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FASCIA SCIENCE AND CLINICAL APPLICATIONS: Original Research

Evaluation of hyaluronan content in areas of densification compared to adjacent areas of fascia

Emmett J. Hughes, Kena McDermott, Matthew F. Funk*

University of Bridgeport College of Chiropractic, USA

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1. Introduction

Epimysial fascia is a type of deep fascia that ensheaths muscles and helps to define their shape and structure. It is continuous with the tendon, allowing it to transmit forces (Stecco et al., 2013; Stecco, 2015). There are three layers: internal, middle and external, each with distinct arrangements of collagen fibers. Each layer of the fascia is comprised of types I and III collagen and elastic fibers. Between each layer is areolar connective tissue rich in hyaluronan (HA) (Bhattacharya et al., 2010). HA is a polysaccharide in the extracellular matrix that provides both lubrication and resistance to compression. Under normal physiological conditions, HA is responsible for normal gliding motion between components of fascia, muscle, nerves, lymphatics and blood vessels (Stecco et al., 2008; Alberts et al., 2002; Dowthwaite et al., 1998).

Centers of coordination (CC), according to Stecco (2004), are specific points where the mechanical forces of muscle contraction converge in epimysial fascia to coordinate the movement of a joint in a specific plane of motion. It is hypothesized that changes in concentration and molecular structure of HA in CC result in a restriction of gliding termed “densifications” (Stecco et al., 2008; Pavan et al., 2014). When subjected to increased stresses involving mechanical, pH or temperature changes, HA polymerizes to form long chain molecules and becomes more viscous (Mattenini et al., 2009). Polymerized HA is thought to provide increased structural protection to myofibrils undergoing stress by creating a densification at CC. While this entanglement of HA protects the myofibrils, it restricts the glide and normal motion, creating dysfunction. There are both myelinated and nonmyelinated nerve fibers distributed throughout the deep fascia (Bhattacharya et al., 2010) which may become sensitized during dysfunctional motion of fascia and contribute to a variety of myofascial pain syndromes (Stecco et al., 2001; Schleip et al., 2010; Kwong and Findley, 2014; Gibson et al., 2009).

Previous experiments using unembalmed cadavers have demonstrated the presence of HA between the layers of the deep fascia as well as in the epimysium, perimysium and endomysium associated with muscles (Stecco et al., 2008). These studies demonstrated HA content but did not examine the differences in concentration between different areas within the fascia.

To our knowledge, no study has yet compared HA content at densified CC to adjacent non-densified areas. The aim of this study was to determine whether there was a visible difference between CC and non-CC sites using histological staining techniques. The hypothesis being tested is that densification at a CC can be identified in an embalmed cadaver through palpation and tissues sampled from a CC will demonstrate greater concentrations of HA than in a non-densified adjacent site.

2. Methods

A CC known as La-Cx, as defined by Stecco in Fascial Manipulation® (Stecco, 2004) was identified anatomically on an embalmed human cadaveric lower extremity in the muscle belly of the tensor fasciae latae. La-Cx was located by an investigator (EJH) trained in Fascial Manipulation® by palpating the left lower extremity of an embalmed cadaver. A densification located at La-Cx was identified by palpation by two investigators (EJH, KM) with Master Class certification in Fascial Manipulation® before dissection. The skin surface was marked above the palpable density and a broad section of muscle and fascia was dissected from the cadaver. The section was laid out on a flat surface and palpated again to confirm that the density was within the dissected tissue. The identified density was

* Corresponding author. 858 Freeman Avenue, Stratford, CT, 06614, USA.
E-mail address: mffunkdc@sbcglobal.net (M.F. Funk).

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then sectioned out of the larger piece of dissected muscle and submitted in two cassettes labeled CC3 and CC4. An area within the same muscle and fascia 2 cm away from the palpated density was palpated and determined to be non-densified. A sample from this non-densified area was submitted in a block labeled NON-CC2.

