

Structural and stereological analysis of elastic fibers in the glans penis of young men

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Abstract

The extracellular matrix is an important element in penile function and pathology, although little is known about its components in human glans. This study evaluates the morphological organization and volumetric density of elastic fibers in the glans penis of young men without any evidence of urogenital disease at autopsy or medical history. Penile glans were obtained from five young men who died of causes not related to the urogenital tract, ranging in age from 18 to 30 years (mean 24 years). Samples were fixed in formalin, embedded in paraffin, and histologically processed. Tissue was analyzed by light microscopy using Weigert's resorcin-fuchsin, after previous oxidation with oxone. The point-counting method was used for morphometrical evaluation. Quantities were expressed as volumetric densities (Vv) and were determined on 25 random fields for each individual. Elastic system fibers were easily identified. These fibers had tortuous profile and surrounded sinusoids in the glans penis. An irregular elastic fibers network was identified in the mucosa, while in the corpus spongiosum the elastic fibers were longitudinally distributed. Volumetric density of elastic fibers in the glans penis is $29.4\% \pm 3.1$. These data could provide valuable information in order to draw parallels regarding patients with erectile dysfunction. Further studies regarding extracellular matrix of the penis are necessary to better elucidate the relation between elastic fibers and erectile dysfunction.

Keywords: extracellular matrix, histology, morphology, penis.

Introduction

Glans penis tissue is composed of smooth muscle cells resting on collagen and elastic system fibers [1, 2]. The exterior structure of the glans consists of mucocutaneous tissue, which is usually covered by foreskin or clitoral prepuce in naturally developed genitalia [3].

The glans is particularly relevant regarding to several pathologies of the penis, e.g. non-specific dermatitis [3] or balanitis, which occurs in 3–11% of males, and up to 35% of diabetic males [4], and cancer [5].

A few studies have been focused on the morphology of glans penis, e.g., the density of genital corpuscles, and the distinct pattern of innervation of the glans [6].

Penile erection is dependent on penile tissue integrity. The mechanism of penile erection includes hemodynamics and a complex physiological process. Besides the well-known vascular problems, other alterations could play a significant role in erectile dysfunction (ED) [1, 7].

An understanding of the elastic system fibers in the glans penis would improve the basic scientific knowledge of the composition and organization of the glans penis structures that are thought to play a key role in the mechanism of erection.

In this study, we characterized and quantified the elastic system fibers in the glans penis of young men. Our analysis was focused on volumetric density by using stereological methods.

Materials and Methods

This study complies with the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh, 2000). Our internal Review Board approved the study guidelines. Also, the protocol received approval by the Ethics Committee on Human Research of the State University of Rio de Janeiro, Brazil.

Fragments of penile glans were obtained from autopsies of five individuals ranging in age from 18 to 30 years (mean 24 years). There was no previous clinical manifestation of erectile dysfunction or balanitis, according to medical history provided by family members and medical examination during autopsy, although is not possible to completely exclude previous inflammatory conditions of the glans in any research involving humans. As well as, the individuals died of traumatic causes not related to the urogenital system. The time elapsed between the individuals' death and the fixation of the material did not exceed 12 hours.

For light microscopy, specimens of 1-cm-thick from

longitudinal axis of glans penis (superficial mucosa-lumen urethral) were fixed in 10% formalin (pH 7.2), processed according to routine histological methods and embedded in paraffin. From the paraffin-embedded samples, 5- μ m-thick sections were initially stained with Hematoxylin and Eosin and were examined by a pathologist to confirm sample adequacy. Weigert's resorcin-fuchsin (Bio-Optica Milano, W01030708, Italy) technique was used for demonstration and evaluation of elastic system fibers that were stained in dark blue, producing a sharp contrast. The complex formed from the basic fuchsin, an iron resorcin lake, binds to the elastic fibers, resulting in the dark blue staining. The nuclear detail is stained with an iron Hematoxylin that will not over differentiate in the acidic elastic stain solution. For the "Weigert's resorcin-fuchsin" staining, the protocol for the working technique can be detected in higher details in previous literature [8, 9].

