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From Cell Biology to Tissue Engineering

An immunohistochemical study of cytokeratins distribution of the human adult male and female urethra

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Summary. Surgical treatment of diseases affecting long urethral areas represents a challenge in urology. Recent developments of tissue-engineered urethral substitutes represent a hope for patients. However finding an ideal tissue source for urethral reconstruction first requires proper understanding of the native human urethra physiology and a deep knowledge of the histological and molecular features of the native human urethra. Here we present a comprehensive characterization of male and female urethra by histological, histochemical and immunohistochemical methods with a panel of 15 antibodies. The results demonstrated that the histology of the male and female urethra depend on the area where the sample is taken along its length. Proximal areas of male and female urethra have differential expression of the epithelial basal and suprabasal layer markers CK14 and CK10 which distinguished the prostatic/membranous and proximal female urethra from the bulbar/penile and distal female areas of the urethra. The distal male (penile) and female may be further divided by the distinct expression pattern of CK19. On the other hand, the expression of CK5/6 and CK19 also make a distinction of the proximal and distal female urethra. These results should facilitate a more informed selection of donor graft tissues for urethral replacement. Besides, novel bioengineered urethral tissue approaches should take into account the characterization of the different areas of the urethra presented in this work.

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Introduction

The urethra is a tubular structure that connects the urinary bladder to the urinary meatus. The male urethra, with a length of about 18-20 cm, is divided into a distal (penile and bulbar) and a proximal (membranous and prostatic) portion (Schenkman and Manger, 2013; del Pozo-Jimenez et al., 2014). The urethra in females is about 4 cm and it may be divided as distal (near to the meatus) and proximal (near to the bladder) (Schenkman and Manger, 2014).

Surgical treatment of diseases affecting long urethral areas is still a challenging problem in urology. Current clinical therapies are based on the utilization of autologous grafts (McAninch, 2014). Various tissues such as preputial mucosa, genital and extragenital skin, buccal mucosa, lingual mucosa, small intestinal submucosa, and bladder mucosa have been proposed for urethral reconstruction (Hampson et al., 2014). However several problems arise when using donor tissue: i) limited donor tissue supply, ii) problems of morbidity in the area of tissue harvesting and damage to the tissue to be grafted, iii) deterioration of the grafted tissue at long term, and iv) healing process of grafted tissue is not equal in all patients and may suffer fibrosis before the final urethral closure proceeds (Barbagli et al., 1995; Mundy, 1995; Dublin and Stewart, 2004). Accordingly, better substitutes for urethral reconstruction are needed,

and for this, a proper characterization of the native human urethra is essential from a clinical standpoint.

Our study aims to contribute to a better knowledge of the adult urethra with a broad characterization of female and male urethra by using histological, histochemical and immunohistochemical methods. The results could be useful for a more adequate selection of graft tissues for urethra replacement.

Materials and methods

Human urethra: tissue samples

Urethral Human Tissue samples were obtained from 8 (3 males and 5 females; 50- to 70- years old) cadaveric donors upon informed consent signature of relatives. Sample tissues were collected within 2-6 hours of death. To obtain urethra samples cystoprostatectomy (males) or cystectomy (in women) with urethrectomy was performed. Four tissue samples (Prostatic, Membranous, Bulbar and Penile urethra) and two urethral samples (Distal and Proximal) were taken from male and female donors, respectively (Fig. 1). This study has been approved by the Regional Transplant Coordination, the Ethics Committee of the Malaga Hospital and the Andalusian Biobank.

Histological analyses

Urethra tissue samples fixed in formaldehyde and embedded in paraffin were transversally cut at 3 μm in all, male and female, urethra samples (Fig.1). Hematoxylin/eosin, Masson's trichrome and PAS staining were used to determine the histology of the urethra under light microscopy.

Immunohistochemistry study

Tissue sections were incubated with primary antibody and stained according to the standard EnVision™ FLEX, High pH (K8010, Dako, Agilent Tecnologies, Denmark) working procedures using the automatic immunostainer Dako Autostainer (Dako).

Ready to use antibody from Dako used: Actin (Alpha-Sr-1), CK34BE12, CK19 (RCK108) and from Master Diagnóstica (Granada, Spain): CK 20 (Ks20.8), CAM 5.2 (clone CAM 5.2), CKAE1/AE3 (clone Y85), cytokeratin types 5 and 6 (D5/16B4), CK7 (OV–TL 12/30), CK10 (SP99), CK13 (KS-1A3), CK14(LL002), CK17 (E3), CK18 (DC-10), involucrin (SY5), GATA 3 (L50-823), Ki-67 (clone SP6).

Primary antibodies were incubated at room temperature and detected by EnVision FLEX/HRP (20 minutes) (Dako). Positive tissue controls were used for all antibodies included in this study (Table S1). Negative controls were performed by replacing primary antibodies with PBS. Results were independently analyzed by 3 researchers. The membrane and/or cytoplasmic expression levels of cytokeratins and the nuclear expression of Ki-67 and GATA3, were recorded and scored considering the staining intensity as low (+), moderate (++) or high (+++) and the area to which they extended (superficial or basal) (Table 1).