Tissues were submitted for routine formalin-fixed paraffin-embedded processing at Marshfield Laboratories (1000 N. Oak Avenue, Marshfield, WI) on blocks labeled CC3, CC4 and NON-CC2, to examine histological properties of the densification from the region known as La-Cx (CC3, CC4) and non-densified tissue and fascia (NON-CC2) within the same region. All tissues were sectioned and stained with Hematoxylin and Eosin. Special stains including Colloidal Iron (Coll Fe), treated and untreated with hyaluronidase, and Alcian Blue (pH 2.5) were performed on serial sections. Alcian Blue, at a pH of 2.5, stains acid mucopolysaccharides blue shades at varying intensity and nuclei stain pink-red by the counterstain nuclear fast red (Sheehan and Hrapchak, 1980). Coll Fe stains acid mucopolysaccharides and sialomucins deep blue and the nuclei and cytoplasm are counterstained pink-red (Carson, 1990). Two additional slides from each block (CC3 and CC4) were stained with the Coll Fe procedure; one slide from each block was first treated with hyaluronidase to digest out hyaluronan (Sheehan and Hrapchak, 1980; Bancroft and Stevens, 1996) and the second slide was left untreated before batch staining with Coll Fe. Marshfield Laboratories was not informed of the hypotheses, what the samples were, or how they were obtained.

The slides were examined using an OMAX 40X-2000X Lab Trinocular Compound LED microscope with 3 MP digital camera and the images were captured using AM Scope 3.7 for digital camera software. All slides were viewed and photographs that were clear of common histological artifacts were chosen.

3. Results

Compared to adjacent tissue, an increased presence of HA was visually confirmed at the CC through Coll Fe staining. Dense blue staining demonstrated a gradual decrease in concentration of positive staining further away from the densification site

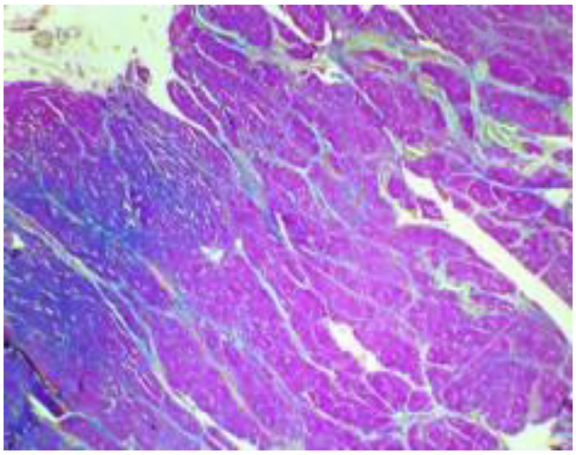

Fig. 1. (CC3 Coll Fe Untreated 4x)
Positive histochemical staining of sample labeled CC3 using Coll Fe stain, untreated with hyaluronidase. Dense blue staining is observed with a gradual decrease in concentration of positive staining further away from the densification. The peripheral area surrounding the densification shows a substantial reduction in concentration of positive staining material. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

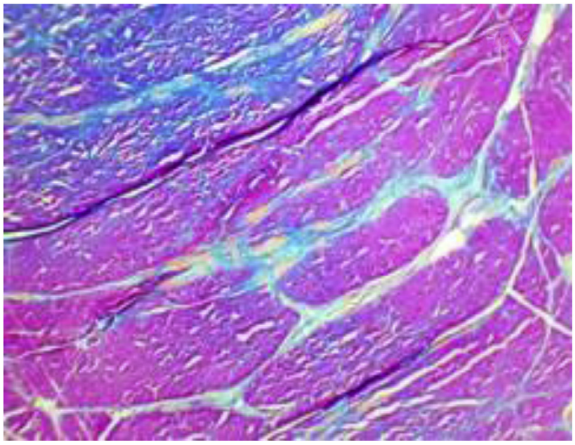

Fig. 2. (CC3 ParaCC Coll Fe Untreated 10x) Image at 10x demonstrating the area immediately adjacent to the densest area of positive staining. Peripheral tissues immediately adjacent to the area of dense positive staining show considerable positive staining surrounding each muscle fiber.

