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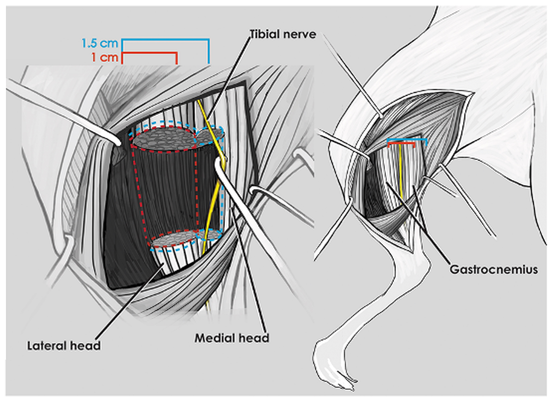
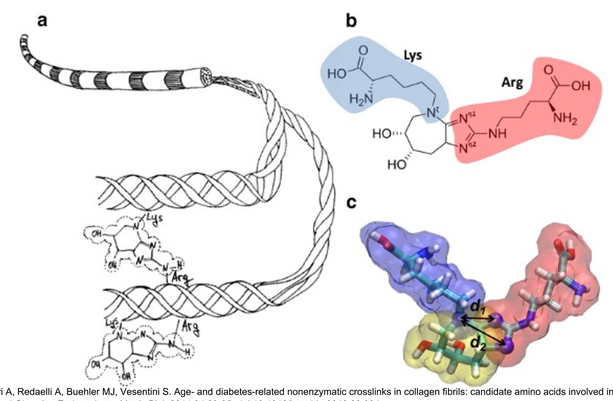
Introduction

Volumetric muscle loss (VML) has been found to overwhelm muscle regeneration, resulting in loss of long-term muscle functionality. Decellularized muscle matrices (DMMs) provide an effective environment for muscle regeneration; however, the age of their source has not been adequately explored for clinical translation. Advanced glycation end-products (AGEs) are chemical cross-links that contribute to the aging process by accumulating on collagen fibers, resulting in a stiffening of the collagenous matrix and an increase in inflammation via the receptor for advanced glycation end-products (RAGE). In previous experiments, we found increased levels of AGE-specific cross-links within DMMs in old mice compared to young as proven by ALT-711 treatment. In this study, we developed a model of aged rat DMMs using AGE cross-links and hypothesized that our AGE-DMM model will contain a higher number of collagen cross-links compared to the control. This AGE-DMM model aims to elucidate the effect of AGEs on muscle regeneration when used *in vitro* or implanted in a volumetric muscle loss model.





Gautieri A, Redaelli A, Buehler MJ, Vesentini S. Age- and diabetes-related nonenzymatic crosslinks in collagen fibrils: candidate amino acids involved in Advanced Glycation End-products. Matrix Biol. 2014;34:89-95. doi:10.1016/j.matbio.2013.09.004

