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Minipig urethra: A suitable animal model *in vitro*

Daniel Joller[a], Shpend Mushkolaj[a], Javier Ratia-Garcia[a], Florian Marti[a], Alexander Bachmann[b] and Bert Müller[a,*]
[a]*Biomaterials Science Center, University of Basel, Basel, Switzerland*
[b]*Department of Urology, University Hospital Basel, Basel, Switzerland*

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Abstract. The study demonstrates that the minipig urethra is an appropriate animal model for *in vitro* experiments and therefore promising for long-term animal tests of artificial urinary sphincter systems *in vivo*. Freshly explanted porcine and minipig urethras were connected to a fluid reservoir that simulated the bladder pressure. This bladder pressure was adjusted by changing the water level (hydrostatic pressure). Specially designed aluminum sphincters loaded with a fluid-filled container were used to close/open the urethras. Results from minipigs and domestic pigs were compared to data published for human urethras. We measured the leak-point pressures (LPP) by adjusting the bladder pressure at a constant sphincter length and by changing the sphincter length at a constant bladder pressure. Because the urethral tissue shows visco-elastic behavior, LPPs for both opening and closing were measured. By fitting the *in vitro* data, we evaluated the three characteristic parameters from the empirical urethra compression model, i.e. wall pressure (p_W), rim force (F_R), and rim length (L_R). From the experimental data we found agreement of mean values between (male) human urethras:

$< p_W >_{\mathrm{opening}} = -(12.9 \pm 0.9)$ cmH$_2$O, $< p_W >_{\mathrm{closing}} = (8.6 \pm 1.1)$ cmH$_2$O,
$< F_R >_{\mathrm{opening}} = (0.06 \pm 0.02)$ N, $< F_R >_{\mathrm{closing}} = (0.10 \pm 0.02)$ N,
$< L_R >_{\mathrm{opening}} = (3.0 \pm 0.3)$ mm, $< L_R >_{\mathrm{closing}} = (5.1 \pm 0.3)$ mm,

and (male) minipig urethras:

$< p_W >_{\mathrm{opening}} = -(13.4 \pm 0.3)$ cmH$_2$O, $< p_W >_{\mathrm{closing}} = -(8.6 \pm 0.4)$ cmH$_2$O,
$< F_R >_{\mathrm{opening}} = (0.19 \pm 0.01)$ N, $< F_R >_{\mathrm{closing}} = (0.21 \pm 0.02)$ N,
$< L_R >_{\mathrm{opening}} = (2.4 \pm 0.1)$ mm, $< L_R >_{\mathrm{closing}} = (3.3 \pm 1.0)$ mm.

These *in vitro* tests quantified by means of the urethra compression model demonstrate that the minipig (especially the male one) represents a suitable animal model for testing artificial urinary sphincters.

Keywords: *In vitro* testing, animal model, artificial urinary sphincter, minipig urethra, urinary incontinence, urethra compression model

1. Introduction

Stress urinary incontinence (SUI) is a worldwide problem that affects millions of people [1]. Up to 50% of all urinary incontinence cases are examples of SUI [1]. In 2003, the median prevalence of genuine

*Address for correspondence: Prof. Bert Müller, Thomas Straumann Chair for Materials Science in Medicine, Biomaterials Science Center, c/o University Hospital Basel, CH-4031 Basel, Switzerland. Tel.: +41 61 265 9660; Fax: +41 61 265 96 99; E-mail: bert.mueller@unibas.ch.

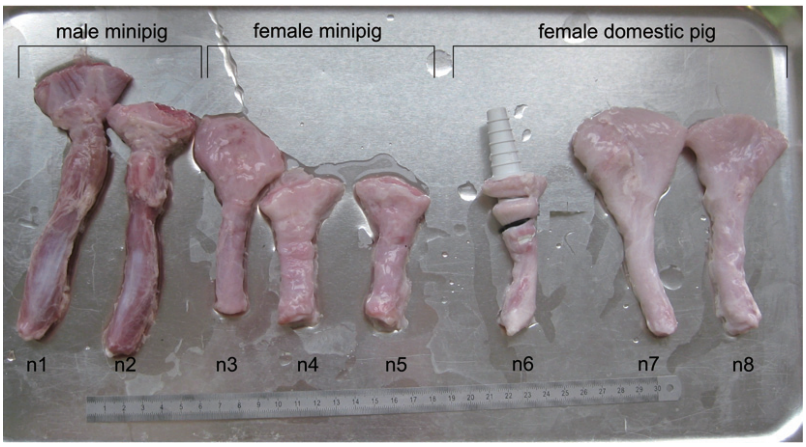

Fig. 1. The photograph shows the explanted porcine urethras after preparation for the study. A part of the bladder serves for fixation to the water reservoir. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/THC-2012-0675)