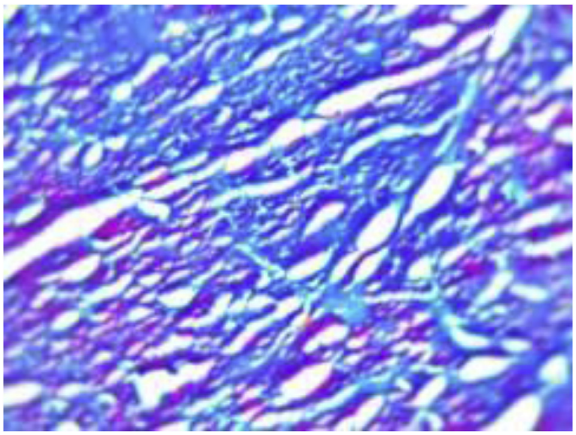

Fig. 3. (CC3 Density Coll Fe Untreated 40x) Close up view of the area of highest density of positive staining.

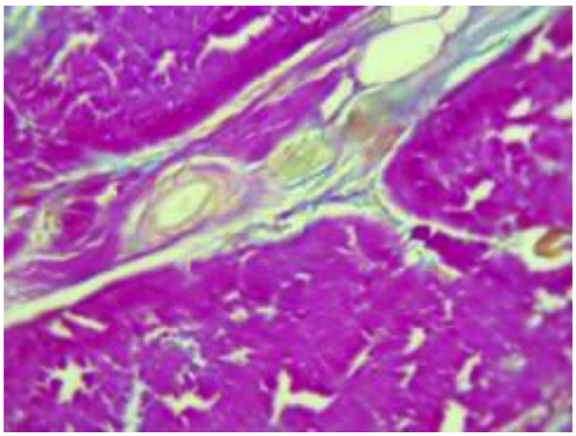

Fig. 4. (CC3 Coll Fe Untreated 40x)
Image showing minimal positive staining in the peripheral tissues furthest from the area of densification. The positive staining is seen mainly surrounding vascular structures.

(Figs. 1–4).

The peripheral area adjacent to the densification showed a substantial reduction in concentration of HA. Peripheral tissues immediately adjacent to the area of dense positive staining demonstrated considerable positive staining surrounding each myofiber (Fig. 2). Minimal positive staining in the peripheral tissues furthest from the area of densification demonstrated staining mainly surrounding vascular structures (Fig. 4).

Dense blue staining was absent in tissues post-treatment with hyaluronidase. This confirmed that the areas of positive staining material were in fact HA (Figs. 1, 2, 5–9). Sections stained with Alcian Blue also confirmed the presence of HA (Fig. 10).

Sections from non-densified tissue demonstrated minimal HA content when compared to the palpated densifications in both CC3 and CC4 (Figs. 11–15).

4. Discussion

This study successfully palpated and sampled an identified CC in an embalmed cadaver. This study confirmed that there is an increased concentration of HA within a densification using Coll Fe, thereby demonstrating the potential value of using this stain in further histological studies evaluating HA content in fascia. Alcian Blue was also used to demonstrate the presence of HA because it has been used in other studies (Stecco et al., 2001). With both Coll Fe and Alcian Blue, the intensity of the final blue color varies with the amount and degree of polymerization of acid mucopolysaccharides. However, Coll Fe demonstrates HA more vividly and with greater contrast, thus making the relative concentrations of HA more apparent. This is due to counter-staining and the intensity of the Prussian blue reaction. Coll Fe was also chosen because it provides more consistent staining with less variability than pH dependent Alcian Blue (Sheehan and Hrapchak, 1980).

The absence of positive staining material in the treated sections in comparison to the untreated sections confirmed that the positive

