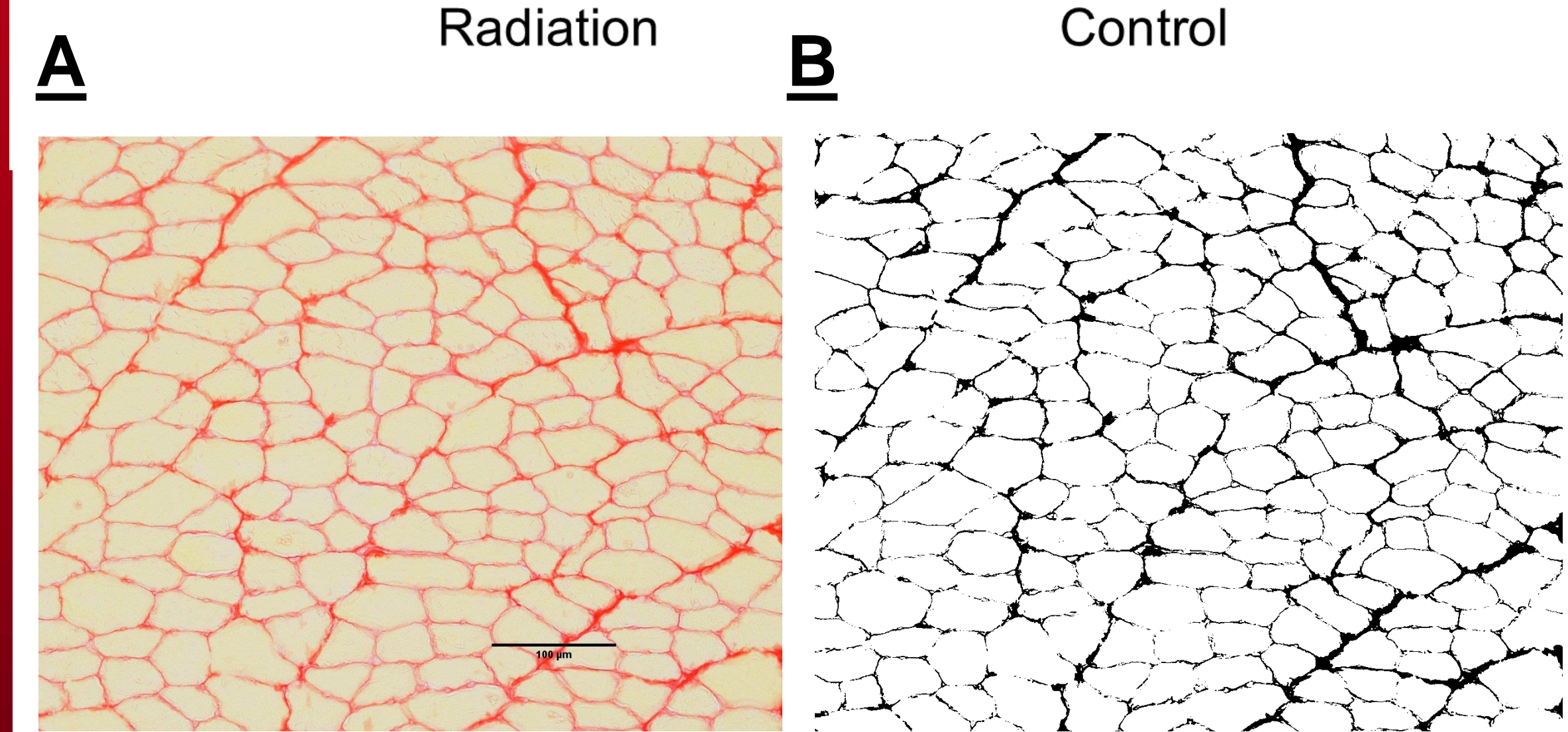