urinary incontinence was 27.6% in females and 10.5% in males [1]. Despite the emergence of many devices on the markets as different male sling procedures, the AMS 800$^{\mathrm{TM}}$ remains one of the most safe and efficient treatment tools for men suffering main from iatrogenic urinary stress incontinence, mostly after radical prostatectomy [2]. In the treatment of severe urinary incontinence, this artificial urinary sphincter system remains the gold standard [3]. The AMS 800$^{\mathrm{TM}}$ is a mechanically driven device with a pressure regulating balloon, an inflatable cuff and a control pump. It has been on the market for more then 20 years and shows an impressive total continence rate after ten years of 73% to 84% [4,5]. During a follow-up ten years later, however, 27% of the AMS 800$^{\mathrm{TM}}$ had to be removed or replaced due to mechanical malfunction and an additional 37% were removed due to infection and erosion [5,6]. The major mechanical problem is the leakage of the system and thus a pressure drop in the cuff [6]. The constant pressure in the cuff often leads to atrophy, erosion, and infection [3] and results in an inability of the AMS 800$^{\mathrm{TM}}$ to react to stress situations and sudden pressure increases, e.g. due to coughing [7]. The success of the AMS 800$^{\mathrm{TM}}$ rises and falls with the patients' ability to handle the device [3].

There are ongoing attempts to develop more sophisticated artificial urinary sphincter systems. The use of shape memory alloys, for example, allows adjusting the pressure onto the urethral tissue as a function of physical activity [8]. Fast enough switching as demonstrated by electrically activated polymers even enables biomimetic functionalities of the sphincter reacting to coughing and Valsalva maneuvers [8].

For *in vivo* testing of the artificial urinary sphincters, before clinical tests can be initiated, an appropriate animal model has to be found. The domestic pig proved to be the solution for short-term studies [9, 10]. Because the domestic pigs grow quickly with respect to the human being, appropriate long-term experiments are impossible [9]. This problem can be mastered using *Göttinger* minipigs as successfully demonstrated for long-term testing of dental implants. This paper aims to examine if the minipigs can be used like-wise for long-term studies of artificial urinary sphincters. Therefore, it is hypothesized that the human urethral tissue exhibits similar mechanical behavior than the urethra of the minipig *in vitro*.

2. Materials and methods

The experiments included five *Göttinger* minipig urethras (two male, three female, each 39 months). The minipigs were sacrificed for an animal experiment of the Dental School of the University of Bern,

