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Gelatin-based biodegradable ureteral stents with enhanced mechanical properties

Alexandre A. Barros[a,b], Carlos Oliveira[b,c], Estevão Lima[b,c], Ana Rita C. Duarte[a,b,*], Rui L. Reis[a,b]

[a] 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4805-017 Barco, Guimarães, Portugal
[b] ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal
[c] Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal

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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

* Corresponding author at: 3B's Research Group – Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4805-017 Barco, Guimarães, Portugal.
E-mail address: aduarte@dep.uminho.pt (A.R.C. Duarte).

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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.

Table 3
Composition of the artificial urine solution (AUS).

	Component	% wt/v
Solution A	Potassium dihydrogen ortho-phosphate	0.76
	Magnesium chloride hexahydrate	0.36
	Urea	1.60
Solution B	Calcium chloride hexahydratate	0.53
	Chicken ovalbumin	0.2
Urease type IX from *Canavalia ensiformis* (Jack Bean)		0.125

immersed in artificial urine solution (AUS) prepared according to Khandwekar et al. [19] with the composition presented in Table 3. Samples immersed were dried and weighed to determine the weight loss, which was calculated according to the following equation:

$$\%\ \text{Weight loss} = \frac{(W_f - W_i)}{W_i} * 100 \quad (1)$$

where W_f is the final weight of the sample (dried after immersion) and W_i is the initial weight of the sample. Each formulation was tested in triplicate.

2.5.1. Gel permeation chromatography (GPC)

5 mg of alginate, gelatin and bismuth were dissolved in 5 ml of an aqueous solution of sodium phosphate dibasic 0.01 M containing 0.1 M of sodium azide (pH 6.6) and used as controls, while the immersion solutions obtained by degradation test of stents formulation 2 at specific time point (1, 3, 6 and 9 days) were lyophilized and then dissolved in 5 ml of the same eluent. The solutions were filtered through a 0.22 μm filter and analyzed on a gel permeation chromatograph (Malvern, Viscotek TDA 305) with refractometer, right angle light scattering and viscometer detectors on a set of four columns: pre-column Suprema 5 μm 8 × 50 S/N 3111265, Suprema 30 Å 5 μm 8 × 300 S/N 3112751, Suprema 1000 Å 5 μm 8 × 300 S/N 3112851 PL and Aquagel-OH MIXED 8 μm 7.5 × 300 S/N 8M-AOHMIX-46-51, with refractive index detection (RI-Detector 8110, Bischoff). Elution was performed at 30 °C using a flow rate of 1 ml min^{-1}. The elution times and the RI detector signal were calibrated with a commercial calibration polysaccharide set from Varian that contains 10 Pullulan calibrants with narrow polydispersity and Mp (molecular mass at the peak maximum) ranging from 180 Da to 708 kDa.

2.5.2. Inductively coupled plasma (ICP)

The immersion solutions from the degradation test of the stents, formulation 2, were filtered and analyzed by inductively coupled plasma (ICP) to follow Bismuth (BI) concentration during the different degradation times. The sample absorption at specific wavelengths (k = 206.17 nm for Bi) was measured, and the bismuth concentration was determined using calibration curves previously obtained with Bismuth standard for ICP (Sigma) (R^2 = 0.96).

2.5.3. Cytotoxicity evaluation of the leachables

The cytotoxicity of the leachable materials during the ureteral stent degradation in AUS was accessed according to ISO/10993 [20]. The cytotoxicity of the samples was assessed using an immortalized mouse lung fibroblasts cell line (L929) purchased from the European Collection of Cell Cultures. First, the immersion solutions obtained by degradation test at specific time point (1, 3, 6 and 9 days) of stents formulation 2 were lyophilized. The leachables were dissolved in basal medium DMEM (Dulbecco's modified Eagle's medium; Sigma–Aldrich, Germany) 10% FBS (heat-inactivated fetal bovine serum, Biochrom AG, Germany), and 1% antibiotic-antimycotic (Gibco, UK). Cells were cultured in a humidified incubator at 37 °C in a 5% CO_2 atmosphere. The effect of the leachables on the cellular metabolism was performed using a standard MTS (Cell Titer 96® Aqueous Solution Cell Proliferation Assay, Promega, USA) viability test. A latex rubber extract was used as positive control for cell death; while cell culture medium was used as negative control representing the ideal situation for cell proliferation. Cell viability was evaluated by the MTS assay after 72 h. This was quantified by UV-spectroscopy, reading the formazan absorbance at 490 nm in a microplate reader (Synergy HT, Bio-Tek Instruments, USA). Each sample formulation and control was tested using 12 replicates.

