

Uncovering the Immunobiology and Biomechanics of Human Fascia Lata as a Biological Graft for Pelvic Organ Prolapse Treatment

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Introduction

- Pelvic Organ Prolapse (POP)** is when the female pelvic organs (bladder, uterus, bowel) descend outside the pelvic cavity.
- It has a **50% prevalence** in parous women, but only **10-20%** seek evaluation for their condition.¹
- It often occurs in post-menopausal women.¹
- POP was previously treated with a polypropylene mesh but was **banned** by the TGA in **2017** due to adverse effects/complications.
- Urogynaecologists currently utilise alternative treatments such as autologous human fascia lata (HFL)

Human Fascia Lata (HFL)

- A dense, fibrous, multi-layered **sheet of connective tissue** that connects the thigh and the hip.²
- It is arranged in an extracellular matrix composed of layers of **fibrillar collagens** and other proteins and is surrounded by loose connective tissue²
- Has a **key role** in **tension regulation** and **joint stabilisation**.²
- Fibrillar collagens and elastin are present in the HFL – these are important for transmitting muscular forces.²
- Post-menopausal women have a decrease in estrogen which could affect HFL biomechanical properties.³



Leg Anatomical Diagram drawn by Lily Anthony

Aim 1: Compare the components of HFL across age groups.

Aim 2: Compare the biomechanical properties of the HFL across age groups.

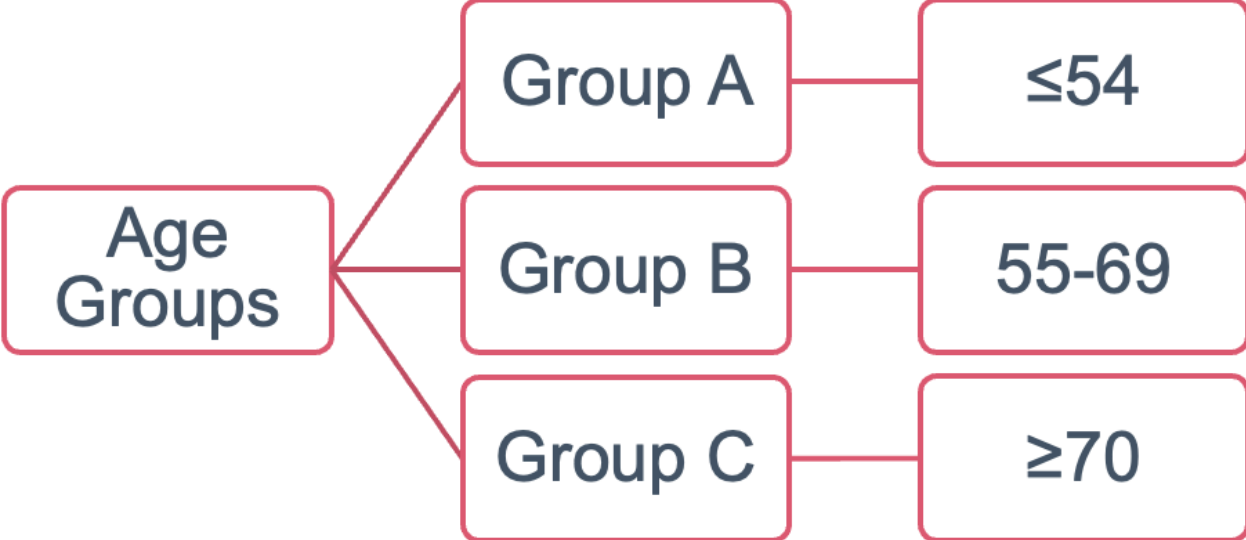
Methods

Tissue Collection

- HFL was harvested using a lateral thigh incision with a 3cm transverse cut, mobilising and dissecting tissue during sacrocolpopexy and a tiny sample is retained post-ethics-approved clinical harvest
- Fascia Lata is stored at -20°C until required
- Is also organised into 3 age groups shown.

Rupture Testing

- Samples were tested using the CellScale UniVert Tensiometer set to rupture settings.
- Data was recorded and transferred to an Excel file, where it was organised into 3 age groups, and its properties were calculated.
- Data was imported into GraphPad Prism 10 and means were compared using a Kruskal-Wallis test with a Dunn's multiple comparison test.