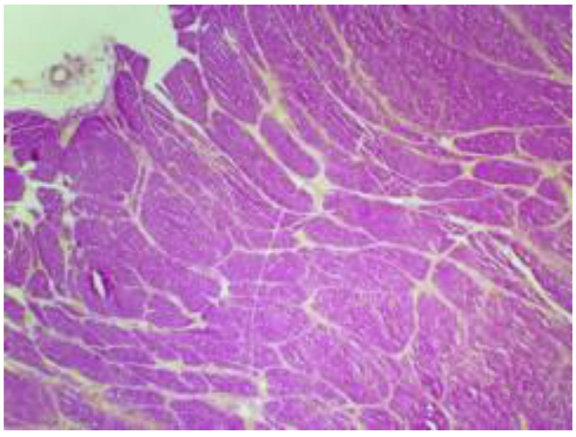

Fig. 5. (CC3 Coll Fe Treated 4x)
Fig. 1 demonstrates positive histochemical staining of sample labeled CC3 using Coll Fe stain, untreated with hyaluronidase. Dense blue staining is observed with a gradual decrease in concentration of positive staining further away from the densification. The peripheral area surrounding the densification shows a substantial reduction in concentration of positive staining material. Fig. 5 is a section from the same sample labeled CC3 that has been treated with hyaluronidase before staining using the same Coll Fe stain as in Fig. 1. The complete absence of blue staining confirms that the positively stained substance in Fig. 1 has been digested by hyaluronidase. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

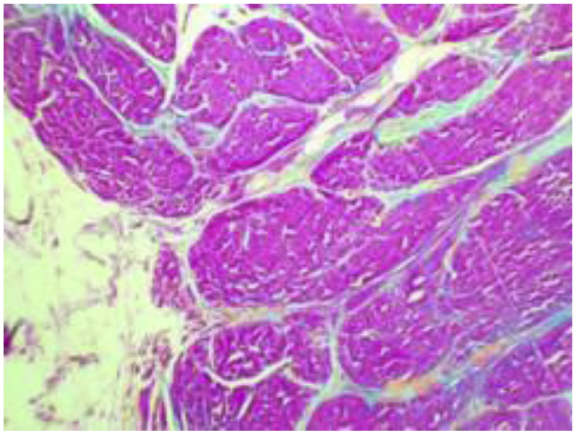

Fig. 6. Demonstrates positive staining of HA in areas throughout the specimen labeled CC3.

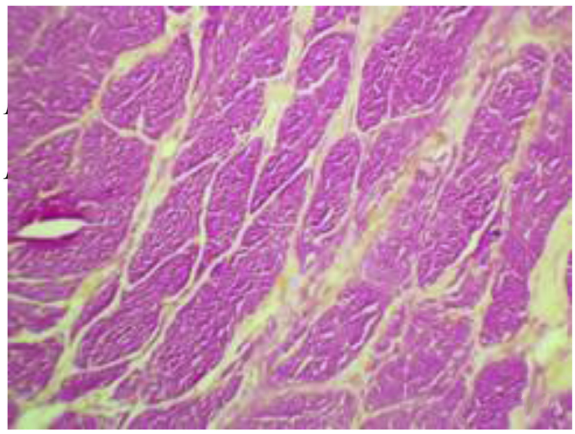

Fig. 7. (CC3 Coll Fe Treated 10x) The section treated with hyaluronidase prior to Coll Fe staining again confirms that HA is the material staining blue in these specimens. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

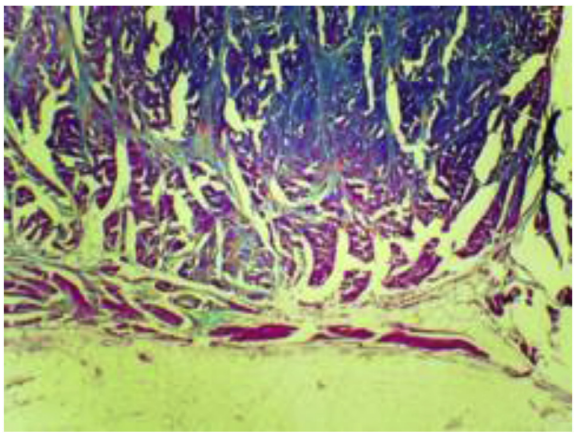

Fig. 8. (CC4 Coll Fe Untreated 4x)
The specimen labeled CC4 also demonstrates a high concentration of HA in an untreated section.