2.6. Tensile mechanical analysis

Tensile mechanical analysis of the biodegradable stents was evaluated using an INSTRON 5540 (Instron Int. Ltd, High Wycombe, UK) universal testing machine with a load cell of 1 kN. The wet samples were hydrated before testing in AUS for 4 h. The dimensions of the specimens used were 5 mm of length, 2 mm width, and 0.5 mm of thickness. The load was placed midway between the supports with a span (L) of 3 mm. The crosshead speed was 1:5 mm min^{-1}. For each condition the specimens were loaded until core break. The results presented are the average of at least three specimens and the results are presented as the average ± standard deviation.

2.7. Surgical procedure and in vivo placement validation

The *in vivo* placement validation study was conducted at Minho University, Braga, Portugal, after formal approval by the institution's review board and in accordance with its internal ethical protocol for animal experiments. Female domestic pigs, weighing ≈30 kg, were used to validate the procedure and the stent degradation. The pigs were not given food or water for 12 h before the procedure. All procedures were performed under general anesthesia and mechanical ventilation as previously described in detail [21,22]. After emptying the bladder, a semi rigid 7 Fr ureteroscope (Karl Storz, Tuttlingen, Germany) was inserted through the urethra and saline solution was instilled. The full procedure was according to the standard technique of ureteroscopy. A 0.035-in. flexible tip guidewire (AQUATRACK® Hydrophilic Nitinol, Cordis®, Johnson & Johnson) was then inserted in the ureters. The biodegradable ureteral stents (6 Fr with 22 cm length) were guided by the guidewire until placed in the right and in the left ureter the commercial stent (Biosoft® duo, Porges, Coloplast) was placed as a control. Conventional ureteroscopy was performed in order to verify the degradation and the presence of any fragment and the morphology of the ureters after 3 and 10 days.

2.8. Statistical analysis

All data values are presented as mean ± standard deviation (SD). Statistical analysis was performed using Graph Pad Prism 6.00 software (San Diego, USA). Statistical significances ($^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$) were determined using one-way analysis of variance (ANOVA) for an average of three to twelve replicates, followed by *post hoc* Tukey's test for all pair-wise mean comparisons.

3. Results and discussion

In our previous work we developed a biodegradable ureteral stent based on different natural polymers. One of the drawbacks of these stents was the poor mechanical properties that result in failure upon *in vivo* implantation.

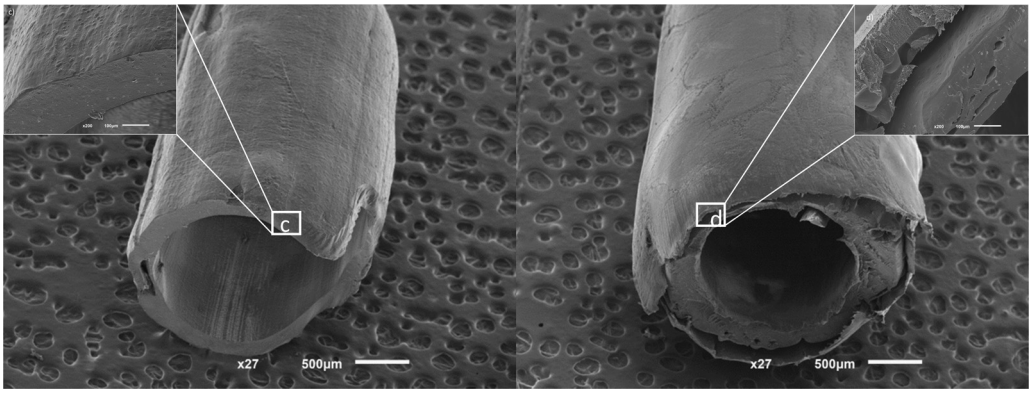

Fig. 1. SEM micrographs of the biodegradable ureteral stent (6 Fr, formulation 2, 0.48 M): (a) before coating, (b) after coating, (c) higher magnificence of one-layer hydrogel and (d) higher magnificence of two layers coating and hydrogel.