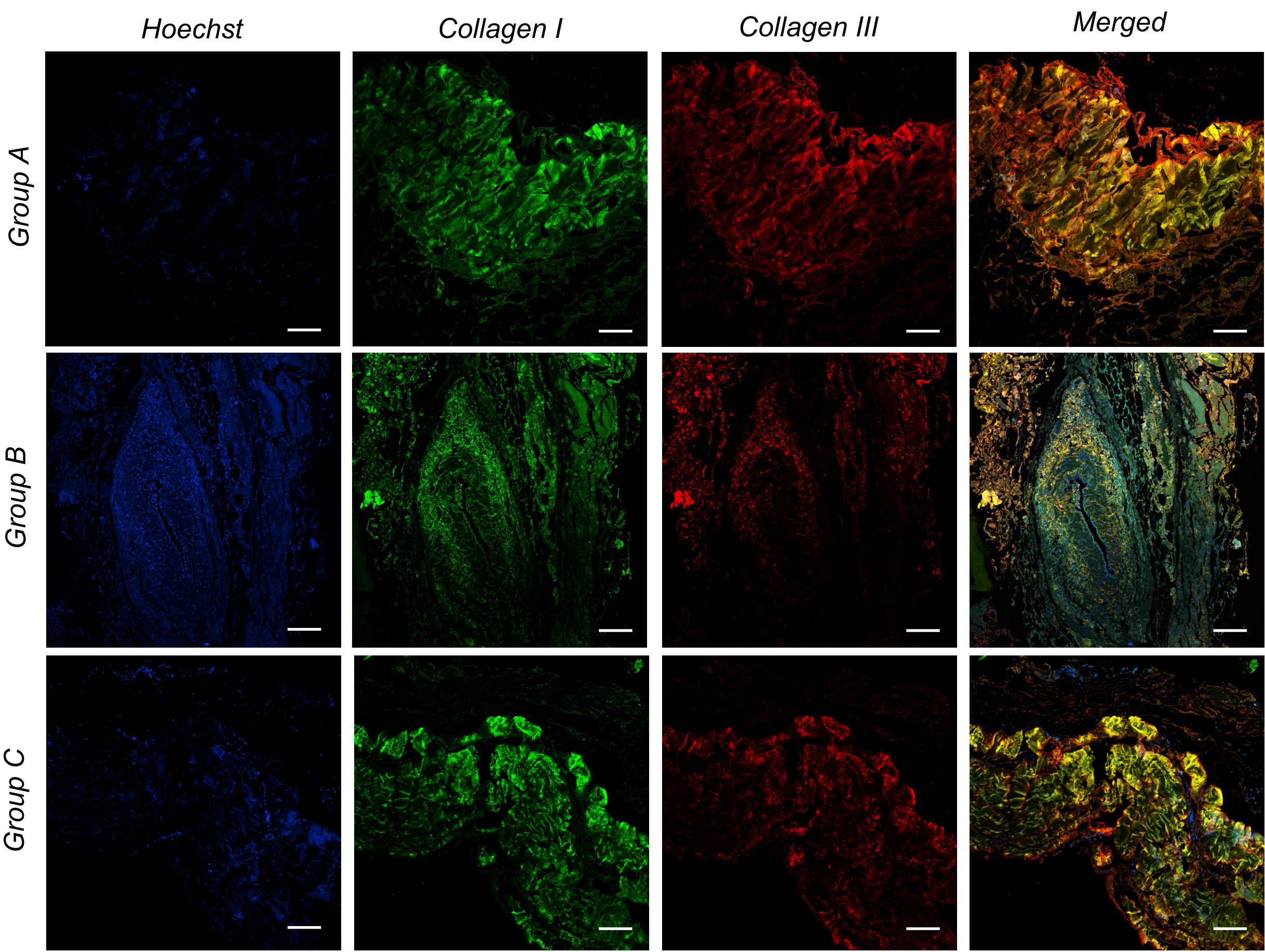
Immunofluorescence

- HFL was processed in FFPE and into slides.
- Slides were stained with their respective antibodies and counterstained with Hoechst 33342
- Stains were checked using fluorescent microscopy, then imaged with confocal microscopy and slides were scanned.
- Scanned slides were then quantified using QuPath 0.6.0, and means were compared on GraphPad Prism 10 using a Kruskal-Wallis test with a Dunn's multiple comparison test.