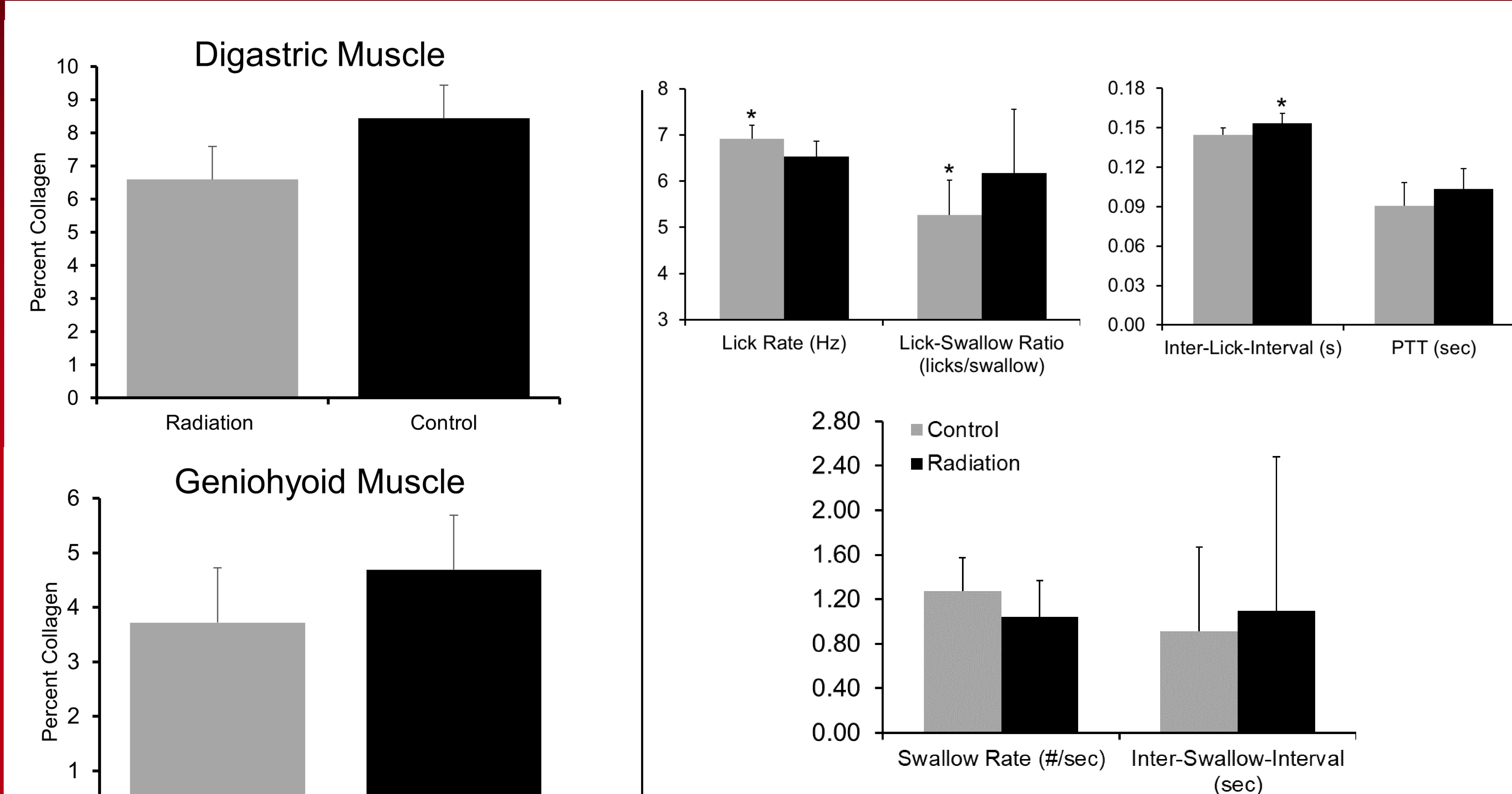