From each individual, five tissue sections were analyzed, and from each section, five random fields were evaluated; therefore, we analyzed 25 test areas for each individual. The analyzed fields were digitized with a final magnification of $\times 400$ by a Sony CCD video

camera (DXC 151-A model) coupled with a light microscope. The quantification was obtained by using an M-42 test grid system on the digitized fields on the screen of a color monitor. Based on the stereological principle, in isotropic tissue, the area distribution of a given structure, as determined on a two-dimensional section of the structure, is proportional to the volume distribution of this structure. The volume density of the histological components was calculated according to the formula $V_v = P_p/P_t$, where V_v is the volume density, p is the tissue component to be taken into consideration, P_p is the number of test points associated with p , and P_t is the number of points of the test system. The stereological methods have been described in detail elsewhere [9].

Results

After careful examination of the slides stained by Weigert's resorcin-fuchsin with previous oxidation with oxone, it was possible to observe the distribution of the elastic system fibers present in the glans penis (Figures 1–4).

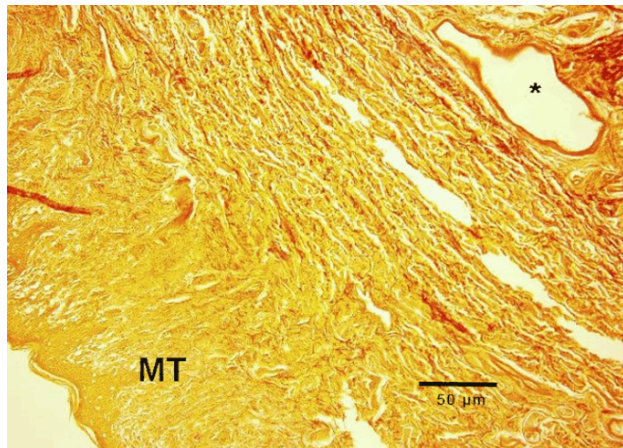


Figure 1 – Penile glans showing the layers, topography of the mucosa and spongiosum part (asterisk). MT: Mucosa topography. Weigert's resorcin-fuchsin. Scale bar = 50 μ m.

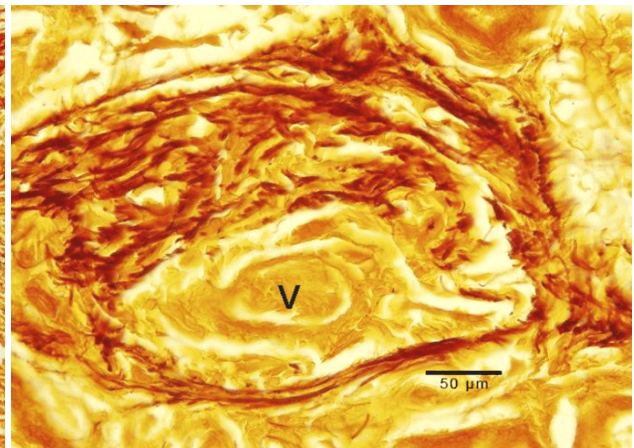


Figure 2 – Elastic fibers surrounding vessels underneath to penile glans of corpus spongiosum. V: Vessel. Weigert's resorcin-fuchsin. Scale bar = 50 μ m.

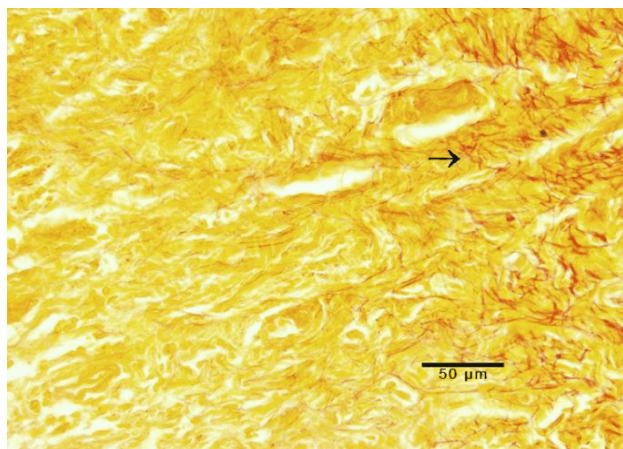


Figure 3 – Irregular elastic fiber network was distributed beneath the penile glans skin (arrow). Weigert's resorcin-fuchsin. Scale bar = 50 μ m.