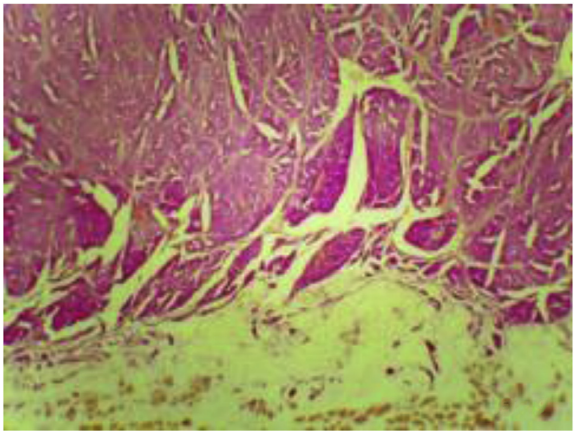

Fig. 9. (CC4 Coll Fe Treated 4x)
A section from the same sample labeled CC4 demonstrates an absence of HA, post-digestion with hyaluronidase, consistent with the sample from CC3.

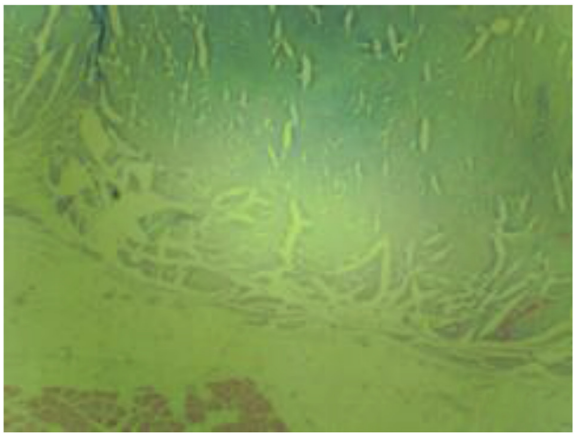

Fig. 10. (CC4 Alcian Blue 4x)
Alcian Blue, pH 2.5 staining of section taken from the same sample labeled CC4 demonstrating dense areas of HA staining. This stain was performed both to demonstrate HA via another method and also because it has been used routinely in other studies demonstrating HA. The Coll Fe stain was chosen because it provides a less temperamental option and demonstrates the HA more vividly and with greater contrast making relevant concentrations of HA more visible. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

stained material was in fact HA. The palpable densification appeared to have increased HA in comparison to non-densified fascia. This study demonstrated the relatively high HA content in one densification of the fascia from the site La-Cx in a single cadaver. The focus of future studies utilizing multiple cadavers and examining multiple densifications involving a variety of CC sites may confirm that these results are consistent with densifications in other sites.

4.1. Limitations

Only two researchers verified the palpable area of densification prior to sampling. The tissues sampled came from only one cadaver and were obtained at only one CC and 2 cm away at the non-CC site. The researchers were aware that slides were either from the CC or the adjacent area and treated or not treated with hyaluronidase.

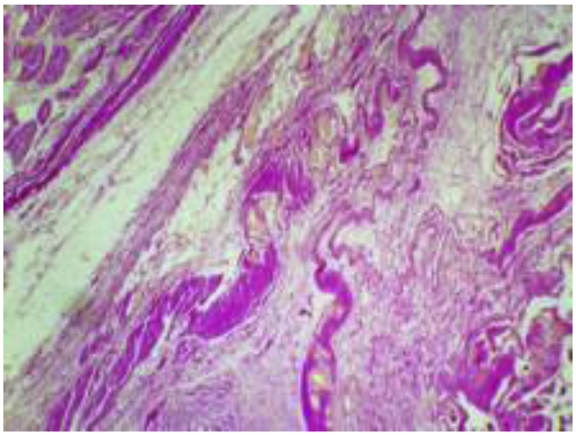

Fig. 11. (Non-CC Coll Fe treated 4x).

