

Quantification by TEM of collagen changes in the anterior vaginal wall of post-menopausal women undergoing cystocele repair

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Statement of Purpose: Transmission electron microscopy (TEM) of human vaginal wall (HVW) has revealed qualitative changes in collagen fibers and bundles configuration; we quantified these changes in the anterior vaginal wall of post-menopausal women.

Methods: Following IRB approval, fresh 1x3cm anterior HVW samples were obtained for biomechanical testing and for TEM analysis. A strict methodology was employed to respect the original tissue orientation. Multiple ultrathin sections were taken at 60nm thick (silver sections), mounted on 200 mesh copper grids, stained by Reynold's lead citrate and uranyl acetate and examined on Zeiss 906E electron microscope. Representative images were taken using a Keenview digital camera with ITEM software (Soft Imaging Systems Inc., Williston, VT) at a magnification of 12960X. Regions with a preponderance of straight fibers were chosen in order to avoid discrepancies in measurements. For each characteristic region the periodicity of the collagen fibers over the entire spread as well as the diameter of the fibers was measured. A total of 200 measurements for the periodicity and diameter of the fibers were obtained for the stretched and the unstretched tissues from control and prolapse samples. Results are reported as mean ± SD. Student's two-tailed t test was used to evaluate the significance of the differences between groups.

Results/Discussion: The average diameter and periodicity of collagen fibers for unstretched and stretched portions of HVW samples from two age-matched post-menopausal patients, -one undergoing a stage IV cystocele repair and another a radical cystectomy (non-prolapse control)-are presented.

Conclusions: A tedious but precise method to quantify TEM changes in HVW collagen was devised. Collagen periodicity increased

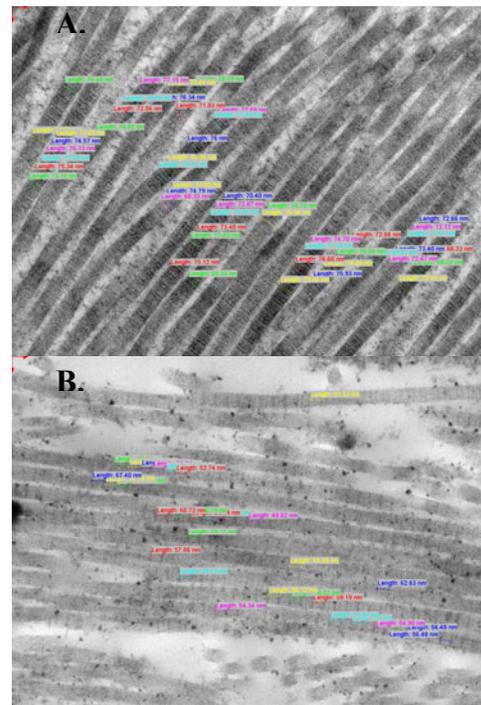
with tension in both samples. This protocol will now be expanded to a larger cohort of patients with both conditions.

| nm,mean± SD | Stretched | Unstretched |
|-------------|----------------------------|-------------|
| Diameter | 63.8±7.3* ^{&} | 62.1±6.8** |
| Periodicity | 62.6±6.6* ^{&} | 60.4±4.4** |

Prolapse ▲ Control ▼

| | Prolapse ▲ | Control ▼ |
|-------------|-----------------------|-----------|
| Diameter | 60.7±5.9 ⁺ | 70.2±6.8 |
| Periodicity | 54.7±3.1 ⁺ | 51.2±4.0 |

* p< 0.0001 stretched prolapse vs. stretched control; ** p< 0.0001 unstretched prolapse vs. unstretched control; & p< 0.0001 stretched prolapse vs. unstretched prolapsed; + p< 0.0001 stretched control vs. unstretched control



Periodicity: A. Control; B. Prolapse