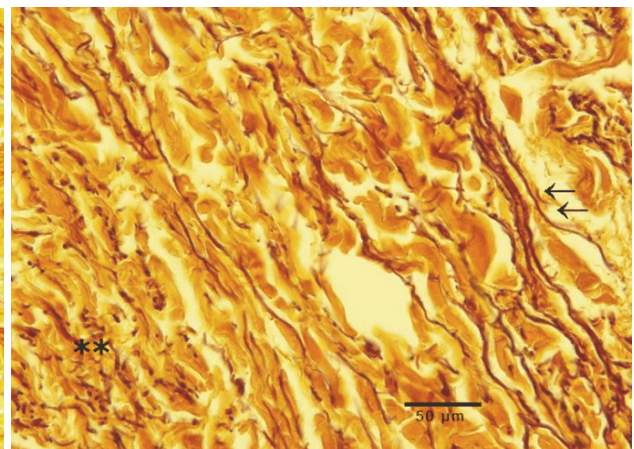


Figure 4 – Elastic fiber system at the penile glans outer periphery. Elastic fibers are interspersed among collagen bundles, forming an inner transverse layer (double asterisk) and an outer longitudinal layer (double arrow). Weigert's resorcin-fuchsin. Scale bar = 50 μ m.

The elastic system fibers were abundant (Figures 1 and 2). These fibers often had a tortuous aspect (Figures 2 and 3) and surrounded the sinusoids (Figure 3) in the glans penis. An irregular elastic fiber network was distributed in the glans penis mucosa (Figure 4), while in the corpus spongiosum, the elastic fibers were observed longitudinally distributed. Similar to collagen, the elastic fibers on the glans are organized into an inner circular layer and into an outer layer with fibers running parallel to the long axis of the glans (Figure 4). The volumetric density of elastic system fibers in the glans penis was $29.4 \pm 3.1\%$.

Regarding to corpora cavernosa, elastic fibers were identified mainly surrounding blood vessels. Still in corpora cavernosa, these fibers presented with a tortuous profile and were seen in less quantity than in the corpus spongiosum.

Discussion

Three types of fibers form the elastic system: oxytalan, elaunin and elastic. The oxytalan fibers are formed exclusively by microfibrils, the elaunin fibers by microfibrils and patches of amorphous material (elastin), and the elastic fibers by a large amount of elastin with microfibrils [10, 11]. The elastin content is delivered to the extracellular matrix as tropoelastin, and the elastic fibers assembly is completed in maturity when tropoelastin synthesis ceases [12].

The aim of our study was to analyze the entire elastic fiber system, and particular emphasis was placed on Weigert's resorcin-fuchsin stain after oxidation, because it highlights all elastic components in the tissues. We also attempted to outline the distribution of elastic system fibers in the glans penis and to characterize their organization in young adult men. Furthermore, the design provided stereological data on the concentration of elastic fibers in the glans penis.

The elastic fibers are active in tissue compliance [13], mainly in organs that change their shape under physiological conditions [12, 14], such as the glans penis. Mechanical forces also induce intense cellular and extracellular changes [15]. As shape and compliance are characteristics attributed to the extracellular matrix, mechanical forces may, in fact, influence shape and tissue compliance.

According to previously reported data [16], the elastic system fibers of extracellular matrix are characterized by major extension qualities and elastic recoil. Tissues that are constantly submitted to tensile strength are rich in elastic fibers [12, 16]. The location and arrangement of fibers are related to their different functionality, which reflects local tissue mechanical properties [16].