Image analyses

200X magnification microscopic images were transformed to 8 bits with Image J program (Schneider et al., 2012) and gray intensity was measured in a 0-255 black-white scale to quantify staining intensity. Image J was also used to count nuclear staining and total number

Figure 1

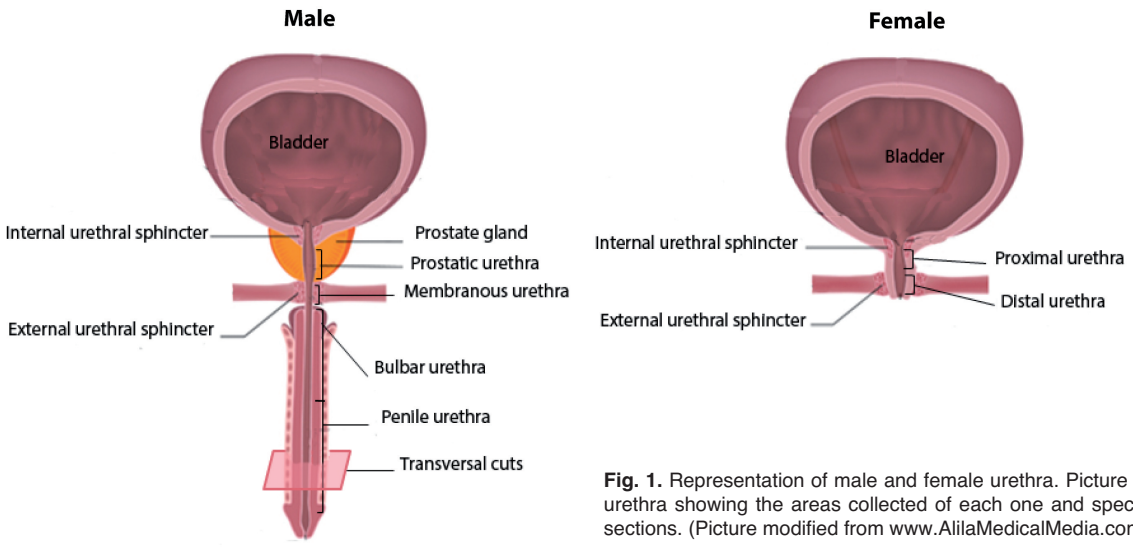

Fig. 1. Representation of male and female urethra. Picture of the male and female urethra showing the areas collected of each one and specifying the orientation of sections. (Picture modified from www.AlilaMedicalMedia.com).

of nuclei per layer in tissue sections stained with Ki 67 or GATA3.

Results

Histological analyses of the human urethra by H&E staining

To characterize epithelial thickness along the male urethral tract, histological sections were evaluated by hematoxylin and eosin (H&E) staining. The proximal (prostatic and membranous) region of the male urethra had few epithelial cell layers (4.26±0.88) corresponding to a transitional epithelium and pseudostratified columnar epithelium, respectively, whereas the distal (bulbar and penile) urethra showed a higher number of cell layers (6.9±1) (Fig. 2i-iv), corresponding to pseudostratified columnar epithelium which then transitions to stratified squamous epithelium distally. The distal female urethra presented a thickness comparable to the distal male urethra; however distal female urethra presents more layers of cells than proximal female urethra, corresponding each area to the transition from pseudostratified columnar epithelium (proximal) to stratified squamous epithelium (distal) (10.1±3.6 cell layers in distal female urethra) (Fig. 2). These results demonstrated that the histology of the male urethra is highly dependent on the area where the sample is taken along its length, from the bladder to the meatus, while female urethra seems to be more homogeneous along its short length

Histochemical analyses of the human urethra connective and muscular layers

To understand variations in tissue architecture along the male urethra, specific staining for the urethral connective and muscular layers were performed. When tissue sections were stained by the Masson's trichrome method (Fig. 3a), we found that all samples had collagen-rich connective tissue (Fig. 3a). As determined by PAS staining higher contents of glycoproteins were observed at the apical epithelial cell layers of the female urethra compared to male urethra (0.1±5.5; 0±3.8; 0.3±9;

Table 1. Semi-quantitative measure of immunostaining of specific antibodies used in male and female urethra.

	Prostatic urethra	Membranous urethra	Bulbar urethra	Penile urethra	Distal urethra	Proximal urethra
CK34BE120	B,SB,C +++*	B,SB,C+++	B,SB,C +++	B,SB,C +++	B,SB,C+++	B,SB,C +++
CKCAM 5.2	B,SB,C +++	B,SB,C+++	B,SB,C +++	B,SB,C +++	B,SB,C+++	B,SB,C +++
CKAE1/AE3	B,SB,C +++	B,SB,C+++	B,SB,C +++	B,SB,C +++	B,SB,C+++	B,SB,C +++
CK5/6	B,C +++	B,SB,C +++	B,SB, C +++	B,SB, C +++	B,SB, C +++	B,C ++
CK7	B,SB,C +++*	B,SB,C +++*	B,SB,C +++*	SB,C ++*	B,SB,C +++	B,SB,C +++
CK10			SB,C +++	SB,C+++	SB,C++*	**SB,C +
CK13	B,SB,C +*	B,SB,C +	B,SB,C +++	B,SB,C +++	B,SB,C +++	B,SB,C ++
CK14	**B,C		B,C++	B,C +++	B,C++	B,C ++
CK17	B,C+	B,C+	B,C++	B,C++	B,C++	B, C ++
CK18	B, SB,C++	B,SB,C ++	B,SB,C ++	B,SBC++	**B,SB,C+	SB,C++
CK19	B,SB,C+++	B,SB,C +++	B,SB,C+++/B,C+	B,C+	**B,SB,C++	B, SB,C +++
INVOLUCRIN	SB, C +	SB,C+	SB,C+	SB,C++	SB,C++	SB,C++
GATA3	**SB,N +	**SB,N +	**B,SB,N ++	**B,SB,N ++	**B,SB,N ++	**B, SB,N ++
Ki67	**B,N +	**B,N +	**B,N +	**B,N ++	**B,SB,N ++	**B,SB,N ++

Type of tissue sample: Human male and female urethra. For each CK, protein expression is scored as slightly positive (+), positive (++), or strongly positive (+++). B: expression in basal layers. SB: expression in suprabasal layers. C: cytoplasmic expression. N: nuclear expression. *: scatered cells are missing the staining; **: only some sporadic cells are stained.