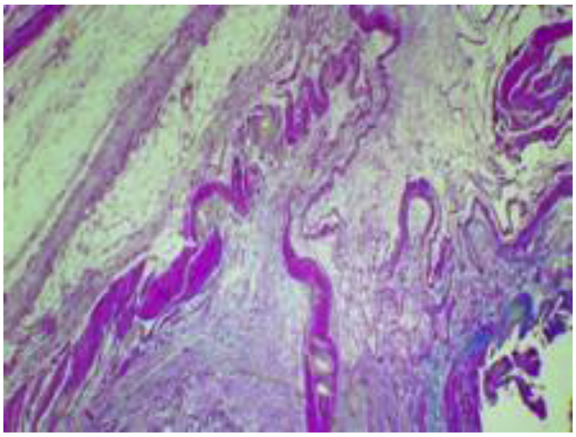

Fig. 12. (Non-CC Coll Fe Untreated 4x)
An area of non-densified tissue was identified via palpation 2 cm away from the palpable densifications in areas labeled CC3 and CC4. Samples from this area were labeled Non-CC and submitted for staining along with CC3 and CC4. Figs. 11 and 12 are from the same tissue block and were stained with and without digestion.

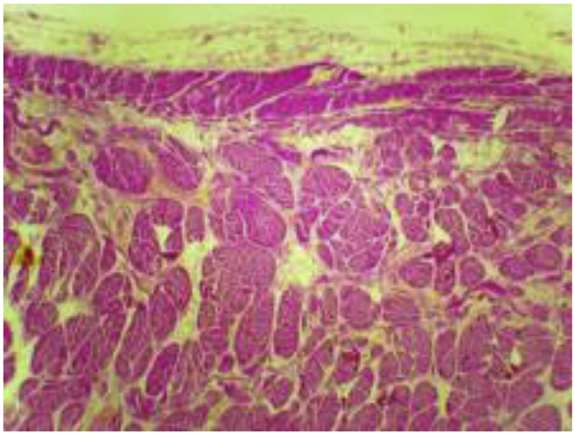

Fig. 13. (Non-CC Coll Fe treated 4x).

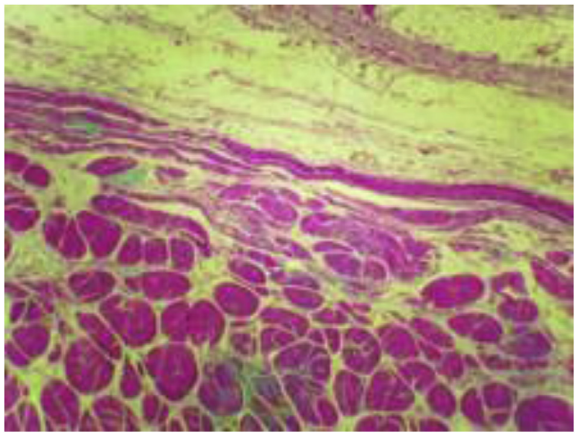

Fig. 14. (Non-CC Coll Fe Untreated 4x)
Figs. 13 and 14 are from the same block. Both samples demonstrate minimal HA content in comparison to the palpated densifications in the CCs.

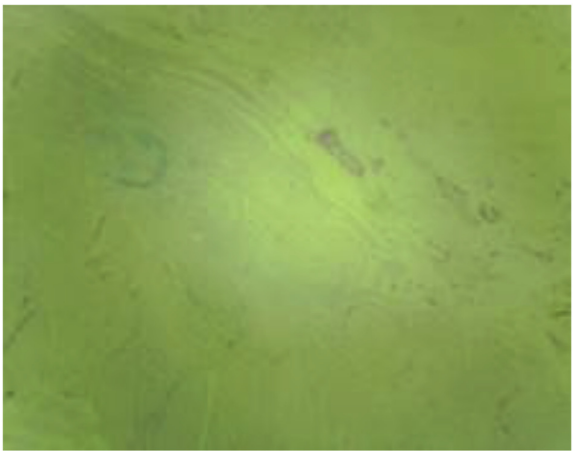

Fig. 15. (Non-CC Alcian Blue 4x)
Stained section from the sample identified as non-densified upon palpation confirms reduced positive staining and minimal HA content in comparison to the sample from a known CC and identified as densified upon palpation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

5. Conclusion

This study is the first known to palpate and sample densified tissue at a CC and an adjacent site from an embalmed cadaver. The hypothesis that a densified CC will contain a greater concentration of polymerized HA than the adjacent non-densified fascia is also supported by this research. HA can be vividly demonstrated in a fascia histology study with Coll Fe.

Disclosures

Declarations of interest

None.

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