

## Analysis of the presence of the hyaluronic acid inside the deep fasciae and in the muscles

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Recent works have demonstrated that the deep fascia is a multilayer structure, formed by different layers of collagen fibers and loose connective tissue (LCT). The aim of this work was to study the layers of LCT inside the deep fasciae, and in particular to evaluate the presence of Hyaluronic acid (HA).

Three fresh not embalmed cadavers were studied. Samples of the deep fascia together with the underlying muscles were taken from the neck over the SCOM, from the abdomen over the rectus muscle and from the thigh over the sartorius muscle. Samples were stained with hematoxylin-eosin, azan-Mallory, Alcian blue and a biotinylated HA-binding protein that has high specificity for HA.

At the microscopic evaluation, the deep fascia was formed of two or three layers of parallel collagen fibre bundles, densely packaged. Each collagen layer presented a mean thickness ( $\pm$  SD) of  $277.6 \pm 86.1 \mu\text{m}$ . Between the different layers, a thin layer of loose connective tissue could be recognized, having mean thickness  $43 \pm 12 \mu\text{m}$ . Staining with the Alcian Blue and with the highly specific HA-binding peptide documented a layer of hyaluronan between fascia and muscle and inside deep fascia, in particular inside the loose connective tissue separating the fibrous sub-layer of the fascia. In some samples, some fibroblast-like cells that stained very well at the Alcian Blue stain were observed. It was postulated that these were specialized cells for the biosynthesis of the HA-rich matrix, that we'll call "fasciocyte". This means that the fascia thus provides an extracellular matrix that is a gliding lubricant over muscle, permitting the free contraction of muscles, but also a unique matrix for its repair and regeneration. Besides, the HA inside the deep fascia facilitates the free sliding of two adjacent fibrous fascial layers, guaranteed the normal functionality of the deep fascia. If the HA assumes a more packed conformation, or more generally if the loose connective tissue inside the fascia alters its density, the behavior of the whole deep fascia and of the underlying muscle could be compromised. This could be at the origin of many myofascial pains.

Keywords: fascia, hyaluronic acid, muscle, loose connective tissue, collagen