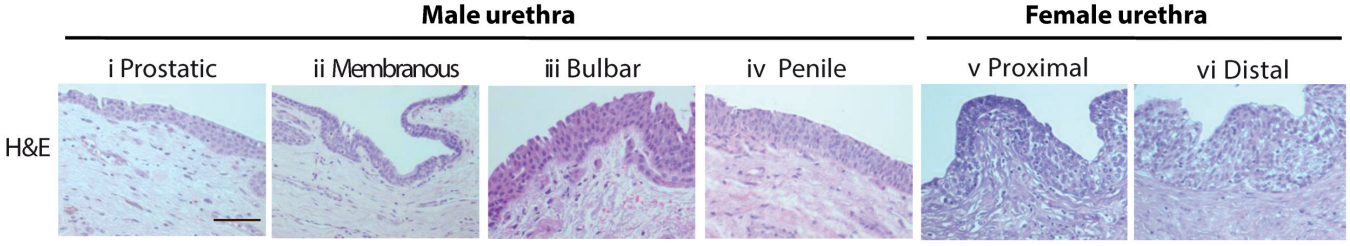

Fig. 2. Histological analysis of the human urethra. H&E staining of sections corresponding to prostatic (i), membranous (ii), bulbar (iii), penile (iv) male urethra and proximal (v) and distal (vi) female urethra showing differences in cell layer numbers. Scale bar: 100 μm.

0.4±10.7 and 0.1±6; 29.7±78 measured in a gray scale in prostate, membranous, bulbar and penile male and proximal and distal female urethra respectively) (Fig. 3b). Immunohistochemical staining for muscle-specific sarcomeric actin revealed that all regions of the male and female urethra had a muscular layer (Fig. 3c).

Immunohistochemical analyses of the epithelial layer with anti-cytokeratin antibodies

To further characterize the apparent differences in epithelial layers of the urethra, a comprehensive panel of cytokeratin-specific antibodies was employed (Fig. 4) and their reactivity patterns analyzed in a semi and quantitative manner (Tables 1, 2). As expected, broad-spectrum anti-cytokeratin antibody CK34BE12 (which recognizes human cytokeratins 1, 5, 10 and 14), CKCAM5.2 (anti-CK7 and CK8) and CKAE1/AE3 (pan-cytokeratin antibody mixture that reacts with 14 CKs of both A and B families) revealed strong cytoplasmic staining in all layers along the male urethra and also in the female urethra (Fig. 4a-c) with the only

Table 2. Quantitative measure of immunostaining of specific antibodies used in male and female urethra.

	Prostatic urethra	Membranous urethra	Bulbar urethra	Penile urethra	Distal urethra	Proximal urethra	Threshold
CK34BE120	32.3±5.7	32.1±5.7	35.4±4.3	33.3±5.3	32.4±5.2	33.8±4.8	0-40
CKCAM 5.2	31.8±8.9	41.4±5.8	41.0±6.3	44.7±4.1	43.5±4.3	41.0±3.8	0-49
CKAE1/AE3	43.5±9.7	46.8±8.4	37.4±9.5	44.4±8.6	38.1±7.5	43.9±9.1	0-61
CK5/6	58.4±9	56.0±9.0	65.2±5.1	59.8±7.8	59.2±8.3	61.0±7.1	0-71
CK7	43.1±13.1	54.8±13.2	51.8±13.6	59.8±17.5	43.3±10.4	50.2±8.4	0-96
CK10	133.1±10.9	118.0±1.9	35.2±2.9	34.3±3.7	120.1±13.2	37.4±7.3	0-68
CK13	73.1±11.5	80.9±6.3	61.1±13.3	50.5±13.4	59.1±9.5	48.8±10.4	0-88
CK14	87.4±10.9	94.9±4	74.0±13.8	66.7±19.5	82.1±7.4	73.1±10.6	0-98
CK17	100.4±7	90.5±13.9	96.8±10.2	92.3±13.6	95.2±9.4	97.4±11.3	0-108
CK18	81.9±9.5	89.2±4.8	83.0±10.9	83.3±10.8	75.0±8.7	86.0±6.9	0-94
CK19	42.3±11.1	49.3±9.0	42.5±9.9	53.4±7.9	41.8±9.8	48.8±10.5	0-64
INVOLUCRIN	71.9±9.0	72.2±9.6	78.5±3.7	73.5±8.0	72.8±11.2	73.9±7.7	0-82
GATA3	17.1±5.1	7.8±2.5	10.1±2.7	3.5±0.9	15.9±3.9	3.8±0.8	
Ki67	2±0.9%	6±1%	6±0.4%	40±1%	37±2.5%	48±3%	

Type of tissue sample: Human male and female urethra. For each CK, staining intensity is scored base on a gray scale from 0 to 255 (white-black) using Image J software. Values are represented ± the standard deviation. For GATA3 the ratio of percentage of stained cells in the apical cell layers/ percentage of stained cells in basal layers was calculated in three different samples. For Ki67 the percentage of stained cells was calculated by counting staining cells and total number of cells per optical area. Three different optical areas in three samples were used. Data are presented as media ± standard deviation in each case.

