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ORIGINAL ARTICLE

Morphological changes in the anterior vaginal wall caused by aging: a scanning electron microscopy study

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Abstract

Introduction and hypothesis Collagen is a protein that confers robustness and resilience to several tissues. In the female reproductive system, collagen plays a critical role in maintaining the health and function of the vaginal walls. Aging leads to collagen reduction, which may cause vaginal dryness, irritation, and prolapse. We aim to analyze the structure and profile of collagen in the anterior vaginal wall of healthy pre-menopausal (pre-M) and post-menopausal (post-M) women under a scanning electron microscope (SEM).

Methods Fragments of the anterior vaginal wall were collected and processed for light and scanning electron microscopy. Histological preparations were performed at first with Weigert's resorcin-fuchsin stain. Decellularized preparations were conducted, and the specimens were placed under an SEM to allow observation of the 3D organization of collagen.

Results Decellularized preparations of the pre-M specimens showed a vaginal wall with an irregular subepithelial layer, organized with ECM projections. The subepithelium evidenced the network of collagen fibrils, which seemed to support the epithelium as a basal layer. In specimens of post-M, a fusion of a network of fibrils from different direction axes was evidenced, with plate formation observed in the subepithelial plane, disfiguring the structural organization of fibrils.

Conclusions Older specimens showed a remodeling of collagen organization in comparison with younger samples of the anterior vaginal wall.

Keywords Collagen · Gynecology · Human · Scanning electron microscopy · Vaginal wall

Introduction

Collagen is an abundant protein found in the extracellular matrix (ECM) that provides strength, elasticity, and support to every tissue in the human body; as such, it plays a significant role in tissue function and architecture [1, 2].

Aging leads to intrinsic changes in the ECM throughout the body, which ultimately decreases the biomechanical function of all tissues. Dysregulation of ECM components leads to aberrant interactions and mechanical impairment, which places these organs at a higher risk for pathogenesis and/or function loss. Thus, the intersection of aging and the ECM, and how each influences the other, is fundamental to understanding numerous processes [3–5].

The morphology and physiology of the vagina undergo characteristic age-related changes. The most prominent physio-pathological changes are linked to menopause [6, 7]. There are several studies in the literature that address collagen organization in patients with and without vaginal prolapse, with conflicting results [8–10].

Few studies reported collagen content in the genital connective tissue under a scanning electron microscope (SEM), in women [4, 11, 12] and men [13, 14]. Collagen is known to be a highly dynamic structure that adapts in both a structural and functional way to increased mechanical loading, as observed in previous studies [3, 15, 16]. It is well known that the increased mechanical loads have been shown to accelerate connective tissue remodeling in soft tissues throughout the body [1, 2].

Studying the relationship between collagen, aging, and the vaginal wall may help researchers to develop new treatments for age-related vaginal issues, such as hormone

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replacement therapy or collagen-based therapies, and can provide a better understanding of pelvic organ prolapse [5].

Therefore, the goal of our study was to describe the three-dimensional ultrastructure of collagen in the vaginal mucosa of pre-menopausal (pre-M) compared with post-menopausal (post-M) women under an SEM. We focus this study on the vaginal subepithelium.

Materials and methods

The present work complies with the provisions of the Declaration of Helsinki of 1964 (revised by the 64th Assembly in Brazil, 2013) for research in humans. The Ethics Committee on Human Research of the Fluminense Federal University reviewed and approved this study (registered as protocol 89868318.6.0000.5243).

Samples of the anterior vaginal wall (AVW) at level I (DeLancey – the anterior third) (3 × 3 cm) were obtained during the autopsy of 10 young (pre-M) and 10 elderly women (post-M). These samples were immediately placed in a 2.5% glutaraldehyde solution buffered in 0.1 M sodium cacodylate buffer (SCB—pH 7.2). The anterior third of the vaginal wall was chosen because of its significant compromise in parturition, hysterectomy, and heavy lifting activities. The sample size was chosen by convenience and was composed only of unclaimed cadavers of women who died as a result of diseases/trauma unrelated to the urogenital tract.

The time elapsed between death and fixation of the samples was <6 h. After the tissues were fixed, a small portion of the sample (1 × 1 cm) was histologically processed. All samples were initially stained with hematoxylin–eosin and examined by a pathologist not involved in the study to detect and exclude foci of carcinoma and to exclude samples with artifacts. Weigert's resorcin-fuchsin counterstained with Van Gieson's was used to observe the elastic fibers of the ECM. Weigert's stain binds with the elastic fibers and usually results in a dark blue color; however, associated with Van Gieson's, elastic fibers are more dark-pink in color whereas collagen is displayed in a bright-pink color. These stains are often used together as they produce a stable compound.

