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Novel injectable urethral bulking agents for the treatment of urinary incontinence

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Stress urinary incontinence is a highly prevalent disorder resulting from weak urethral closure mechanisms. Endoscopic injection of a urethral bulking agent (UBA) under the urethral mucosa increases coaptation, which improves continence. Collagen is an efficient agent, although its effects are limited in time. Other materials still suffer either from a short-lasting effect or migration in distant organs.

We evaluated here novel UBAs using an *ex vivo* model, with respect to criteria of ease of injection, ability to form a high and stable tissue bulking, implant elasticity and tissue reaction. One approach involves solutions of polymers in water-miscible organic solvents that precipitates *in situ*. In this manner, high and stable bulks were routinely obtained using various commercial polymers. Selected solvents reduced the tissue reaction to the implant. Microsphere suspensions in hydrogels also proved to be efficient UBA, although less stable bulks were obtained. Thermosetting chitosan hydrogels showed promising results with respect to bulk stability and isoelasticity with surrounding tissues. Different strategies have thus been compared and optimised *ex vivo*. Further experiments are required to compare the ability of these materials to induce a sustained *in vivo* bulking effect.

Introduction

Stress urinary incontinence results from a weak musculature of the bladder neck and urethral sphincter; it is a highly prevalent disorder in women but also concern men after prostatectomy. Endoscopic injection of an urethral bulking agent (UBA) under the urethral mucosa is an attractive minimally invasive procedure to treat stress urinary incontinence. The bulking agent produces a local tissue elevation which improves mucosal coaptation, hence urethral closure and continence. Collagen has gained acceptance as a safe and effective UBA, despite its short-lasting effect [1]. Various other materials have been proposed such as fat, particles suspensions made of poly(tetrafluoroethylene) [2], silicone [3] or carbon-coated zirconium [4]. However, these agents are not ideal due to short-lasting effect, particle migration [5–10], granuloma formation [5, 11], immunogenicity or volume loss [12]. The ideal material has yet to be developed [13].

We propose here different new strategies to produce urethral implants. These injectable products are: (i) charged latexes that coagulate in presence of physiological fluids, (ii) thermosetting hydrogels, (iii) microspheres suspended in a hydrogel carrier and (iv) solutions of preformed polymers in organic solvents that precipitate when in contact with water-containing tissues. Using an *ex vivo* model, we compared these new UBAs to collagen.

Material and methods

A commercial collagen-based UBA served as a control (Contingen, Bard, UK). The latex was a poly(vinyl acetate) (PVAc) aqueous suspension obtained by emulsion polymerisation of vinyl acetate, and subsequently purified by a two-day dialysis against water [14]. The highest latex concentration (20%) required the addition of 10% Lutrol F68 for stabilisation. The thermosetting hydrogel was based on chitosan and β-glycerophosphate, a compound liquid at room temperature that undergoes physical gelling when submitted to body temperature [15]. Poly(hydroxyethyl methacrylate) (PHEMA) or poly(hydroxyethyl methacrylate-*co*-methyl methacrylate) 20 : 80 (HEMA-co-MMA) microspheres were

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TABLE I Properties of selected urethral bulking agents. Bulk flattening is defined as the ratio of height after 4 h over initial height; n.m. stands for not measurable

UBA	Seringability	Backward flow	Bulking height (mm, mean ± std)	Young's modulus (kPa)	Bulk flattening (%)
Collagen control	2	3	5.8 ± 0.5	12.8	94
Latex PVAc 10%	3	3	4.3 ± 0.3		83
Latex PVAc 20%	3	2	5.0 ± 0	7.1	91
Thermoset chitosan 5% β-GP	2	2.5	7.0 ± 0.2	12.0	100
Thermoset chitosan 10% β-GP	2	1.8	5.5 ± 1.5	n.m.	100
HEMA-*co*-MMA 20 : 80 10% suspension	2.7	3	6.0 ± 0.5	n.m.	77
HEMA 10% suspension	2	2.5	3.8 ± 0.7	n.m.	57
EMA-*co*-MMA in DMSO	3	2	5.3 ± 0.4	21.5	103
EMA-*co*-MMA in NMP	3	2	5.0 ± 0.2	54.3	97
PMMA in GF 75	2	2	5.8 ± 0.3	28.8	96

produced by suspension polymerisation. HEMA was chosen for its biocompatibility with urethral tissue [16]. The microspheres were sieved to retain only those in the 80–120 μm range in order to avoid *in vivo* migration. The microspheres were suspended in different carrier gels or excipients: chitosan, sodium alginate, glycerol, hyaluronic acid or dextran 70.

