­­Table 0: Relative Protein staining intensities

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Species | Subdivision\* | | Total Collagen6 | Collagen Type I6 | Collagen Type II6 | Collagen Type III6 | Procollagen I6 | MMP-16 | Elastin3 | | Fibrillin-13 | Tropoelastin3 | Hyaluronan3 |
| Human | 1 | Basement Membrane Zone | ++++ | +++++ | - | +++++ | +++++ | +++++ | ++++ | ++++ | | +++++ | + |
| 2 | Superficial Third | ++ | ++ | - | +++ | +++ | ++ | + | +++ | | ++ | + |
| 3 | Intermediate Third | + | + | - | ++ | ++ | ++++ | +++++ | + | | +++++ | ++++ |
| 4 | Deep Third | +++++ | ++++ | - | ++++ | ++++ | ++ | ++++ | +++ | | ++++ | ++ |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Species | Subdivision\* | | Versican8 | Decorin8 | Heparan Sulfate8 | Chondroitin 4-Sulfate8 | Biglycan8 | Agrecan8 |
| Human | 1 | Basement Membrane Zone | +++ | +++++ | ++++ | ++++ | +, +++ | - |
| 2 | Superficial Third | + | +++ | + | ++ | +, +++ | - |
| 3 | Intermediate Third | +++ | + | + | ++ | + | - |
| 4 | Deep Third | +++ | +++ | ++ | +++ | ++++ | - |

Numeric relative staining assignments translated into +’s and –‘s with 0 = - , and 5 = +++++

N/A – not assessed

\*MATLAB defined regions do not mimic SLP, ILP, DLP exactly but sections the tissue into approximate thirds

[3] Hahn, Mariah S., James B. Kobler, Barry C. Starcher, Steven M. Zeitels, and Robert Langer. "Quantitative and comparative studies of the vocal fold extracellular matrix I: elastic fibers and hyaluronic acid." *Annals of Otology, Rhinology & Laryngology* 115, no. 2 (2006): 156-164.

[6] Hahn, Mariah S., James B. Kobler, Steven M. Zeitels, and Robert Langer. "Quantitative and comparative studies of the vocal fold extracellular matrix II: collagen." *Annals of Otology, Rhinology & Laryngology* 115, no. 3 (2006): 225-232.

[8] Hahn, Mariah S., James B. Kobler, Steven M. Zeitels, and Robert Langer. "Midmembranous vocal fold lamina propria proteoglycans across selected species." *Annals of Otology, Rhinology & Laryngology* 114, no. 6 (2005): 451-462.

Table 1: Collagen Concentration by VF layer

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| VF Layers\*\* | | Fractional Collagen | Quantitative Collagen | Collagen Fractional Area | Collagen Type2 | Organization2 | Notes |
|  | | % Collagen of human LP TP^ | mg collagen per mg of TP^ | Mean ± SEM |  |  |  |
| LP | Superficial Third | 43.4 ± 2.6 %6 | 0.276 | 0.307 ± 0.009 6 | I, III | Strong birefringent | * Narrow band below basement membrane of epithelium * Reticular fibers |
| Intermediate Third | 0.326 | 0.360 ± 0.005 6 | I, III | Weakly birefringent | * Less densely organized * Reticular fibers, decreasing towards superior portions |
| Deep Third | 0.716 | 0.333 ± 0.005 6 | I, III | Strongly birefringent | * Densely organized * Penetrate superficial muscle bundles of vocal muscle |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| VF Layers\*\*\*\* | | Fractional Collagen | |  |  |  |
|  |  | 9, \*\*\* | % area\*\*\*\*\* |  |  |  |
| Superficial LP  Deep LP | 0-20% | 3,565 | 17.8 |  |  |  |
| 20-40% | 3,165 | 15.8 |  |  |  |
| 40-60% | 3,582 | 17.9 |  |  |  |
| 60-80% | 4,892 | 24.4 |  |  |  |
| 80-100% | 6,687 | 33.4 |  |  |  |

\*\* Superficial, intermediate, Deep LP regions do not mimic SLP, ILP and DLP exactly but rather section the LP tissue into approximate thirds

\*\*\* arbitrary collagen concentration units, range from 0-20,000 (0 = field measured contained no collagen, 20,000 = completely filled with collagen).

\*\*\*\* LP divided into 5 sections, each approximately 20% of LP)

\*\*\*\*\* assume arbitrary collagen concentration is linear to % area occupied

^ TP = total protein

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[2] Prades, Jean-Michel, Jean Marc Dumollard, Sébastien Duband, Andrei Timoshenko, Céline Richard, Marie Dominique Dubois, Christian Martin, and Michel Peoc’h. "Lamina propria of the human vocal fold: histomorphometric study of collagen fibers." *Surgical and radiologic anatomy* 32, no. 4 (2010): 377-382.