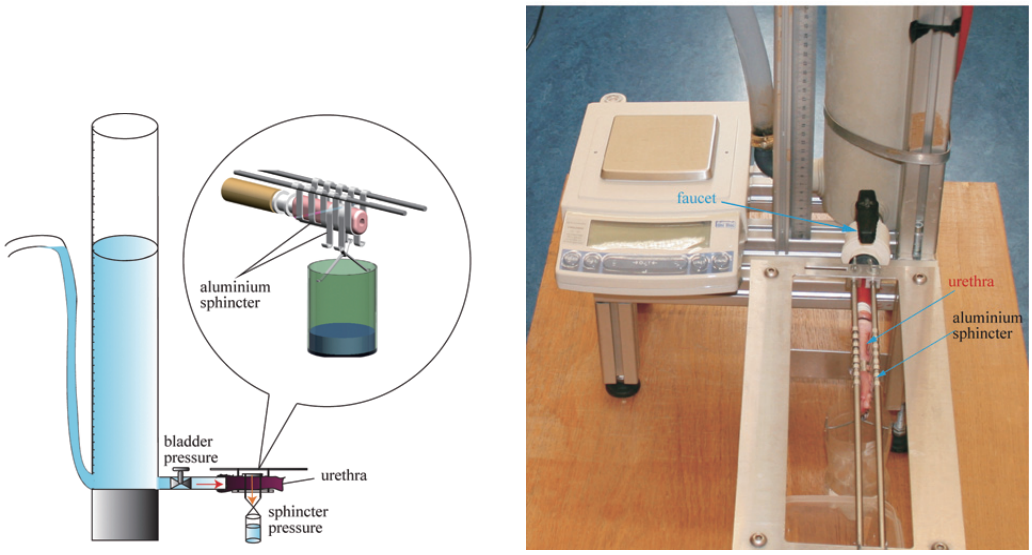

Fig. 2. The experimental setup for *in vitro* characterization of the explanted urethras consists of a water-filled cylinder attached via faucet served as artificial bladder to load the urethras. The selected water levels corresponded to pressures applied. The urethras were mounted onto special aluminum brackets. A second, weight-loaded aluminum bracket was placed on top to build a simple sphincter structure. The application of different sphincter lengths ranging from 2.0 to 22.5 mm allows simulating different sphincter geometries. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/THC-2012-0675)

Switzerland, according to the Declaration of Helsinki and the local ethical guidelines. Three domestic pig urethras (female, three months) sacrificed for food production served as control. The urethras were extracted along with the bladder. Subsequently, the bladder and excess parts including fat were carefully removed. The prepared urethras, as shown in Fig. 1, were stored in a 0.9% saline solution at a temperature of 5 to 6°C in a refrigerator. The saline solution was changed every two days to reduce degeneration.

In order to extract urethral tissue parameters according to the published compression model [10], a water-filled cylinder attached via faucet served as an artificial bladder to load the urethras hydrostatically (see Fig. 2). The selected water levels corresponded to physiologically relevant pressures between 1.2 and 6.7 kPa (12.5 to 68.3 cmH_2O). The urethras were connected to the faucet after horizontal placement onto special aluminum brackets [10]. Another, similar bracket was placed on top to build a sphincter structure for the *in vitro* study, where the occlusion length could be varied [10]. The application of different sphincter lengths ranging from 2.0 to 22.5 mm allows for the simulation of different occlusion lengths important for optimizing artificial sphincters. Note that such variations are impossible for the AMS 800$^{\mathrm{TM}}$, which has a given length. For the experiments we placed the aluminum-sphincter at the bladder neck to replicate the position of the AMS 800$^{\mathrm{TM}}$ [11]. The second bracket was loaded using water-filled container to generate the closing force by means of gravity. The amount of water in the container was adapted taking advantage of a 60 ml syringe. Subsequently, the weight of this water-filled container was measured to calculate the force applied. The experiments were performed at room temperature keeping the urethras moist by periodic sprinkling with water. The start of leakage corresponds to the situation, when the first droplet becomes clearly visible at the end of the urethra.

Each urethra underwent three series of *in vitro* experiments. The first series corresponded to the determination of sphincter pressures as a function of the applied bladder pressure at fixed sphincter length. The second series investigated the relationship between the sphincter pressure and the sphincter length at selected bladder pressures. To check the reproducibility, a third series of experiments was

Table 1
Characteristics of the explanted minipig and domestic pig urethras

Urethras	Length/mm	Diameter/mm	Wall thickness (muscle/epithelium)/mm	Circumference/mm
minipig ♂ (n1)	120	12	3/2–5	55
minipig ♂ (n2)	100	12	3/2–3	60
minipig ♀ (n3)	60	12	3–4/< 1	42
minipig ♀ (n4)	45	14	5/< 1	50
minipig ♀ (n5)	45	15	3–4/< 1	44
dom. pig ♀ (n6)	55	15	2/4	45
dom. pig ♀ (n7)	70	14	2/4	43
dom. pig ♀ (n8)	60	14	1–2/3–4	48

dom. = domestic.

