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Gelatin-based biodegradable ureteral stents with enhanced mechanical properties

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ABSTRACT

A first generation of biodegradable ureteral stents based on natural origin polymers developed in a previous work has proven to be an interesting alternative to conventional stents, but it has however demonstrated to fail upon the first *in vivo* validation in a pig model. In this work, with the objective to overcome the low mechanical performance encountered and to make the biodegradable ureteral stents by origin polymers a success *in vivo*, four formulations with different concentrations of gelatin and alginate and different concentrations of crosslinking agent were tested in order to obtain higher mechanical properties. Bismuth was added to confer radiopaque properties to the stent. Not only a new formulation was developed but also the processing method to fabricate the stents was optimized. The biodegradable ureteral stents were coated with a biodegradable polymer. X-ray scan demonstrated the radiopacity of this second generation of biodegradable stents. The degradation of the biodegradable ureteral stents was assessed in artificial urine solution and it was observed that the degradation of the materials occurs *in vitro* between 9 and 15 days. Degradation was followed by weight loss of the samples and by chemical analysis of the solutions both by inductive couple plasma (ICP) and gel permeation chromatography (GPC). Formulation with highest amount of gelatin has shown good mechanical performance in terms of tensile properties when compared with the commercial stent (Biosoft® duo, Porges, Coloplast), and the crosslinking concentration has shown not to have a great influence on the mechanical behavior of the stents. The *in vivo* performance of this second-generation of the ureteral stents was herein validated. The biodegradable ureteral stents were placed in the ureters of a female pig, following the normal surgical procedure. The animals remained asymptomatic, with normal urine flow, the stents remain intact during the first 3 days and after 10 days the ureteral stents were totally degraded. This new formulation combined with a new production process overcomes the problems verified with the first generation of natural-based biodegradable stents.

1. Introduction

The most frequent adverse effects reported by patients experiencing ureteral stenting are pain and difficulties in urinary tract [1]. These problems can significantly impact patient quality of life with loss days of working, urinary leakage and sexual difficulties [2]. In last years, new ureteral stent designs have been tested with novel polymers, coatings and the incorporation of active compounds in an attempt to significantly reduce the most common problems like bacterial infection and encrustation [2–4]. Lange et al. [1] in a recent review concluded that the stent of the future will be degradable, in a control manner, and possible to coat or elute active compounds. No biodegradable ureteral stent is currently available on the market, although in the past years there has been a crescent interest in this field [1]. Polymers like polylactic acid, polyglycolic acid, poly(lactic-co-glycolic acid) and alginate-based materials have been used to develop the biodegradable ureteral stents [5–9]. Lumiaho et al. reported an *in vivo* study in pig model using polylactic acid and poly(lactic-co-glycolic acid) based stents which have shown good properties like antireflux properties and favorable drainage but the biocompatibility and

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the degradation profile were proven to be insufficient for clinical use [5,10,11]. The same ureteral stents showed a different behavior in a canine model, presenting a good biocompatibility and degradation which occurred in 12 weeks [12]. Other studies using poly(lactic-co-glycolic acid)-based ureteral stents reported favorable radiopaque and drainage properties, but the biocompatibility was compromised, according to what is reported in the literature [5,13–15]. The degradation of the ureteral stents must be uniform and homogenous or dissolving based on directionality, preventing the formation of fragments during the degradation process that can block the ureter [1,6,16]. Uriprene stent (Poly-Med, USA), a radiopaque, glycolic–lactic acid based stent has been designed to degrade in the direction of the bladder coil to renal coil preventing ureteral obstruction secondary to degrading stent fragments [1]. The *in vivo* pig model studies of Uriprene reported a good stability and biocompatibility, with a predictable degradation during 2–4 weeks while maintaining drainage. In our previous study, we reported an ureteral stent produced with natural based polymers processed by critical point drying with carbon dioxide [16]. This study was however not the first in literature to report alginate-polymer-based temporary ureteral stents. Lingeman et al. [9,17] showed in phase I and phase II clinical trials that these ureteral stents were designed to be intact at least 48 h before degradation with facilitated urinary drainage, favorable tolerability and safety profiles. The problem of these alginate-based stents is the fact that it presented a nonhomogeneous dissolution profile and fragmentation resulting in the need for secondary procedures to remove fragments in some patients.