For a better analysis of the ECM, the processing of specimens was performed via alkali–water maceration (decellularized preparations) following standard protocols previously described in the literature [17] and fixed in a 2.5% glutaraldehyde solution buffered in 0.1 M SCB for 48 h at 4°C, and then incubated in 40 ml of 2 M NaOH at room temperature for 8 days.

Then, the samples were washed in 0.1 M SCB pH 7.2 and coated with a thin silver layer 20–30 nm thick ("sputtering", ©Leica EM SCD 500, Wetzlar, Germany). The samples were placed for 1 h on a solution of 2% osmium tetroxide in 0.1 M SCB for 2 h. Afterward, the samples were washed again in 0.1 M SCB for 3 × 3 min and incubated in 1% aqueous tannic acid solution for 45 min. Between these steps, the samples were always washed 3 × 10 min in ultrapure water (UPW), incubated again in 2% osmium tetroxide for 5 min, and washed 3 × 10 min in UPW. After the final washing steps, the samples were dehydrated using ice-cold solutions of 30%, 50%, 70%, 90%, and 2 × 100% ethanol (anhydrous), for 30 min each [18].

They were dried in a critical-point dryer with CO_2 (Balzers CPD 030, Schaumburg, IL, USA) and examined using a JEOL SEM (Neoscope JCM-6000Plus, Portsmouth, NH, USA) with an acceleration voltage of 10–15 kV.

Measures and photographs of the approximate linear thickness were taken to better characterize the fibrous components of the stroma and to allow comparison. This SEM technique was also used to monitor the efficiency and extent of the cellular solubilization process [18].

Results

The mean age of the women who were pre-M was 30 ± 4 years old, whereas the mean age of the women who were post-M was 75 ± 6 years old. The reported findings are exclusively from the mucosa (subepithelial) of the anterior vaginal wall (level I DeLancey).

For the histological analysis, the 5-µm sections were stained with Weigert's resorcin-fuchsin and counterstained with Van Gieson's solution. Light microscopy showed a well-defined, uniform distribution of elastic fibers in the mucosal layer of the pre-M women with delicate fiber networks that serve as the supportive stroma between smooth muscle and connective tissue (Fig. 1A).

In SEM analysis, the decellularized samples showed a smooth and spongy fibrous sheet that lined the surface of the vaginal mucosa (Fig. 1B), forming a dense and supportive network for the vaginal mucosa wall (subepithelium), as seen in transverse sections (Fig. 1B). These collagen fibrils were mostly distributed in concentric layers under the lamina propria. A spongy organization with a thin but dense lamella delimited empty spaces on the deeper ECM portion of the fibrous septa.

Light microscopy also revealed that the concentration of elastic fibers was higher in post-M women (Fig. 2A) in comparison with pre-M women (Fig. 1A). This could be observed in the lamina propria and submucosa layers. A significant increase was observed in the volumetric density of the connective tissue of the post-M women (Fig. 2B) in comparison with the pre-M samples in SEM analysis (Fig. 1B).

It was observed in the pre-M samples that the structural framework was well delimited and organized in bundles that were distributed in a wavy pattern in two distinct axes (transverse and horizontal), forming a true support network for the subepithelium (Fig. 3A). The further from the luminal surface, the more condensed and thicker the ECM was.