Figure 1. No changes in collagen content 2-months after 64Gy of fractionated radiation. (A) Cross sections of the digastric muscles show collagen in red and myofibers in yellow (Bar 100µm) 2-months after treatment. Histological images were analyzed using ImageJ software using threshold settings. (B) Display of the collagen present and the results were shown as the area and integrated intensity.

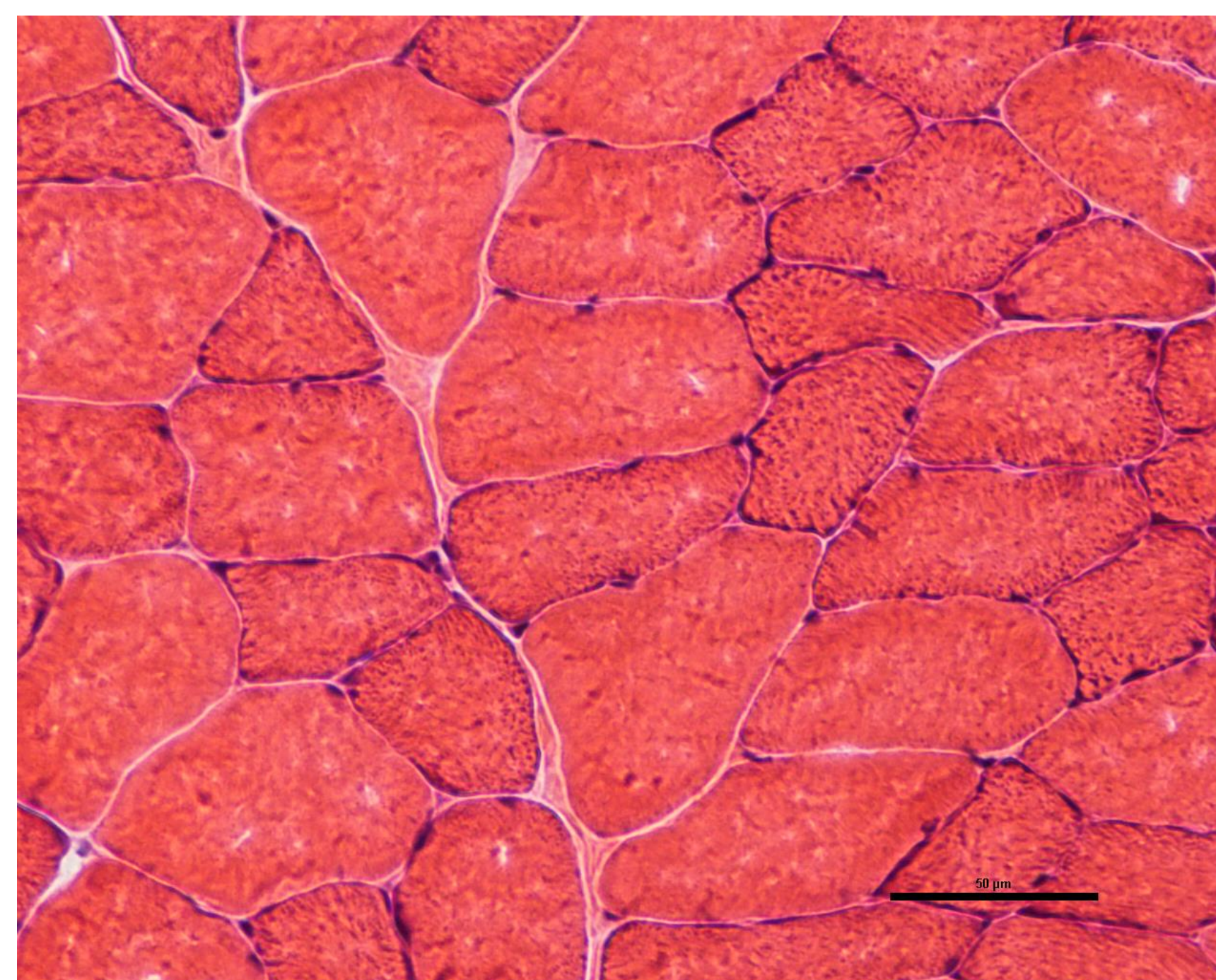


Figure 3. H&E Staining was conducted to ensure tissue sections were taken from the mid-belly area of the digastric and geniohyoid muscles. A cross sections of the digastric muscle shows nuclei stained in blue/purple whereas the cytoplasm and extracellular matrix have varying degrees of pink staining (Bar 50 µm).

Figure 2. Radiation to submental muscles results in changes in oral and pharyngeal swallowing at 2-months post treatment. Radiographic videos of rats during voluntary drinking were analyzed using prototype JawTrack™ software. Statistical significance of $p < 0.05$ is shown as * and error bars indicate standard deviations.

Results

- No measurable changes in collagen content within the digastric and geniohyoid muscles 2-months post radiation
- No other significant changes were found with collagen testing
- Significant changes in swallowing function were observed after treatment including:
 - Decreases in licking rate
 - Increases in lick-swallow ratio
 - Increases in inter-lick intervals

Conclusions

Results showed that there was no correlation between histological changes in swallowing muscles and swallowing functions post radiation. However, results indicate that licking function was affected by radiation to the submental muscles expressed in the decrease in licking rate, an increase in lick-swallow ratio, and an increase in inter-lick intervals. Our results demonstrate that radiation to the submental muscles has a negative impact on rat's swallowing mobility. Results showed no significant changes in collagen content which could be due to analysis taking place during acute stages post-radiation, therefore it is unlikely that changes in muscle structure i.e. fibrosis had developed. Further research is needed to explore possible relationships associated with fibrosis and swallowing functions.

Acknowledgements

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