[3] Hahn, Mariah S., James B. Kobler, Barry C. Starcher, Steven M. Zeitels, and Robert Langer. "Quantitative and comparative studies of the vocal fold extracellular matrix I: elastic fibers and hyaluronic acid." *Annals of Otology, Rhinology & Laryngology* 115, no. 2 (2006): 156-164.

[6] Hahn, Mariah S., James B. Kobler, Steven M. Zeitels, and Robert Langer. "Quantitative and comparative studies of the vocal fold extracellular matrix II: collagen." *Annals of Otology, Rhinology & Laryngology* 115, no. 3 (2006): 225-232.

[9] Hammond, Thomas Hale, Steven D. Gray, and John E. Butler. "Age-and gender-related collagen distribution in human vocal folds." *Annals of Otology, Rhinology & Laryngology* 109, no. 10 (2000): 913-920.

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| --- | --- | --- | --- | --- | --- | --- |
| VF Layers\*\* | | Fractional Elastin \* | Quantitative Elastin Levels \* | Fractional Area3 | Organization | Notes |
|  | | % Elastin of human LP TP^ | mg elastin per mg TP^ | Mean ± SEM |  |  |
| LP | Superficial Third | 8.5 ± 2.13 | 0.333,5 | 0.312 ± 0.006 | Oxytalan | * Superficial localization of fibrillin-1 relative to elastin * Higher relative tropoelastin to elastin staining; higher rater of turnover per elastin molecule |
| Intermediate Third | 3.193,5 | 0.354 ± 0.006 | Mature elastic fibers | * Strong elastin staining and weak fibrillin-1 |
| Deep Third | 11.893,5 | 0.335 ± 0.01 |  |  |

Table 2: Elastin Concentration by VF layer

\* levels of desmosine (amino acid only found in crosslinked elastin) quantified, for human used conversion factor 0.030 to relate picomoles of desmosine to mg of elastin5.

\*\* superficial, intermediate, Deep LP regions do not mimic SLP, ILP and DLP exactly but rather section the LP tissue into approximate thirds

^TP = total protein

[3] Hahn, Mariah S., James B. Kobler, Barry C. Starcher, Steven M. Zeitels, and Robert Langer. "Quantitative and comparative studies of the vocal fold extracellular matrix I: elastic fibers and hyaluronic acid." *Annals of Otology, Rhinology & Laryngology* 115, no. 2 (2006): 156-164.

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|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| VF Layers\*\* | | Fractional HA\* | Quantitative HA | Fractional Area3 | Organization | Notes |
|  | | % HA of human LP TP^ | μg HA per mg TP^ | Mean ± SEM |  |  |
| LP | Superficial Third | 0.82 ± 0.11 | 7.43 | 0.312 ± 0.006 |  | * Lowest HA staining |
| Intermediate Third | 8.73 | 0.354 ± 0.006 |  | * Highest HA staining |
| Deep Third | 8.53 | 0.335 ± 0.01 |  |  |

Table 3: HA Concentration by VF layer

\* Assuming linear microscope, camera operation and stoichiometric staining. Fractional integrated optical density (IOD) linearly related to regional mass. Fractional HA approximated by (Fraction elastin region = mass elastin region / mass elastin LP = IODregion / IOD region)

\*\* superficial, intermediate, Deep LP regions do not mimic SLP, ILP and DLP exactly but rather section the LP tissue into approximate thirds

^ TP = total protein

[3] Hahn, Mariah S., James B. Kobler, Barry C. Starcher, Steven M. Zeitels, and Robert Langer. "Quantitative and comparative studies of the vocal fold extracellular matrix I: elastic fibers and hyaluronic acid." *Annals of Otology, Rhinology & Laryngology* 115, no. 2 (2006): 156-164.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| VF Layers\*\* | | sulfated GAG content\* |  |  |  |
|  | | μg sulfated GAG per mg TP |  |  |  |
| LP | Superficial Third | 14.7 ± 2.1 |  |  |  |
| Intermediate Third |  |  |  |
| Deep Third |  |  |  |

Table 4: sulfated GAG content by VF layer

[8] Hahn, Mariah S., James B. Kobler, Steven M. Zeitels, and Robert Langer. "Midmembranous vocal fold lamina propria proteoglycans across selected species." *Annals of Otology, Rhinology & Laryngology* 114, no. 6 (2005): 451-462.