As for the precipitating polymers, we used commercially available poly(methyl, ethyl or butyl methacrylate) (PMMA, PEMA or PBMA), copolymers of ethyl acrylate and methylmethacrylate (EMA-*co*-MMA), vinyl polymers (polyvinyl acetate) (PVAc), ethylene-*co*-vinyl alcohol (EVAL), and cellulose esters (cellulose acetate, CA, or cellulose acetate butyrate, CAB). The water-miscible organic vehicles have pharmaceutical or veterinary precedence. Dimethyl sulfoxide (DMSO) was used as a reference for its clinical precedence [17, 18], in addition to pharmaceutical excipients showing a reduced toxicity [19]: *n*-methyl pyrrolidone (NMP, ISP Technologies), dimethyl isosorbide (DMI, Uniquema), and Glycofurol 75 (GF75, gift from Roche, Basle, Switzerland).

The *ex vivo* model consisted of female porcine bladders with their urethra. We injected a constant volume (1.5 ml) through a 26-gauge endoscopic needle that is commonly used for submucosal peri-urethral injections. The bladders were immersed in saline at room temperature, or 37 °C for the thermosetting chitosan. In order to evaluate UBA efficiency, we measured the bulk dimensions up to 4 h after injection. We requested low bulk height variations (< 10%), and defined the bulk flattening as the ratio of the bulk height after 4 h over the initial height. We also required an easy injection and a limited backward flow of the polymer solution after needle withdrawal. The ease of injection or seringability was subjectively rated on a 3–0 scale (3: easily injectable, 2: injectable with little effort, 1: difficult to inject, 0: not injectable). Backward flow was rated similarly (3: no flow, 2: small tolerable flow, 1: important flow, 0: important flow leading to bulk flattening). The retrieved implants were examined by scanning electron microscopy, their elastic modulus was measured as the slope of the stress–strain curve for strains smaller than 10%. Following a 4-h incubation in an organ preservation solution (low-potassium dextran), selected formulations were submitted to histological examination.

Results

PVAc latex were easily injectable due to their low viscosity. Latex concentration of 10% did not lead to very stable bulks (83% of the initial height after 4 h, Table I) whereas 20% resulted in a more stable tissue elevation (91%). The bulks appeared as soft whitish mass (Fig. 1), with an elastic modulus of 7.1 kPa and dimensions comparable to collagen. However, the retrieved implants were also brittle, which would exclude them from clinical use.

Chitosan thermosetting gels were easily injectable and produced high bulks which were translucent (Fig. 1) due to the high water content. The β-glycerophosphate concentration impacted on the UBA performances, 5% being preferable for seringability and bulking effect (Table I). No bulk flattening was noticed. Elastic hydrogel implants were obtained (elastic modulus = 12 kPa).

To be injectable, the microspheres were suspended in a carrier viscous solution. We tested in preliminary