Methods

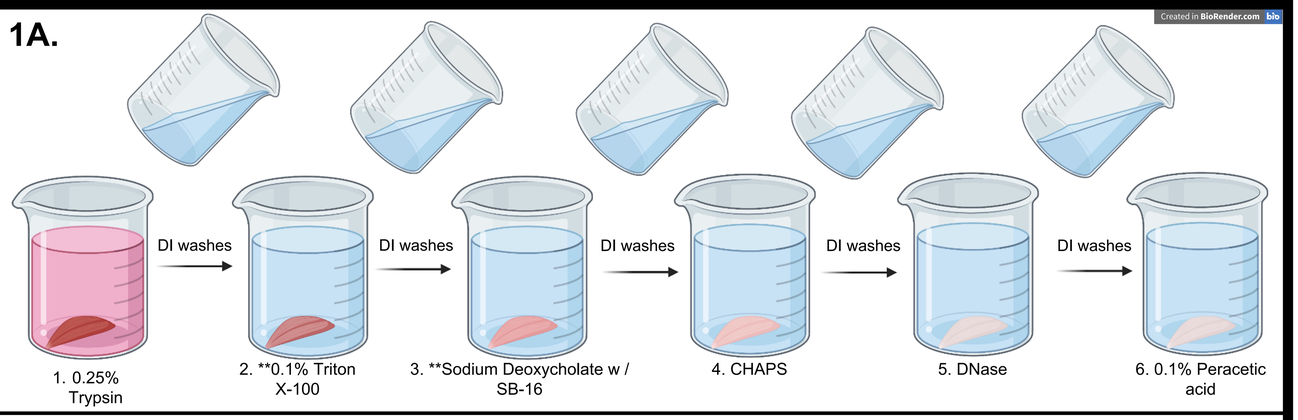
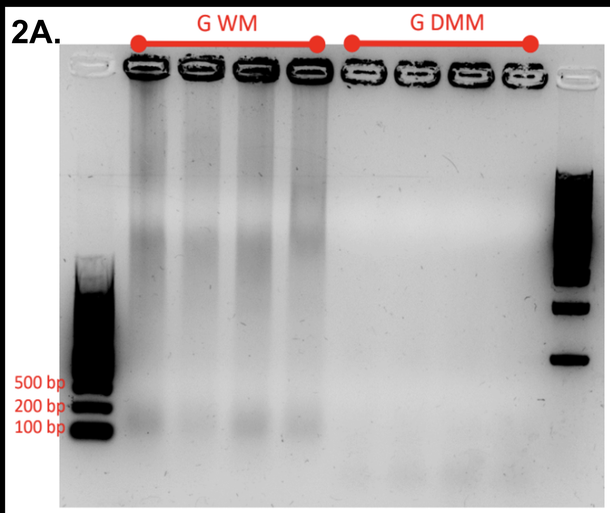


Figure 1: A) Rat latissimus dorsi (LD) muscles were decellularized using 0.25% trypsin and several detergent steps. Muscles were washed in 80 U/mL DNase to eliminate DNA, followed by placement in a final 0.1% peracetic acid wash for sterilization. Each step occurred in a 4℃ fridge for 23 hours, and was followed by three 15-minute washes with DI water before solutions were changed. DMMs were run on a 1% agarose gel to compare DMM DNA fragmentation to a control whole muscle (WM) biopsy and stained with Masson's Trichrome stain and Hoechst DNA stain. This entire process was repeated with rat gastrocnemius (G) muscles. **B)** DMMs were biopsied and placed in 100- and 250-mM D-ribose solution for 5 days at 37°C with 5% CO_2.Three 5-minute washes with DI water on a plate shaker followed ribose placement prior to AGE ELISA analysis. Results were normalized to wet weight, total protein, and hydroxyproline levels.