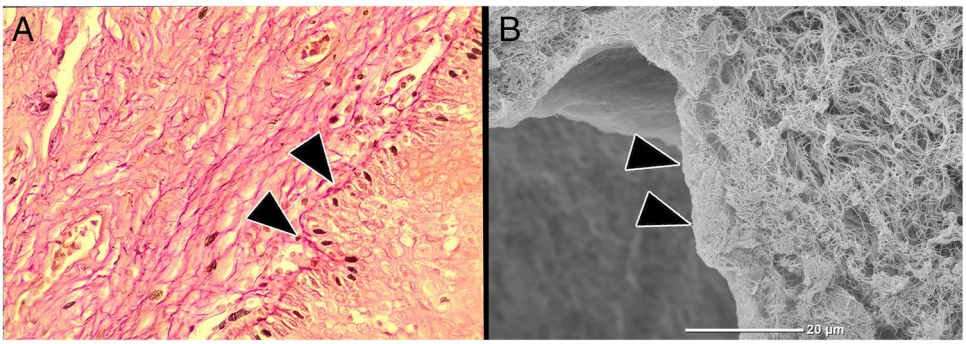

Fig. 1 **A** Histological micrograph of vaginal mucosa (pre-menopausal), showing a nonkeratinized stratified squamous epithelium and an underlying lamina propria, as seen by light microscopy. It is possible to observe a loose irregular connective tissue with many elastic fibers, stained in a dark color (*arrowheads*), whereas collagen of a pink-ish color is seen. Weigert's resorcin-fuchsin counterstained with Van Gieson's (×200, original magnification). **B** Scanning electron microscopy analysis (pre-menopausal) of the underlying lamina propria with collagen fibrils and elastic fibers in their natural locations and shapes in 3D (×1,500, original magnification)

Samples in the post-M women revealed that the collagen concentration was higher (Fig. 3B) than in pre-M women (Fig. 2B) in SEM analysis. This could be observed in the lamina propria and submucosa layers. The fibers appear to be arranged in layers with alternating orientations. Within each layer, the fibers assumed an approximately parallel alignment. At higher magnifications (Fig. 3B) the smaller fibers appear as a randomly oriented network, as shown by SEM.

Discussion

Initially, the functions performed by collagen were considered of minor importance and highlighted only the mechanical support and filling of the interstitial spaces, being seen as a practically inert biological component. Nowadays, the role of ECM is recognized in practically all tissue phenomena, constituting the substrate on which cells migrate, proliferate, and differentiate [1, 19, 20].

The role of the anterior vaginal wall in female sexual activity is prominent. Therefore, the hypothesis that structural alterations of this organ could account for sexual problems in pre-M and post-M women is reasonable [11, 21]. However, over the last few years, much has been done to increase our knowledge of female genital anatomy [22–25].

To date, investigations into the changes in the vaginal wall that occur with prolapse are limited and more commonly seen in animals [5]. Currently, the vast majority of studies comparing the vagina of women with and without prolapse have used biopsies of the vaginal wall or vaginal epithelium and a variable amount of subepithelial tissue that has been removed after repair of an anterior or posterior vaginal wall defect (e.g., a colporrhaphy). Very few of the studies [3, 11] have confirmed the target tissue under study histologically, making it difficult to determine exactly which portion of the vaginal wall is being analyzed.

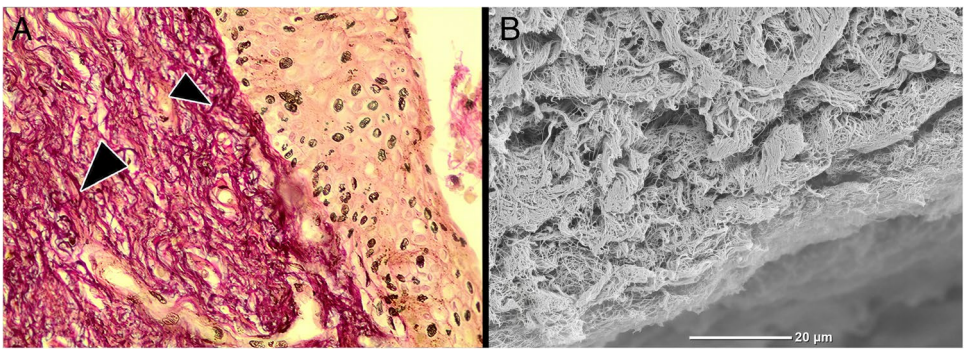

Fig. 2 **A** Histological micrograph of vaginal mucosa (post-menopausal), showing a nonkeratinized stratified squamous epithelium and an underlying lamina propria. A dense and irregular connective tissue with many elastic fibers (darker, *arrowheads*) and collagen can be seen. Weigert's resorcin-fuchsin counterstained with Van Gieson's stain (×200, original magnification). **B** SEM analysis (post-menopausal) of the underlying lamina propria and submucosa layer with a visual increase in collagen (×1,500, original magnification)