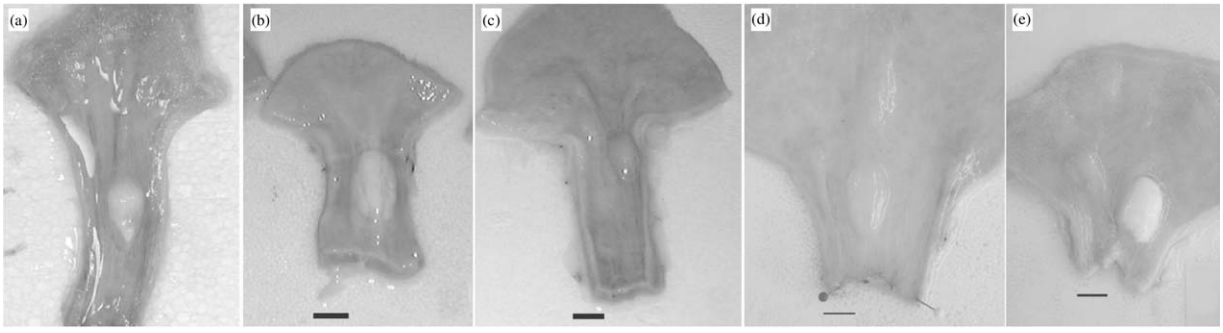

Figure 1 Macroscopic aspect of the urethras injected with (a) the collagen control, (b) a solution of EMA-*co*-MMA in NMP (c) a suspension of poly(HEMA-*co*-MMA) microspheres, (d) a thermosetting chitosan and (e) a PVAc latex. Scale bar = 1 cm.

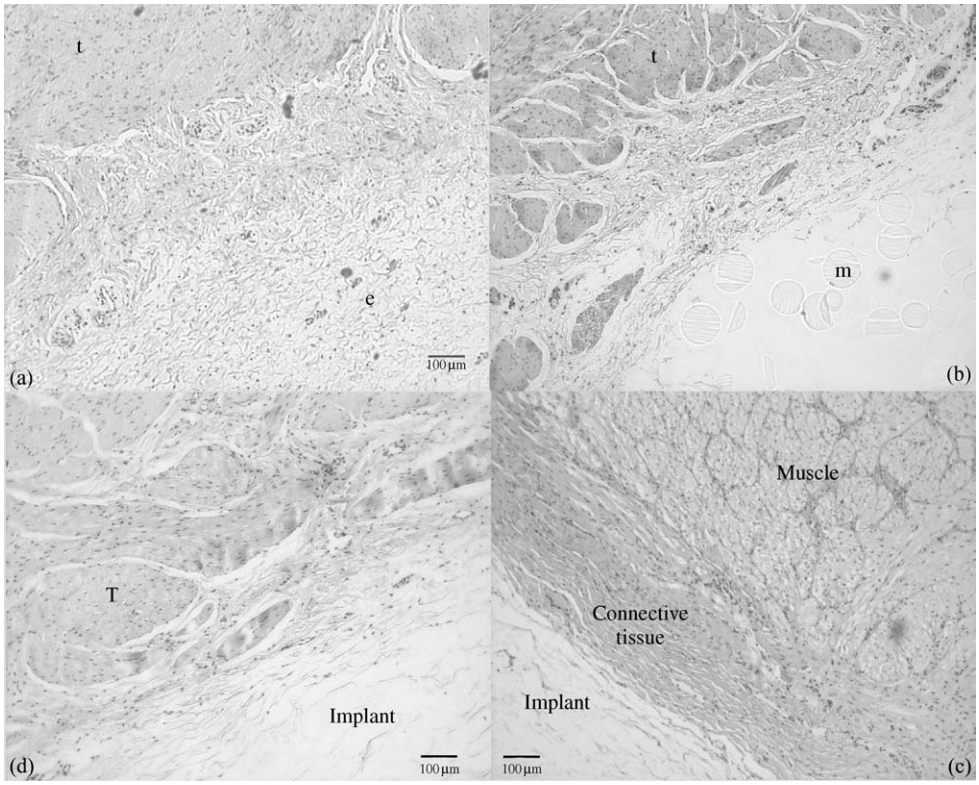

Figure 2 Histological sections stained with haematoxylin–eosin of: (a) a saline control, (b) a suspension of poly(HEMA-*co*-MMA) microspheres in dextran, precipitating polymers based on (c) cellulose acetate butyrate in DMSO and (d) EMA-*co*-MMA in NMP. Black arrows indicates a microsphere in (b). Scale bar = 100 µm.

experiments various solutions at different concentrations, based on sodium alginate, hyaluronic acid, chitosan, dextran 70 and glycerol. We selected an aqueous dextran solution with a concentration of 20% for its seringability, its ability to create high bulks over 4 h and to undergo autoclaving sterilisation without noticeable viscosity decrease. In addition, dextran is a well-documented pharmaceutical excipient devoid of adverse reactions. Suspension of either 10% microspheres made of HEMA-*co*-MMA (20 : 80) or made of pure PHEMA produced high tissue elevations comparable to collagen. Higher concentrations could not be injected through the 26-gauge endoscopic needle. Bulk flattening was noticeable (typically ranging from 60 to 80%) with these suspensions. No elasticity could be measured since the UBA remained viscous following injection.