Penile erection and detumescence processes require a complex structure. These processes are closely related to the distribution of fibrous connective tissue elements, mainly the elastic system fibers and collagen bundles that also have a stretching function during erection [2]. The penile function also relies on the elastic system fibers [1]. Previous studies have shown a decrease in elastic fibers concentration in patients with ED from vascular diseases, which is the most important cause of

ED in senescent individuals [2]. In addition, researches revealed a reduction in concentration of elastic fibers in men with ED from any cause, after adjustment for age [17].

However, despite elastic fibers that endow glans connective tissue with the critical properties of elasticity and resilience, to our knowledge, no previously reported study has assessed the quantification of elastic fibers in the glans penis. Therefore, we present original data concerning this aspect in young adult men. In the present study, we verified that the elastic fibers were abundant in glans penis, reaching a volumetric density of 29.4%.

The elastic system fibers play an important role in the firmness of the glans penis during erection and sexual intercourse. It could resist less to dilation during erection, which results in a decrease in pressure, and then ED would occur. However, the disposition of these fibers remains the same, that is, more numerous in the corpus spongiosum and around blood vessels of the corpora cavernosa [2, 17, 18]. These observations suggest that the elastic system fibers might be one of the important factors in the development of ED.

Although the likely efficacy of new drugs has been demonstrated in certain dysfunction disorders, the real solutions for all changes involving ED remain unknown. Further studies regarding the specific structure and function of the different components of the penis would be an essential step in answering many questions on the pathophysiology of erection and eventually could be helpful to resolve the problem of certain forms of male impotence [17].

Our results show elastic system fiber concentration and distribution in glans penis of young men and, in spite of being essentially quantitative, they provide evidence for closer relationships between biomechanical modifications and erectile dysfunction.

Concerning the morphometric analysis, most of the studies attempting to quantify linear structures primarily use area density since the advent of computer-aided image analysis programs [2, 19, 20]. These programs use an element of color properties (pixels) of the image to determine a threshold level for inclusion. This is a rapid procedure, although for most linear structures, it is not an appropriate tool, since the most significant increase occurs in their length and not in their volume. Moreover, in rather thin linear structures, such as the elastic fiber system under analysis, the contrast between the fibers and the background is low and the error introduced using color intensity, as the method of measurement is too high. Thus, this renders it impossible to use the volume density as a reliable method of study [21].

The point counting method (stereology) used in our study proved to be quite effective in avoiding the bias that frequently occurs with computerized image analyses, which may overestimate or underestimate the analyzed structures. These methods have been used to quantify and particularly to determine the amount (%) of the elastic system fibers in the penis [12, 22, 23] and other extracellular matrix elements [21, 24, 25]. This procedure has been recommended by several authors [12, 22, 26].

✉ Conclusions

The elastic fibers in the glans penis are organized in an inner circular and an outer longitudinal layer, and its volumetric density was $29.4 \pm 3.1\%$.