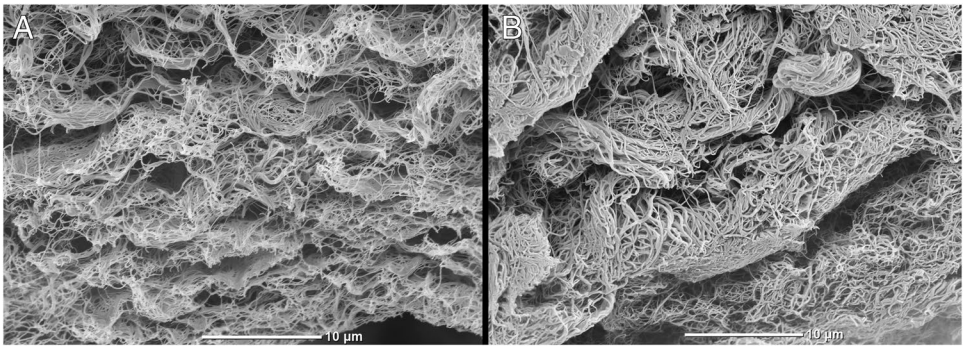

Fig. 3 Scanning electron micrograph of vaginal mucosa (pre-menopausal), showing the surface of the subepithelium in a decellularized preparation. **A** Ridges of the subepithelium (×270, original magnification). **B** Ridges of the subepithelium at higher magnification (×900, original magnification)

Although there has been some recent progress, advances in understanding the male sexual function and dysfunction have not been paralleled by similar advances in understanding the female sexual function, even in basic anatomy and physiology [14, 15]. There are still many areas in the field of female sexual physiopathology that remain to be fully studied [4, 16], among which are disturbances in the relationship between the tissue composition and anterior vaginal wall prolapse [3, 11].

The stroma of the vaginal wall is composed of muscle cells, associated connective tissue (with a large proportion of collagen fibers, elastic fibers, and undifferentiated fibroblasts), vessels, and nerves [20]. Smooth muscle, an integral part of the vaginal wall, and endopelvic structures that support the pelvic viscera, have also been implicated in the pathophysiology of POP [26, 27]; however, with few morphological details. Our previous work showed a reduction in smooth muscle content in the AVW caused by aging, which is in line with the high prevalence of POP in older women [3].

The present work was aimed at observing the ultrastructural aspect of the ECM in decellularized samples of the anterior vaginal wall obtained from young and old cadavers. We observed in general a more collagenous content present in the ECM as well as a more disorganized pattern in samples of the post-M women.

We observed that this architecture presented itself differently in the post-M group: it showed a condensation and disorganization in the collagen bundles, which at times were well-oriented and in a wave-like formation; as well as a sensitive increase in the presence of collagen in the subepithelial mucosa with fibril fusion and in the mucosa-occupying spaces where they were once formed by vessels and cells.

These findings may suggest that there is a disturbance in the organization of the collagen/elastic fibers, associated with a disturbance in their volumetric density–as shown by a previous stereological study [3]. The increase in collagen and reduction of elastic fibers caused by senescence may indicate tissue fibrosis, which is in contrast to the broad knowledge of the relationship between collagen and ageing. However, it can be speculated that our findings are related to the higher incidence of POP in elderly women and is worthy of further investigation.

One study compared the vaginal wall morphology between healthy pre-M women and pre-M women undergoing POP surgery. The authors observed an increase in type III collagen and elastin in women with POP [28]. The authors proposed that these changes were an acquired effect, rather than an intrinsic defect in the connective tissue.

However, a recent study [3] showed otherwise, as there were significant changes in the ECM of the vaginal wall in older women in comparison with younger ones. The results presented herein contribute to the understanding of POP and the AVW. The reduction in SM and the increase in fibrous tissue with aging can be one of the causes of the high prevalence of POP in older women [29]. Another study observed that local estrogen therapy played a significant role in reducing the degradation of the ECM caused by menopause and POP [30], which is also in agreement with our results.

The results of the present study support the hypothesis that atrophy of the vaginal mucosa might have its origin, in part, in the imbalance and disarrangement of the ECM in the anterior vaginal wall, mainly in the mucosa and submucosa. This is caused by senescence, as seen in the studied samples.

To our knowledge, this is the first study evaluating "postmortem" ultrastructural anterior vaginal wall tissue in pre-M and post-M women. Therefore, expanding morphological knowledge is essential to understand the mechanism that involves the development of prolapse, which is multifactorial [29].

The limitations concerning the present study are mainly regarding the selected sample/individuals, as there was a lack of data regarding comorbidities, tabagism, number of pregnancies, hormone replacement therapy, and the presence of genetic disease that affected the ECM. Furthermore, no quantitative methods of analysis or immunohistochemistry or collagen differentiation were performed.

Conclusion

Decellularized preparations and ECM analysis of the AVW showed remodeling of collagen organization in older samples in comparison with younger ones. The changes caused by aging may play a role in POP.

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Declarations

Conflicts of interest None.

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