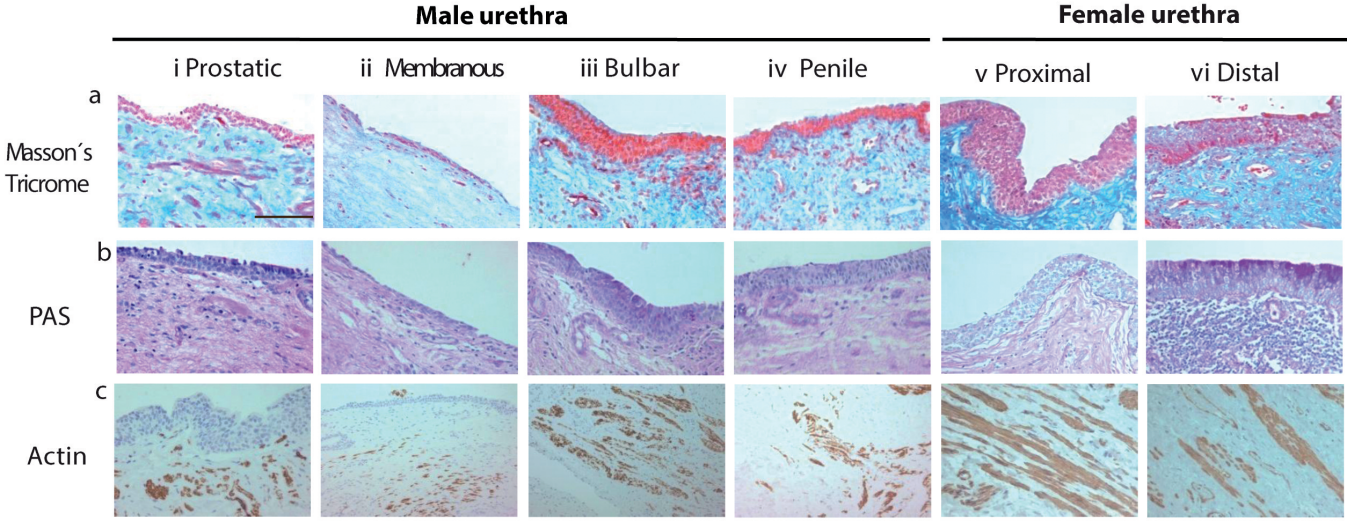

Fig. 3. Histochemical analysis of the human urethra connective and muscular layers. **a.** Masson´s trichrome staining. **b.** PAS staining. **c.** Immunohistochemistry staining against actin. Staining of sections corresponding to prostatic (i), membranous (ii), bulbar (iii) and penile (iv) male urethra and proximal (v) and distal (vi) female urethra. Scale bar: 100 μm.