To avoid these problems, we hypothesized the use of two biodegradable materials instead of one, with the objective to reinforce the mechanical properties of the stent [18]. Additionally, the combination of template gelation with critical point carbon dioxide drying also contributes to enhance the features of the stent. In the first generation biodegradable ureteral stents were produced using alginate, gellan gum and a blend of these with gelatin. The bacterial adhesion of Gram-positive and Gram-negative was assessed and compared with a commercial stent (Biosoft® duo, Porges, Coloplast) showing a decrease of adhesion. The biodegradation profile was observed to be highly dependent on the composition of the stent, with a complete dissolution of alginate-based during 14 days and the gellan gum-based up to 60 days [16]. A first generation of biodegradable ureteral stents based on natural origin polymers developed previously has proven to be an interesting alternative, but it has however demonstrated to have mechanical properties upon the first *in vivo* validation. Following these results, we developed a second generation of these ureteral stents. Gelatin was used as a base material for these stents and a hydrophobic coating was applied to improve the mechanical properties and allow the placement of the stent *in vivo* by the conventional surgical procedure. A preliminary *in vivo* validation was performed in a pig model.

2. Materials and experimental

2.1. Materials

Alginic acid sodium salt, gelatin, urea, urease type IX from Canavalia ensiformis (Jack Bean), calcium chloride, chloroform, ethanol, bismuth (III) carbonate basic, sodium phosphate dibasic and sodium azide were purchased from Sigma–Aldrich (Germany). Potassium dihydrogen ortho-phosphate and magnesium chloride hexahydrate were obtained from Riedel-de Haën (Germany). Bismuth standard for ICP was obtained from Sigma–Aldrich (Germany). Polycaprolactone resin PCL 787, commercially available as TONETM polymer, was obtained from Union Carbide Chemicals and Plastics Division, Bound Brook, New Jersey. Carbon dioxide (99.998 mol%) was supplied by Air Liquide (Portugal). All reagents were used as received without any further purification.

Table 1
Summary of the formulations tested to prepare the different biodegradable ureteral stents.

Formulation	Material conc. (wt. %)			
	1	2	3	4
Alginate	10	30	45	50
Gelatin	85	65	50	45
Bismuth (III) carbonate basic	5	5	5	5
Coating	10% PCL resin PCL 787			

2.2. Preparation of second generation of biodegradable ureteral stents

Polymers were dissolved in hot distilled water (70 °C) at different concentrations as described in Table 1. The solutions were stirred for 1 h and the polymeric solution was injected in a mold to obtain a tubular structure. After 1 h the piece was taken out of the mold and placed in an alcohol solution (100% ethanol) for 1 h. The stents were then transferred into a crosslinking solution of calcium chloride ($CaCl_2$), with different concentrations (Table 2) at room temperature. After crosslinking, the stents were relocated in an alcoholic solution (100% ethanol) to obtain an alcohol gel which can be dried in a high-pressure vessel with supercritical carbon dioxide ($scCO_2$) under controlled pressure (100 bar) and temperature (40 °C) and a continuous flow of the $scCO_2$ during 90 min. Finally, the dried stents were immersed in distilled water for 30 min and in ethanol 100%, for 1 h, to remove the template. The stents were finally dried at room temperature conditions, during 1 day. The coating of the stents was performed by immersion in a 10% of polycaprolactone (PCL) resin 787 (Mw 80,000 g mol^{-1}) dissolved in chloroform. The stents were dried at ambient conditions overnight. Commercial Biosoft® duo, Porges, Coloplast used as a control in this study is also shown.

2.3. Scanning electron microscopy

The morphology of the biodegradable stents was analyzed on a JEOL SEM, model JSM-6010LV. The samples were fixed with mutual conductive adhesive tape on aluminum stubs and covered with gold/palladium using a sputter coater.

2.4. Postoperative X-ray

The radiopaque characteristics of the biodegradable ureteral stent developed were evaluated in a postoperative X-ray equipment located at the Department of Imaging Hospital de Braga, Portugal. The radiographs were taken in abdomen mode with magnification of 0.27×.

2.5. Degradation study

The degradation of biodegradable stents was measured as function of the weight loss of the samples. Samples (10 mg) were

Table 2
Crosslinking agent concentrations used to prepare the different biodegradable ureteral stents.

		Crosslinking agent conc. (M)		
Crosslinking agent	$CaCl_2$	0.24[a]	0.48[b]	1[a]

[a] Formulation 2.
[b] Formulation 1, 2, 3 and 4.