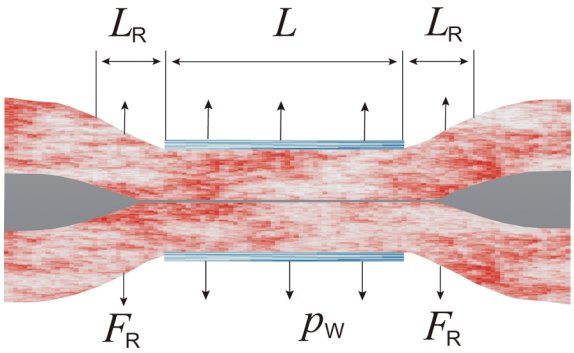

Fig. 3. The schematic cross-section of the compressed urethra shows the denotation of the three characteristic parameters from the empirical urethra compression model, namely the wall pressure p_W, rim force F_R, rim length L_R, and the length of the sphincter L. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/THC-2012-0675)

performed changing both bladder pressure and sphincter length. All tests included the measurement of sphincter pressures for closing and opening. Between the measurements we kept a continuous flow of water to minimize the differences between opening and closing states. The series of experiments were performed twice on each urethra within two or three days to detect potential changes of the urethras with time.

3. Results

There are distinct morphological and mechanical differences between the male and female *Göttinger* minipig urethras. The more muscular male urethras are significantly longer and stiffer. The morphological characteristics are quantitatively summarized in Table 1. The urethra consists of different layers (cp. wall thickness) including the *tunica mucosa* and the *tunica muscularis* [12]. This layered structure is similar for the domestic female porcine urethras and the male minipig urethras but different for the female minipig urethras.

The scheme in Fig. 3 shows the meaning of the three parameters from the empirical *urethra compression model*, i.e. the wall pressure (p_W), rim force (F_R) and rim length (L_R). The parameter p_W stands for the pressure the urethral wall generates. The rim force signifies the stress of the urethra at both ends of the sphincter. The rim length labels the length difference between the actual sphincter length and length affected by the artificial sphincter. As expected from the compression model, a linear dependence was found between the external urethral leak-point pressure (euLPP) – sphincter pressure – and the