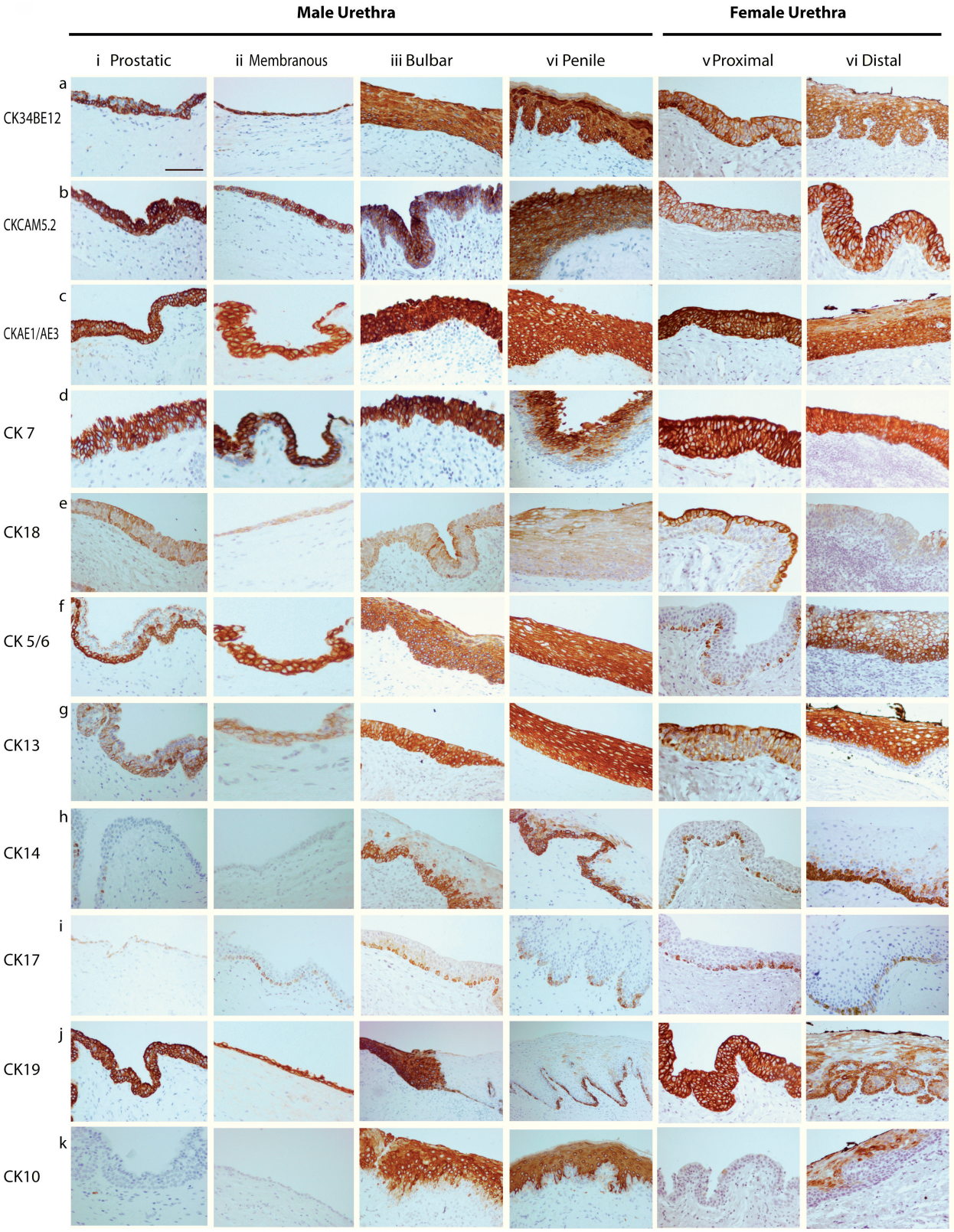

Fig. 4. Immunohistochemical analysis of cytokeratins of the human urethra epithelial layer. Immunodetection of a battery of cytokeratins was performed in tissue sections of prostatic (i), membranous (ii), bulbar (iii) and penile (iv) male urethra and proximal (v) and distal (vi) female urethra. Antibodies against broad-spectrum anti-cytokeratin antibodies CK34BE12 **(a)**, CKCAM 5.2 **(b)** and AE1/3 **(c)** showed cytoplasmic staining in all layers. Specific cytokeratin antibodies against CK7 **(d)**, CK18 **(e)**, CK5/6 **(f)**, CK13 **(g)**, CK14 **(h)**, CK17 **(i)**, CK19 **(j)** and CK10 **(k)**. Scale bar: 100 μm.

exception of some suprabasal layer cells of the prostatic urethra that appeared negative for CK34BE12 (Fig. 4a). To characterize expression of simple epithelia and urothelium-specific cytokeratin markers, immune detection of cytokeratins 7 and 18 was performed. Generalized CK7 and CK18 staining in all layers in male prostatic, membranous and bulbar urethra epithelium was seen (Fig. 4, d-e). Sporadic cells of the male prostatic and membranous urethra showed no expression (Fig. 4, d-e). In the penile urethra, CK7 was observed in suprabasal layers but not in the first basal layer (Fig. 4d), and CK18 was more intensely expressed in suprabasal layers (Fig. 4e). Proximal female urethra showed a very intense staining of CK18 in the suprabasal layers while distal female urethra showed very light staining for both cytokeratins and only in sporadic cells of all layers (Fig. 4, d-e). To characterize CK5 and CK6 expression, CK5/6 antibody staining was used. Staining of CK5/6 revealed intense cytoplasmic staining in all samples from male urethra (Fig. 4f), although restricted to the basal layers in the prostatic urethra (Fig. 4f). A very similar profile was found for CK13 expression, which was mostly localized in the basal cell layer of the prostatic urethra and expanded to all layers once the urethra moved away from the prostate (Fig. 4g). The distal female urethra had a similar staining profile for CK5/6 to bulbar and penile urethra (Fig. 4f-g). However, proximal female urethra showed CK5/6 expression only in the basal cell layer. The expression of CK13 was also localized in all the layers in the distal and proximal female urethra. Interestingly, basal cell-specific CK14 was expressed only by basal cells of the bulbar and penile urethra, and also the female urethra (distal and proximal), but not the prostatic and membranous urethra (Fig. 4h). Remarkably, CK17 was expressed only by distinct basal layer cells in all areas of the male urethra and in the female urethra (Fig. 4i). As for CK19, we found strong cytoplasmic staining in all layers of the male prostatic and membranous urethra (Fig. 4j). However, a striking difference was seen in the CK19 pattern of the bulbar epithelium (which was intensely positive in all layers) and the penile urethra (negative in suprabasal layers; the abrupt transition from one area to the other may be clearly seen in panel iii of Fig. 4j). Distal female urethra had strong CK19 staining in the basal layer and very light sporadic staining in some suprabasal cells, however, proximal urethra showed a strong expression of CK19 in all layers (Fig. 4j). CK20

Table S1. Positive controls used for the different antibodies included in this study.

Antibodies	Human tissue
CK34BE12	Tonsil
CKCAM5.2	Prostate
CKAE1/AE3	Breas
CK5/6	Skin and prostate
CK7	Breast and lung
CK10	Skin
CK13	Skin
CK14	Skin
CK17	Skin
CK18	Liver
CK19	Tonsil and liver
CK20	Appendix
Involucrina	Skin
Ki-67	Tonsil
GATA3	Prostate