Results



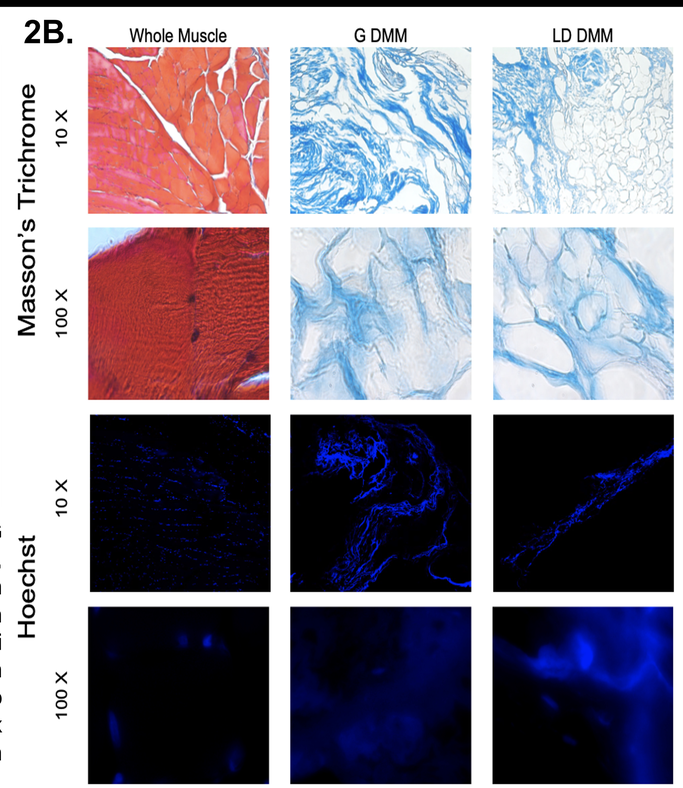


Figure 2: A) Gel electrophoresis ladders of LD and G DMM found fragmentation to be under 100 base pairs, indicating significantly less DNA content in DMMs in comparison to WM control. **B)** Masson's Trichrome stain revealed a collagenous matrix in the DMM, and no nuclei were observed at 100x magnification. Further, though background Hoechst staining was observed compared to no stain controls, no nuclei were observed at 100x magnification as staining intensity was markedly less in DMM lanes compared to WM.

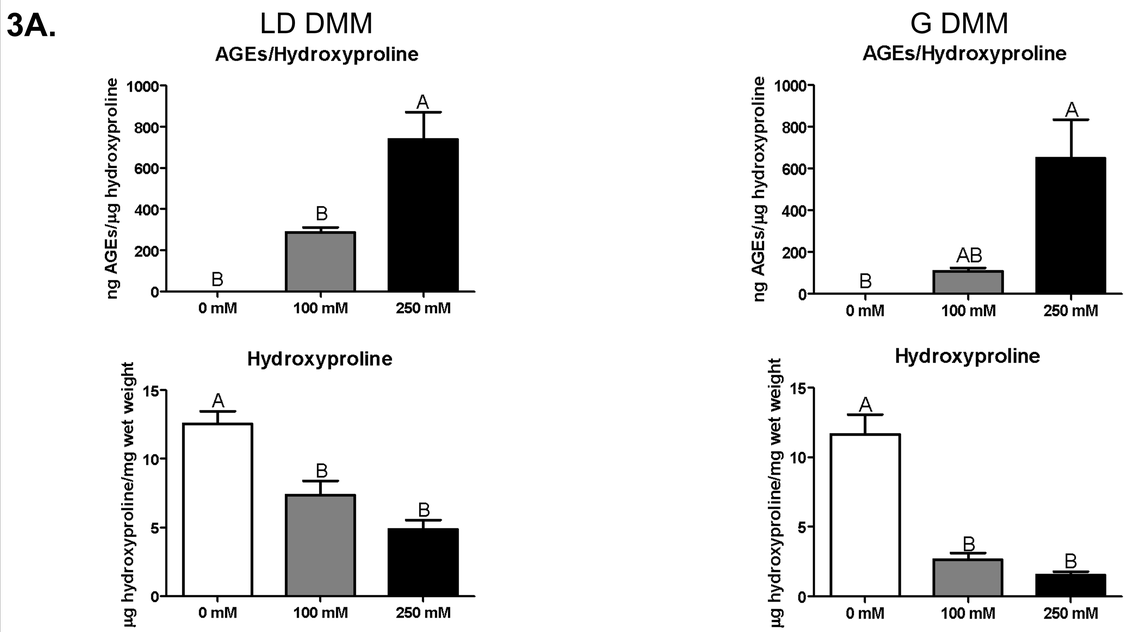


Figure 3: A) Analysis of the AGE ELISA showed increased AGE counts per hydroxyproline on both LD and G DMMs at 100 and 250 mM. Additionally, AGE induction on LD and G DMMs illustrated increased AGE counts via wet weight and total protein levels at 100 and 250 mM. Hydroxyproline, which is known to be inversely related to collagen cross-linking, also decreased at 100 and 250 mM, indicating the presence of collagen cross-links on both the LD and G DMMs.

Acknowledgements

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Conclusions

- We developed a model that aims to resemble aging phenotypes by inducing AGE cross-links on young DMM.
- We observed a higher number of collagen cross-links on young DMM as compared to control DMM.
- Maisson's Trichrome and Hoechst staining confirmed DNA absence through the staining intensity of the DMM relative to the WM control.