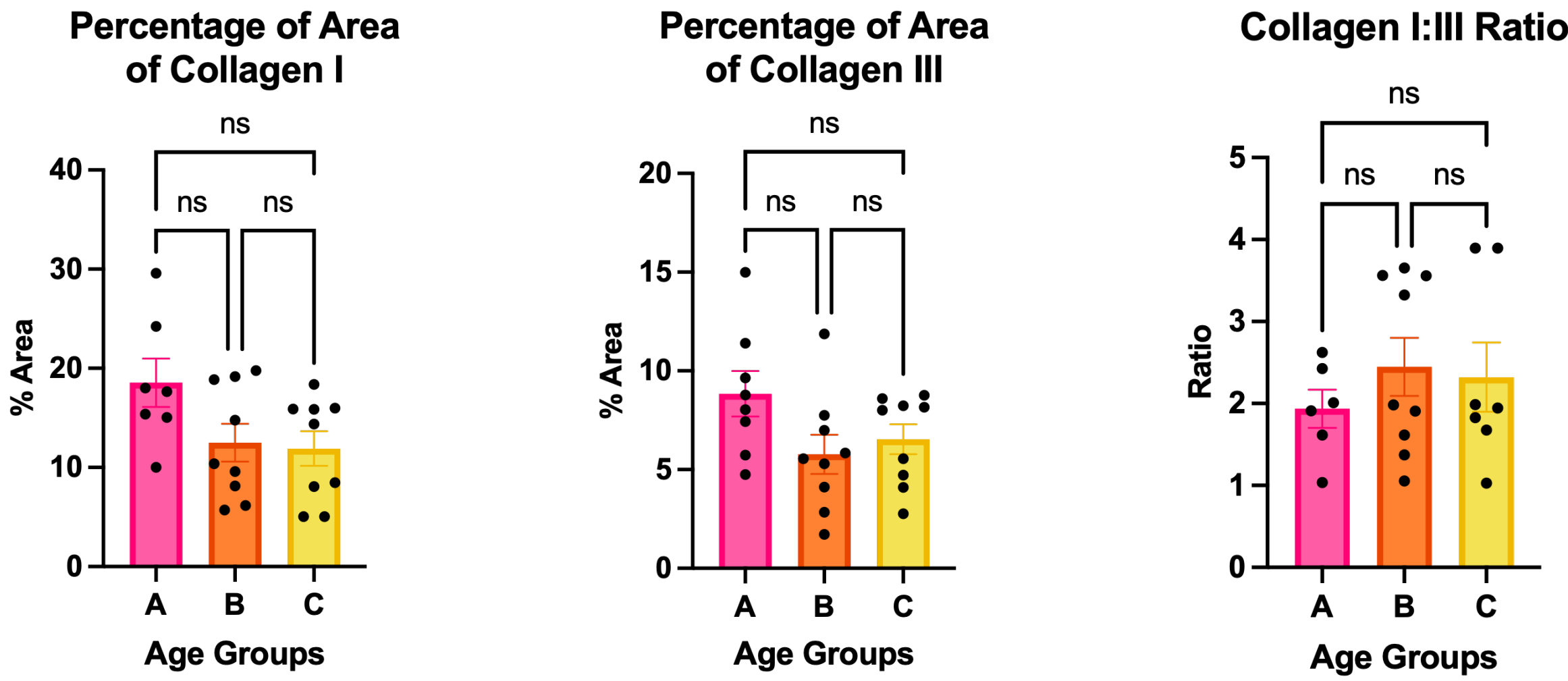
Results

Aim 1

HFL Collagen I & III Immunofluorescence



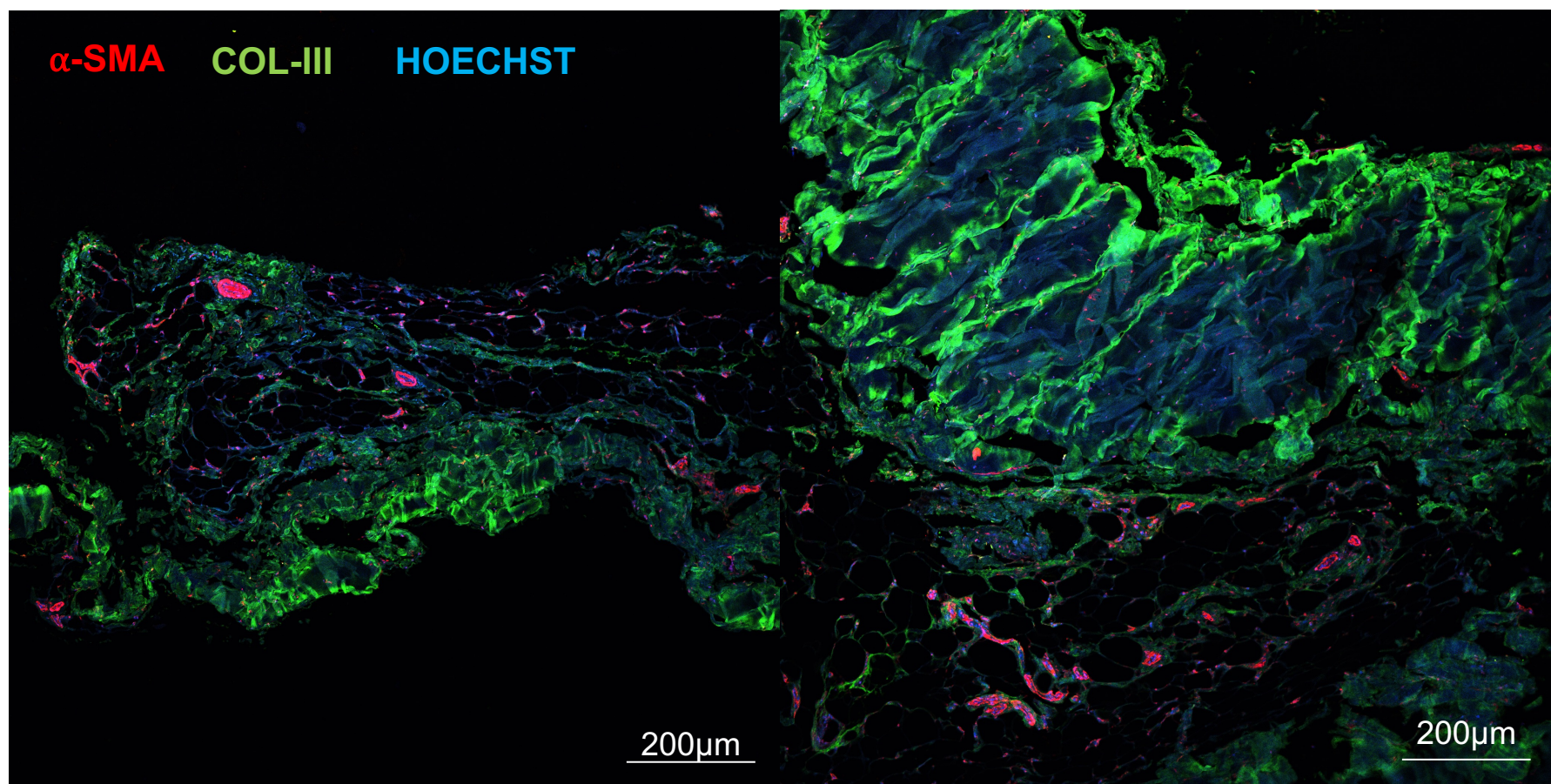
Images were taken on a confocal microscope. Magnification of images is 10x, and scale bars represent 200µm. HFL was stained with collagen I with Alexa Fluor 488 (green), collagen III with Alexa Fluor 568 (red) and Hoechst (blue). Scale bars are 200µm



HFL Collagen Quantification between 3 age groups. Comparison of the means between Group A (<54, n=7) and Group B (55-69, n=9) and Group C (>70, n=19). Graphs depict mean ± SEM. Ns = no significant difference