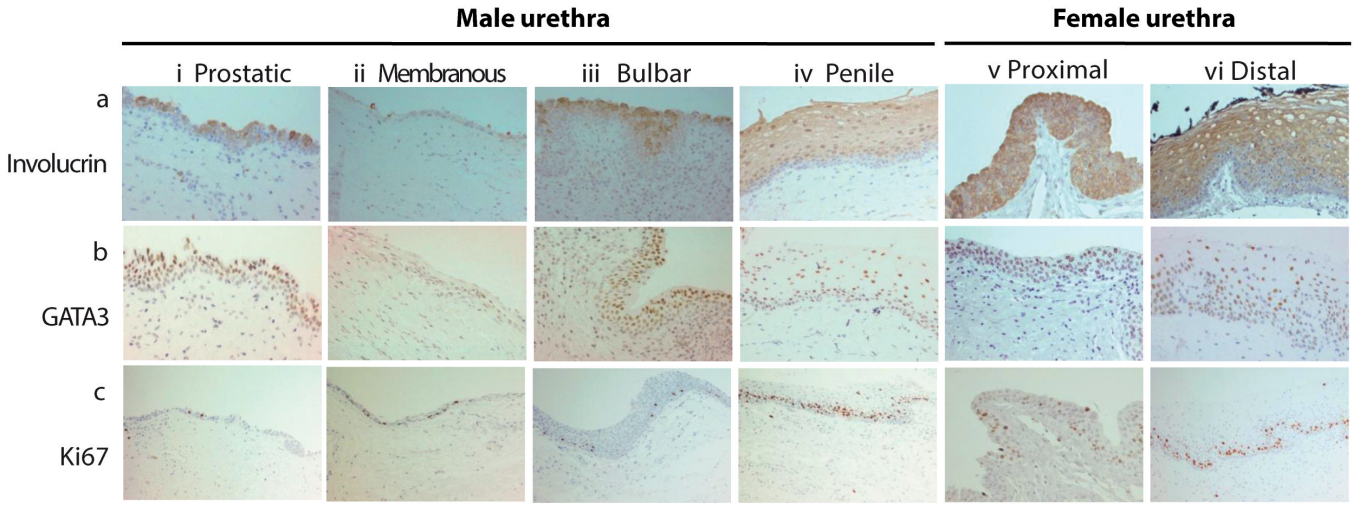

Fig. 5. Immunohistochemical analysis of epithelial differentiation, urothelial and proliferation markers. **a.** Involucrin antibody staining. **b.** GATA3 staining. **c.** Proliferation marker Ki67 staining. Scale bar: 100 μm.

immunostaining resulted negative for all male and female samples (data not shown). Finally, the suprabasal layer marker CK10 was completely absent in the prostatic and membranous urethra, but was intensely present in all suprabasal layers of the bulbar and penile urethra (Fig. 4k). Female urethra showed only some scattered positive cells in suprabasal layers of the distal urethra (Fig. 4k). The expression of CK10 was very low and only present in sporadic cells in the proximal female urethra. In summary, the immunohistochemical analysis of cytokeratin expression in the human male and female urethra revealed important differences among the different areas of the samples.

Immunohistochemical analyses of the epithelial layer with proliferation and differentiation markers

As would be expected, involucrin, an epithelial differentiation marker showed slightly positive expression at the suprabasal cell layers of the male and female urethra, but not at the basal layers (Fig. 5a; Tables 1, 2). With respect to GATA3, a marker for urothelial umbrella cells, slight positive staining was present in the nucleus of scattered cells in all layers of the male and the female urethra (Fig. 5b; Tables 1, 2). Remarkably, numbers of stained cells were higher in the apical cell layers (Ratio of percentage of stained cells in the apical cell layers/ percentage of stained cells in basal layers= 17.1±5.1; 7.8±2.5; 10.1±2.7; 3.5±0.9 and 15.9±3.9 and 3.8±0.8 in prostatic, membranous, bulbar, penile and female proximal and distal urethra respectively). Finally, the proliferation marker Ki67 was present in basal layers of all regions of the male urethra and in the female urethra, and the number of positive cells was higher in areas in which the epithelium had a higher number of cell layers (2±0.9%; 6±1%; 6±0.4%; 40±1% and 37±2.5 and 48±3% of Ki67 positive cells in prostate, membranous, bulbar, penile and proximal and distal female urethra respectively) (Fig. 5c, Tables 1, 2).

Discussion

A histological, histochemical and immunohistochemical characterization of the epithelial lining of the normal adult human female and male urethra is presented in this study.

Previous studies describe a variation of the lining epithelium among different regions of male urethra with distal areas showing a stratified squamous epithelium, and proximal areas being more similar to the bladder epithelium. In the female, it is likely that the whole urethra is very homogeneous, although proximal female urethra is lined by urothelium, followed by pseudostratified columnar epithelium and only distal (1/3) is lined by stratified squamous epithelium (Ross and Pawlina, 2015). Our work corroborated the heterogeneous nature of the urethral epithelium in the different zones of the male urethra and the difference between male and female urethra (Ross and Pawlina, 2015). In fact, as expected we found that proximal areas (prostate and membranous) of the male urethra had a lower number of epithelial layers, whereas the distal areas (bulbar and penile) had more cell layers. Even though both male and female have stratified squamous epithelium in distal areas, the female seems to be thicker than male urethra and with more cell layers, however with no statistical differences.

The current treatment for pathologic conditions involving long segments of the urethra often rely on reconstructive procedures. The most common clinical practice is to use buccal mucosa, preputial mucosa or other sources of tissue, although all of these tissues present limitations compared to the autologous urethral tissue (de Kemp et al., 2015), due to the histological and gene expression differences among tissues (Djordjevic, 2014). Therefore, the search for ideal and fully biomimetic alternative sources of tissue for use in urethral reconstructions is the subject of numerous research studies (de Kemp et al., 2015). We have shown that all parts of the urethra both male and female have a muscular component highlighting the need to incorporate not only urothelial cells when developing new bioengeeniering urethras, but also all the components of a real control urethra including the muscular tissue. The content of collagen highlights the importance of the elasticity and robustness of the urethral tissue (Lalla et al., 2010) and therefore the substitute tissues should have similar content of collagen or similar properties.