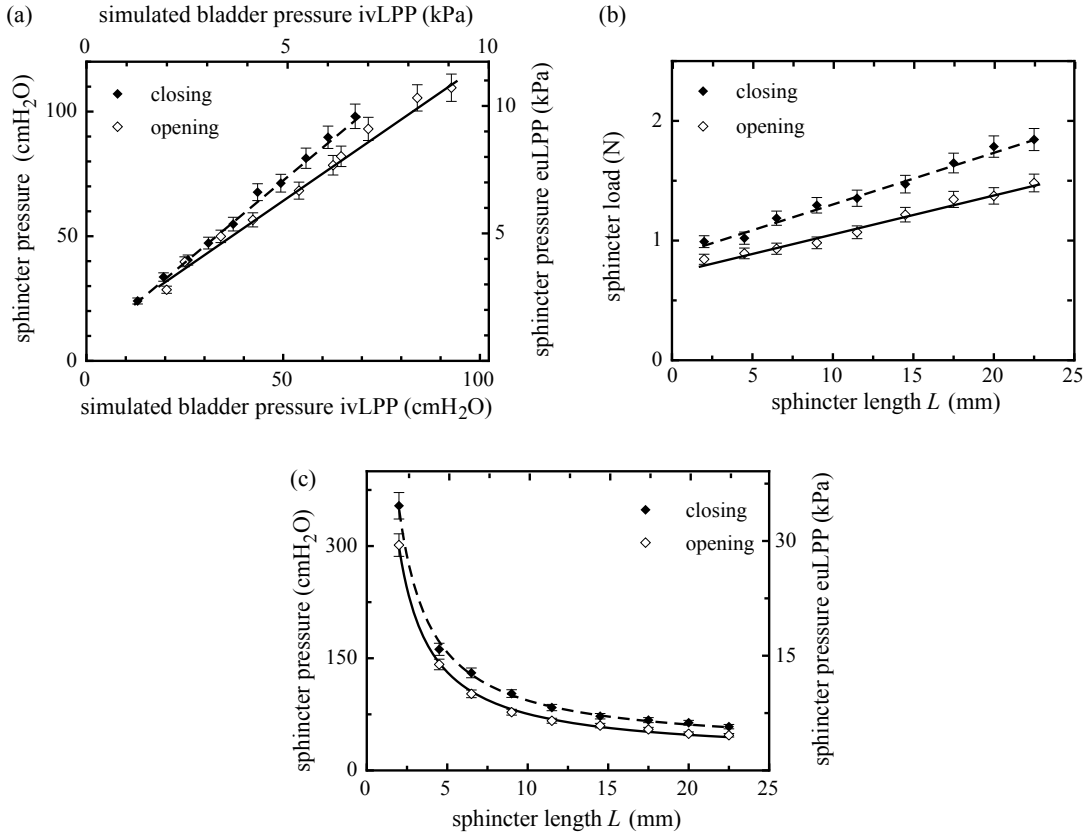

Fig. 4. The experimental data follow the predictions of the compression model: (a) linear dependence of sphincter pressure on bladder pressure; (b) linear dependence of sphincter force on the sphincter length; and (c) inverse proportionality of sphincter pressure versus sphincter length. The solid and dashed lines represent three-dimensional fits with the three independent parameters to the measured data.

intravesical leak-point pressure (ivLPP) – bladder pressure – (cp. Fig. 4a) as well as between the external urethral leak-point force F_{euLPP} and the sphincter length L (Fig. 4b). Furthermore, as shown in Fig. 4c, the euLPP is inversely proportional to the sphincter length L. These experimental findings strongly indicate the applicability of the compression model. Consequently, the acquired data were fitted in the three-dimensional space of the three quantities, euLPP, ivLPP, and L, to extract the three independent parameters of the urethra compression model [10], namely wall pressure, rim force and rim length, with highest achievable precision by means of the software proFit 6.1.10 (Quantumsoft, Uetikon am See, Switzerland), which also yields the error bars accounting for the errors and the distribution of the measured quantities. It is based on the Levenberg-Marquardt algorithm.

The three characteristic parameters p_W, F_R, and L_R for the porcine urethras are listed in Table 2. With those three parameters the required external force of the sphincter can be predicted for any selected bladder pressure [10]. For comparison, the measured parameters for explanted human urethras published [10] are included in Table 2. Note that one obtains different sets of parameters for closing and opening.

The measurements were well reproducible. No significant differences were detected between the measurements on freshly explanted urethras and the ones stored up to three days in 0.9% saline solution at a temperature of 5 to 6°C.

Table 2
Evaluated parameters of the urethra compression model for opening and closing

urethra	state	p_W/cmH_2O	F_R/N	L_R/mm	R/mm
n1	opening	-12.4 ± 0.3	0.21 ± 0.006	1.57 ± 0.07	7
n1	closing	-8.1 ± 0.4	0.21 ± 0.009	3.54 ± 0.09	7
n2	opening	-14.4 ± 0.4	0.17 ± 0.006	3.21 ± 0.06	7
n2	closing	-9.1 ± 0.5	0.22 ± 0.157	3.02 ± 1.80	7
n3	opening	-11.0 ± 0.3	0.11 ± 0.004	1.57 ± 0.06	5.5
n3	closing	-5.2 ± 2.1	0.11 ± 0.031	2.93 ± 0.43	5.5
n4	opening	-6.4 ± 0.4	0.10 ± 0.006	2.70 ± 0.08	5.5
n4	closing	-2.5 ± 0.5	0.13 ± 0.008	2.98 ± 0.11	5.5
n5	opening	-7.7 ± 0.5	0.14 ± 0.065	1.91 ± 1.05	5.5
n5	closing	2.5 ± 0.5	0.02 ± 0.001	5.14 ± 0.11	5.5
n6	opening	-6.3 ± 0.4	0.11 ± 0.007	3.67 ± 0.09	5.5
n6	closing	-5.1 ± 0.6	0.14 ± 0.01	6.56 ± 0.12	5.5
n7	opening	-39.2 ± 0.3	0.38 ± 0.004	-0.57 ± 0.06	5.5
n7	closing	-16.4 ± 0.6	0.31 ± 0.008	4.07 ± 0.12	5.5
n8	opening	-17.9 ± 0.3	0.14 ± 0.004	3.90 ± 0.07	5.5
n8	closing	-10.8 ± 0.5	0.24 ± 0.008	4.66 ± 0.11	5.5
human1	opening	-12.6 ± 0.9	0.10 ± 0.02	2.6 ± 0.3	5.5
human1	closing	-8.1 ± 1.1	0.11 ± 0.02	4.7 ± 0.3	5.5
human2	opening	-13.4 ± 0.9	0.03 ± 0.02	3.3 ± 0.3	5.5
human2	closing	-9.5 ± 1.0	0.09 ± 0.02	4.6 ± 0.3	5.5
human3	opening	-11.7 ± 0.9	0.05 ± 0.02	3.1 ± 0.3	5.5
human3	closing	-8.4 ± 1.1	0.09 ± 0.02	5.9 ± 0.4	5.5