Aim 1

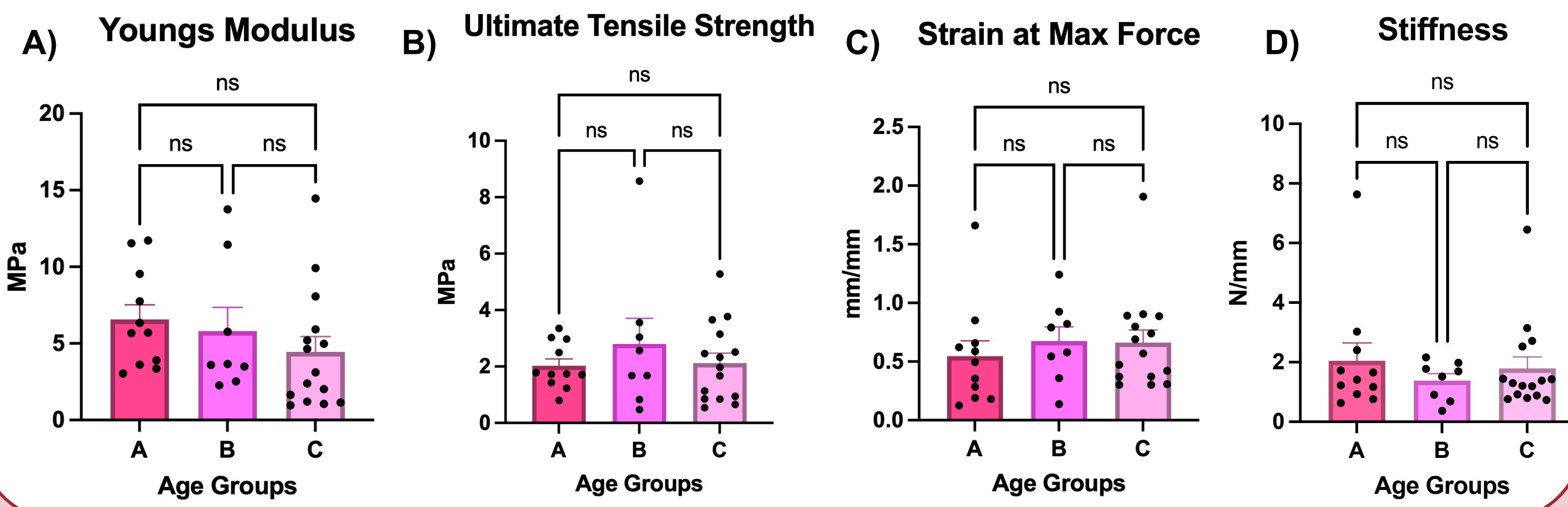
Expression of α-SMA in HFL



Images were taken on a confocal microscope. Magnification of images is 10x, and scale bars represent 200µm. HFL was stained with collagen I with Alexa Fluor 488 (green), α-SMA with Alexa Fluor 568 (red) and Hoechst (blue). Scale bars are 200µm

Aim 2

HFL Rupture Test Results Across the 3 Age Groups



HFL Rupture Test Results between 3 age groups. Comparison of the means between Group A (<54, n=11) and Group B (55-69, n=8) and Group C (>70, n=15). Graphs depict mean ± SEM. Ns = no significant difference

Overall Findings

Differences between age groups were **not statistically significant** in all results

Aim 1

- Collagen I and III **decreased** with age
- Slight increase** in collagen I:III ratio with age – more collagen I than collagen III
- α-SMA** expression was identified in HFL – indicates presence of myofibroblasts

Aim 2

- Strain at Max Force **slightly increased** with age, but no significant difference
- Young's modulus **decreased** with age, but no significant difference

Implication

- Indicates HFL retains its structural and functional integrity even in elderly patients
- These findings are insightful for clinicians to form an evidence-based approach to pelvic floor reconstructive surgery.

References

- Barber, M.D., Maher, C. (2013) Epidemiology and outcome assessment of pelvic organ prolapse. *Int Urogynecol J*
- FEDE, C. et al. (2021) A Closer Look at the Cellular and Molecular Components of the Deep/Muscular Fasciae. *Int J Mol Sci*, 22.
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