Cytokeratins are intermediate filaments characteristic of epithelial tissues. Specific combinations of cytokeratin expression are related to the specific epithelial cell type or pathological conditions (Moll, 1993; Paramio, 2006). Previous works based mostly on two-dimensional gel- electrophoresis reported a biochemical characterization of the cytokeratins of the diverse epithelia of the urogenital human male tract or of urothelial cell cultures (Quinlan et al., 1985; Rheinwald and O'Connell, 1985). However, this technique provides information of total epithelia but not of specific cell types. Afterwards, Schaafsma et al. identified by immunohistochemistry 7 of the 11 CKs reported by Achtstaetter et al. Besides, more recently some reports identified relevant spatiotemporal markers of the male (Pechriggl et al., 2013a) and female (Pechriggl et al., 2013b) urethra during development, pointing to the importance of the differential spatial expression pattern in the urethra. Our present work supports that different zones of the male urethra show different profiles of epithelial and stromal components, which may reflect specific tissue functions. We also extend the Achtstaetter et al. study to 11 CKs and differentiation and proliferation markers.

Our results suggest that proximal zones (prostatic and membranous) of the male urethra tended to express CKs that are typical of non-keratinized epithelia such as CK7 and CK19, whereas the distal zones -bulbar and penile- showed intense expression of markers of

keratinized and well-differentiated stratified epithelia such as CK14, CK10 and involucrin. These results are in agreement with the Dr. Schaafsma report and partially agree with reports by Dr. Achtstatter (Achtstatter et al., 1985; Schaafsma et al., 1989). This expression pattern could be explained by the location of each type of epithelium and the closer relationship of the bulbar and penile urethra with the external environment, but also by the more powerful urodynamic forces at the bulbar and penile urethra. Moreover, the expression of markers such as CK14, CK17, CK5 at the basal layers of the epithelium is consistent with the results previously reported for other types of epithelia such as the human skin, bladder or oral mucosa (Smedts et al., 1992), as well as urothelium (Hatina and Schulz, 2012). The increased presence of positive expression of basal cell markers CK14, CK5 (Moll, 1993) and proliferation marker Ki67 (Krishna et al., 2016) in the squamous epithelia of the male bulbar and penile urethra and in the distal female urethra reflects an increase in the proliferation rates in these areas that could explain the increase in number of cell layers. Besides, CK17 has been proposed as a marker for epithelial stem cells in complex epithelia (Troyanovsky et al., 1992). Moreover, during development CK17 is expressed in the urethral plate as well as in the urethral groove and epithelial cells surrounding the prostate. At the beginning of the organogenesis of the urethra, CK17 immunoreactivity is restricted to the luminal cells and in later stages is present exclusively in the basal cells of the epithelium (Pechriggl et al., 2013a).

It is worth mentioning the expression pattern of CK19 found with a clear abrupt expression in the transition from bulbar to penile urethra. The consensus for normal adult urothelium in urinary tract areas other than the urethra is that CK19 is expressed throughout all urothelial cell layers (Southgate et al., 1999; Sartoneva et al., 2011). Our results are in agreement with Schaafsma et al. who reported a considerable decrease in the distal part of the male urethra and extensive expression in the proximal part of the male urethra (Schaafsma et al., 1989). However, we observed in this area more homogeneous staining than Schaasfma et al. This is an important fact when developing artificial urethras as tissue substitutes for specific areas. In fact, in culture, human urothelial cells from ureter which are fairly similar to cells in the proximal part of the urethra were positive for CK19 staining during the whole cell culture period when culturing them on synthetic PLCL (poly-L-Lactide-co-ε-caprolactone) and cell culture plastic, while when culturing them on human amniotic membrane, CK19 expression of the urothelial cells decreased with time (Sartoneva et al., 2011).

Interestingly, the expression profile shown by proximal female urethra tended to resemble the profile of the prostatic and membranous male urethra, while distal female urethra was more similar to the bulbar and penile male urethra for most specific markers. These results are congruent with the coincidence in the epithelium type of each area in male and female urethra. The advances in bioengeeniering are focused on the development of new artificial constructs based on scaffolds with or without cell-seeded. However, the clinical experience is still relatively scarce and no general conclusions can be drawh due to the different etiology of the urethral pathology and probably due to the differences in the areas treated in those studies. Based on our results, the specific CKs expression pattern depending on the area of the human urethra to treat could determine the cells to culture and the biomaterials to be used as scaffold. Therefore, a histological analysis of new constructs should be made and compared to the control expression pattern specific to the area to be treated to guarantee the substitute most similar to the original tissue. In summary, this study highlights the heterogeneity of the urethral epithelium along the length of the male and female urethra. However, we analyzed a limited number of specimens and this study will only be the starting point for future investigations. In addition, the novel bioengineered urethral tissues (Orabi et al., 2013, de Kemp et al., 2015) should take into account the results found in the present work and generate a human urethra substitute able to reproduce the histological and histophysiological characteristics of the human urethra uncovered in this report.