References

- [1] Liu LC, Huang CH, Huang YL, Chiang CP, Chou YH, Liu LH, Shieh SR, Lu PS, *Ultrastructural features of penile tissue in impotent men*, Br J Urol, 1993, 72(5 Pt 1):635–642.
- [2] Sattar AA, Wespes E, Schulman CC, *Computerized measurement of penile elastic fibers in potent and impotent men*, Eur Urol, 1994, 25(2):142–144.
- [3] Birley HD, Walker MM, Luzzi GA, Bell R, Taylor-Robinson D, Byrne M, Renton AM, *Clinical features and management of recurrent balanitis; association with atopy and genital washing*, Genitourin Med, 1993, 69(5):400–403.
- [4] Edwards S, *Balanitis and balanoposthitis: a review*, Genitourin Med, 1996, 72(3):155–159.
- [5] Grayson W, Loubser JS, *Eccrine porocarcinoma of the penis*, J Urol, 2003, 169(2):611–612.
- [6] Halata Z, Spaethe A, *Sensory innervation of the human penis*, Adv Exp Med Biol, 1997, 424:265–266.
- [7] Moreland RB, *Pathophysiology of erectile dysfunction: the contributions of trabecular structure to function and the role of functional antagonism*, Int J Impot Res, 2000, 12(Suppl 4):S39–S46.
- [8] Cotta-Pereira G, Guerra Rodrigo F, Bittencourt-Sampaio S, *Oxytalan, elaunin, and elastic fibers in the human skin*, J Invest Dermatol, 1976, 66(3):143–148.
- [9] Bancroft JD, Cook HC, *Manual of histological techniques and their diagnostic application*, Churchill Livingstone, Edinburgh, 1994, 35–67.
- [10] Mandarim-de-Lacerda CA, *Stereological tools in biomedical research*, An Acad Bras Cienc, 2003, 75(4):469–486.
- [11] Kielty CM, Sherratt MJ, Shuttleworth CA, *Elastic fibers*, J Cell Sci, 2002, 115(Pt 14):2817–2828.
- [12] Bastos AL, Silva EA, Silva Costa W, Sampaio FJ, *The concentration of elastic fibres in the male urethra during human fetal development*, BJU Int, 2004, 94(4):620–623.
- [13] Parks WC, Secrist H, Wu LC, Mecham RP, *Developmental regulation of tropoelastin isoforms*, J Biol Chem, 1988, 263(9):4416–4423.
- [14] Huang K, Rabold R, Schofield B, Mitzner W, Tankersley CG, *Age-dependent changes of airway and lung parenchyma in C57BL/6J mice*, J Appl Physiol, 2007, 102(1):200–206.
- [15] Jackson ZS, Gottlieb AI, Langille BL, *Wall tissue remodeling regulates longitudinal tension in arteries*, Circ Res, 2002, 90(8):918–925.
- [16] Kreis T, Vale R, *Guidebook to the extracellular matrix and adhesion proteins*, Oxford University Press, Oxford, 1993.
- [17] Costa WS, Carrerete WG, Horta WG, Sampaio FJ, *Comparative analysis of the penis corpora cavernosa in controls and patients with erectile dysfunction*, BJU Int, 2006, 97(3):567–569.
- [18] Adsan O, Oztürk B, Cetinkaya M, Kulaçoğlu S, Memis A, Güner E, *The value of cavernous body biopsy in evaluating of impotent men*, Arch Ital Urol Androl, 1997, 69(3):151–153.
- [19] Rotten D, Gavignet C, Colin MC, Robert AM, Godeau G, *Evolution of the elastic fiber network of the human uterine cervix before, during and after pregnancy. A quantitative evaluation by automated image analysis*, Clin Physiol Biochem, 1988, 6(5):285–292.
- [20] Flotte TJ, Seddon JM, Zhang YQ, Glynn RJ, Egan KM, Gragoudas ES, *A computerized image analysis method for measuring elastic tissue*, J Invest Dermatol, 1989, 93(3):358–362.
- [21] Battlehner CN, Caldini EG, Pereira JC, Luque EH, Montes GS, *How to measure the increase in elastic system fibres in the lamina propria of the uterine cervix of pregnant rats*, J Anat, 2003, 203(4):405–418.
- [22] Pinheiro AC, Costa WS, Cardoso LE, Sampaio FJ, *Organization and relative content of smooth muscle cells, collagen and elastic fibers in the corpus cavernosum of rat penis*, J Urol, 2000, 164(5):1802–1806.
- [23] Babinski MA, de Brito-Gitirana L, Chagas MA, Abidú-Figueiredo M, Costa WS, Sampaio FJ, *Immunohistochemical analysis of smooth muscle cells and volumetric density of the elastic system fibers of wild boar (Sus scrofa) penis*, Anim Reprod Sci, 2005, 86(3–4):317–328.
- [24] Chagas MA, Babinski MA, Costa WS, Sampaio FJ, *Stromal and acinar components of the transition zone in normal and hyperplastic human prostate*, BJU Int, 2002, 89(7):699–702.
- [25] Costa WS, de Carvalho AM, Babinski MA, Chagas MA, Sampaio FJ, *Volumetric density of elastic and reticular fibers in transition zone of controls and patients with benign prostatic hyperplasia*, Urology, 2004, 64(4):693–697.
- [26] Cruz-Orive LM, Weibel ER, *Recent stereological methods for cell biology: a brief survey*, Am J Physiol, 1990, 258(4 Pt 1):148–156.

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