4. Discussion

A recent report [2] about the options of surgical treatment of stress incontinence in men has shown that the situation has been much improved with male sling procedures for mild and moderate stress incontinence, but for the severe form of stress incontinence in men the artificial urinary sphincter remains the treatment of choice. As the complication rate for the AMS 800$^{\mathrm{TM}}$ is still high, further investigations and evaluations of appropriate methods are required. For *in vitro* and *in vivo* testing of alternative approaches a proper animal model is needed, especially for long-term *in vivo* studies. There are anatomical and physiological similarities between human and porcine tissues that include the lower urinary tract and the kidney [9]. Except for size and growth rate, there is a close resemblance between the anatomy and physiology of the domestic pigs and minipigs. A study of the histological properties of ureters of humans and various animals has suggested that the ureters of minipigs are the best model for human ureters, followed by those of domestic pigs [13]. Therefore, the hypothesis that minipigs should be adequate animal models for the test of artificial urinary sphincters sounds reasonable. As the minipigs grow much slower and their organs and tissues alter less with time, the minipigs seem to allow for long-term animal studies to search for potential changes in the artificial sphincter *in vivo* and tissue alterations as a result of sphincter pressure.

The *in vitro* tests of explanted urethras quantified by means of the urethra compression model demonstrate that the female domestic pig is an appropriate animal model mimicking the behavior of the human urethra [10]. The present study validates the hypothesis that the minipig urethras, especially the male ones, can be used likewise for *in vitro* experiments to test the performance of artificial urinary sphincters. Such *in vitro* studies can include dynamic measurements [14] to simulate stress incontinence.

The wall pressure is the most important parameter, because it reflects the stiffness of the urethral tissue. The mean values for opening and closing measurements for male minipig urethras are -1.3 kPa and

-0.84 kPa (-13.4 cmH_2O and -8.6 cmH_2O), respectively. For human urethras the mean p_W-values for opening and closing measurements are -1.35 kPa and -0.84 kPa, respectively, which agree surprisingly well with the values for minipig urethras. As a result of the soft tissue of the female minipig and female domestic pig urethras, the p_W-values tend to fluctuate more than for the male ones. Thus, one may conclude that these urethras are less satisfactory for sphincter tests.

It is important to point out that minipig is not only an adequate animal model for long-term studies in fields such as cardiology [9], dental science [15], and toxicology [16] but also for urology. Previous urodynamic experiments have shown that minipigs can mimic the response of the human bladder to obstruction [17]. The minipig as a model allows for the *in vivo* testing of different sphincter systems for the treatment of urinary incontinence over a long period of time. It enables testing of the functionality of the implant in both normal and stress situations as well as an intensive examination of the tissue changes due to the implant, as the tissue can be extracted and histologically analyzed under a microscope after sacrificing the animal. Because of the evident similarities between human and minipig urethras, the minipig might also be an appropriate model for medical training in artificial sphincter placement.

One should be aware that the data of the *in vitro* study cannot be applied one-to-one to the *in vivo* situation. Potential differences can originate from the absence of blood flow and pressure, temperatures lower than 37°C and changes in humidity. Nevertheless, the main mechanical properties of the urethral tissue *in vitro* seem to resemble the physiological situation *in vivo* as also indicated from preliminary experiments by means of the aspiration method *in vitro* and *in vivo*.

In conclusion, the results of the *in vitro* study based on the *urethra compression model* confirm that the minipig and especially the male minipig represents a suitable animal model for testing artificial urinary sphincters in short-, mid- and long-term trials.

Acknowledgments

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