The precipitating polymer solutions, once injected, produced microporous foams with pore size ranging from 10 to 100 µm. Various formulations resulted in efficient UBAs: PMMA in DMSO, DMI or GF 75; EMA-*co*-MMA copolymer in DMSO, NMP or GF 75 as well as EVAL and CAB in DMSO produced stable bulks comparable to the collagen control. A distinctive feature of the precipitating polymer solutions is the absence of bulk flattening after 4 h. The elastic modulus was slightly higher than with the aqueous-based solutions. It remained however in the 20–50 kPa range, still comparable to urethral tissue (13 kPa). In addition, we have shown that these implants did not loose their elastic properties after one year in saline (data not shown).

In order to assess possible tissue damage related to the presence of the organic vehicle, we carried out histological analysis of selected precipitating polymer solutions, using saline and microsphere suspensions as controls. Saline injection induced an oedema (e) that preserved normal subepithelial tissues (*t*, Fig. 2(a)), and suspension of poly(HEMA-*co*-MMA) in dextran did not either induce subepithelial tissue damage (Fig. 2(b)). Using DMSO-based solutions, some subepithelial tissue swelling was observed (Fig. 2(c)), whereas NMP, GF 75 and DMI seemed to preserve the tissues around the implants (Fig. 2(d)). Although thinner mucosae were generally observed around these implants, no mucosa rupture was seen.

Discussion

Despite significant advances in the field of urethral implants, current bulking agents still suffer from either a too short effect or migration of particles in distant organs. We evaluated here different new strategies for urethral bulking using a simple *ex vivo* model that allowed to select promising agents. The implants resulting form precipitating polymer solutions were generally stiffer than those obtained with aqueous-based implants (hydrogels, chitosan, latex, microsphere suspensions) and were shown to keep their elastic modulus over one year *in vitro*. In addition, they formed cohesive foams as opposed to the brittle material obtained with PVAc latexes. Permanent, biostable implants created by injection of precipitating polymers may therefore be good candidates to induce a sustained bulking effect. Our histological study indicated that pharmaceutical excipients such as DMI, GF 75 or NMP may be a valid alternative to the previously proposed DMSO [20] in order to decrease the short-term local reaction to the solvent. Similar implants based on NMP were shown to

be biocompatible when injected subcutaneously or intramuscularly [21].

Permanent implants may also induce a chronic, local reaction. Chronic granulomatous reaction of the giant cell type are known to contribute to the therapeutic success of implants for urinary incontinence [22], although this reaction may not be controlled on the long term and lead to implant failure [5, 11]. Biodegradable materials may therefore be of interest, more specifically if they induce and sustain the formation of new tissue. Chitosan is a biodegradable material of interest for bulking the urethra, since it is known for its wound healing properties and its stimulation of connective tissue formation [23]. Similarly, particle suspensions contain only a few percent of permanent materials that are expected to form new collagen and subsequent tissue bulking. The initial flattening of the bulk, that was attributed to the diffusion of the gel carrier, may be reduced *in vivo* due to local tissue reactions. The different strategies evaluated herein have been shown to induce short-term bulking effects on an *ex vivo* model. Their long-term fate *in vivo* has still to be confirmed in animal models.

Conclusion

Different strategies for urethral bulking were evaluated using a simple *ex vivo* approach. Solutions of preformed polymers in organic solvents resulted in permanent, porous implants. Thermosetting hydrogels and microsphere suspensions did also lead to efficient bulking, although their long-term fate *in vivo* will depend on their ability to induce a substantial long-lasting tissue proliferation. Further *in vivo* experiments will be dedicated to this issue. Novel formulations may induce a durable bulking effect, paving the way to clinically efficient agents.

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