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References

Achtstatter T., Moll R., Moore B. and Franke W.W. (1985). Cytokeratin polypeptide patterns of different epithelia of the human male urogenital tract: Immunofluorescence and gel electrophoretic studies. J. Histochem. Cytochem. 33, 415-426.

Barbagli G., Palminteri E., de Stefani S. and Lazzeri M. (2006). Penile urethroplasty: Techniques and outcomes using buccal mucosa grafts. Contemporary Urology 18, 25-33.

de Kemp V., de Graaf P., Fledderus J.O., Ruud Bosch J.L. and de Kort L.M. (2015). Tissue engineering for human urethral reconstruction: Systematic review of recent literature. PloS One 10, e0118653.

del Pozo-Jimenez G., Jara Rascon J., Aragon Chamizo J., Blaha I., Hernandez Fernandez C. and Lledo Garcia E. (2014). Anatomy and vascularization on the male urethra and penis. Archivos Españoles Urologia 67, 5-11 (in Spanish).

Djordjevic M.L. (2014). Graft surgery in extensive urethral stricture disease. Curr. Urol. Rep. 15, 424.

Dublin N. and Stewart L.H. (2004). Oral complications after buccal

mucosal graft harvest for urethroplasty. BJU Int. 94, 867-869.
Hampson L.A., McAninch J.W. and Breyer B.N. (2014). Male urethral strictures and their management. Nat. Rev. Urol. 11, 43-50.
Hatina J. and Schulz W.A. (2012). Stem cells in the biology of normal urothelium and urothelial carcinoma. Neoplasma 59, 728-736.
Krishna O.H., Kayla G., Abdul Aleem M., Malleboyina R. and Reddy Kota R. (2016). Immunohistochemical expression of ki67 and p53 in wilms tumor and its relationship with tumor histology and stage at presentation. Pathol. Res. Int. 2016, 6123951.
Lalla M., Gregersen H., Olsen L.H. and Jorgensen T.M. (2010). In vivo biomechanical assessment of anterior rabbit urethra after repair of surgically created hypospadias. J. Urol. 184, 675-680.
McAninch J.W. (2014). Urotrauma guidelines. J. Urol. 192, 336.
Moll R. (1993). Cytokeratins as markers of differentiation. Expression profiles in epithelia and epithelial tumors. Veroffentlichungen Pathologie 142, 1-197 (In German).
Mundy A.R. (1995). The long-term results of skin inlay urethroplasty. Br. J. Urol. 75, 59-61.
Orabi H., Bouhout S., Morissette A., Rousseau A., Chabaud S. and Bolduc S. (2013). Tissue engineering of urinary bladder and urethra: Advances from bench to patients. Scient. World J. 2013, 154564.
Paramio J.M. (2006). Intermediate filaments. Springer.
Pechriggl E.J., Bitsche M., Blumer M.J. and Fritsch H. (2013a). The male urethra: Spatiotemporal distribution of molecular markers during early development. Ann. Anat. 195, 260-271.
Pechriggl E.J., Bitsche M., Blumer M.J., Zwierzina M.E. and Fritsch H. (2013b). Novel immunohistochemical data indicate that the female foetal urethra is more than an epithelial tube. Ann. Anat. 195, 586-595.
Quinlan R.A., Schiller D.L., Hatzfeld M., Achtstatter T., Moll R., Jorcano J.L., Magin T.M. and Franke W.W. (1985). Patterns of expression and organization of cytokeratin intermediate filaments. Ann. NY Acad. Sci. 455, 282-306.
Rheinwald J.G. and O'Connell T.M. (1985). Intermediate filament proteins as distinguishing markers of cell type and differentiated state in cultured human urinary tract epithelia. Ann. N.Y. Acad. Sci. 455, 259-267.
Ross M.H. and Pawlina W. (2015). Histology: A text and atlas: With cell and molecular biology. Wolters Kluwer.
Sartoneva R., Haimi S., Miettinen S., Mannerstrom B., Haaparanta A.M., Sandor G.K., Kellomaki M., Suuronen R. and Lahdes-Vasama T. (2011). Comparison of a poly-l-lactide-co-epsilon-caprolactone and human amniotic membrane for urothelium tissue engineering applications. J. R. Soc. Interface 8, 671-677.
Schaafsma H.E., Ramaekers F.C., van Muijen G.N., Ooms E.C. and Ruiter D.J. (1989). Distribution of cytokeratin polypeptides in epithelia of the adult human urinary tract. Histochemistry 91, 151-159.
Schenkman N. and Manger J. (2013). Male urethra anatomy. Medscape.
Schenkman N. and Manger J. (2014). Female urethra anatomy. Medscape.
Schneider C.A., Rasband W.S. and Eliceiri K.W. (2012). NIH image to imagej: 25 years of image analysis. Nat. Methods 9, 671-675.
Smedts F., Ramaekers F., Troyanovsky S., Pruszczynski M., Robben H., Lane B., Leigh I., Plantema F. and Vooijs P. (1992). Basal-cell keratins in cervical reserve cells and a comparison to their expression in cervical intraepithelial neoplasia. Am. J. Pathol. 140, 601-612.
Southgate J., Harnden P. and Trejdosiewicz L.K. (1999). Cytokeratin expression patterns in normal and malignant urothelium: A review of the biological and diagnostic implications. Histol. Histopathol. 14, 657-664.
Troyanovsky S.M., Leube R.E. and Franke W.W. (1992). Characterization of the human gene encoding cytokeratin 17 and its expression pattern. Eur. J. Cell Biol. 59, 127-137.

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