To prepare the second generation of biodegradable ureteral stents made by origin polymers new formulations were tested and the method of injection molding and drying was optimized. Gelatin and alginate are very hydrophilic polymers. In order to delay the hydration of the materials upon implantation we decided to coat the hydrogel with a polymeric layer of polycaprolactone resin PCL 787. Polycaprolactone resin PCL 787 was chosen as it is a safe material and has a fast degradation in comparison with other biodegradable polymers. The biodegradable ureteral stents are prepared from an initial aqueous solution of alginate-gelatin from which gelation is induced by decreasing the temperature followed by an ionic crosslinking with a $CaCl_2$ solution. Gelatin and alginate were chosen because of their versatility to form gels and the results obtained in the previous study [16] combining gelatin with other polysaccharides it is possible to induce changes in the water uptake, degradation profile and particularly were benefices regarding bacteria adhesion. In this work we have added bismuth to the formulation. The use of bismuth in the new formulation provides radiopaque properties to the ureteral stent due the inherent radiopaque characteristics of this compound. This material was already used and proved to be safe and it is already FDA approved [23]. After crosslinking a combination of steps in ethanol and supercritical carbon dioxide was further employed to dry the biodegradable ureteral stents. Supercritical drying process parameters were kept as in the first version of these stents as they had already been optimized supercritical fluid drying process used is a process in which the matrices do not undergo any phase transition and therefore the integrity of the lumen of the stents is not compromised [24]. Different other drying methods were tested, namely air drying but the integrity of the lumen of the stents was compromised, unlike what was observed when using supercritical fluid CO_2.

3.1. Morphology

Fig. 1 presents the SEM images of the cross-sections of biodegradable ureteral stent developed according to the formulation 2. In Fig. 1a we can see the uncoated stent and in Fig. 1b the stent with PCL coating. Fig. 1c and d are the magnifications of stent wall. It is possible to distinguish the two layers, outer layer from PCL coating and the inner layer the alginate-gelatin plus bismuth matrix. From the SEM images we can observe a poor interfacial bonding between the polymers (alginate/gelatin and PCL 787). The inner diameter, *i.e.* the lumen of the stent is 2 mm. The inner and outer diameters and the length of the stents are only dependent on the injection mold used to prepare them, and do not depend on the formulation tested. Like in the first-generation of the biodegradable ureteral stents the surface obtained without coating is similar [16].

3.2. X-ray validation

An important feature of the ureteral stents is its radiopacity. The possibility to assess by postoperative X-ray, localize the stent in the body and follow the degradation during time is of major importance and for this we used a standardized product, namely bismuth (III) carbonate basic, however, others can be used. In Fig. 2 it is possible to confirm the radiopacicity, in wet state, of the biodegradable ureteral stent developed (Fig. 2b) in comparison with commercial stent (Fig. 2a). In this work we used a lower concentration of this compound in the formulation as compared to the Lingeman et al. [23], demonstrating that low amounts are suitable to provide this feature to the stent.

3.3. In vitro *degradation study*

The *in vitro* degradation of the biodegradable ureteral stents with the different formulations and different concentrations of crosslinking agent was assessed measuring the weight loss of the samples. The weight loss, measured as the percentage of mass lost when immersed in AUS for a predetermined time period is presented in Fig. 3. All the conditions tested demonstrated *in vitro* that no degradation occurs during the first 3 days of immersion. After 9 days the stents have shown complete degradation. Comparing the different formulations tested, the results suggest that

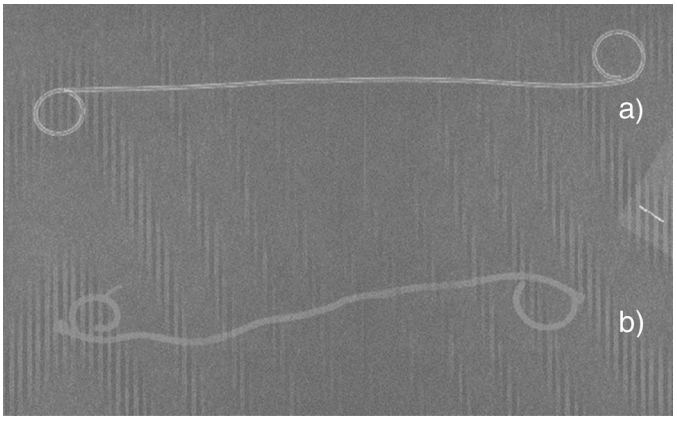

Fig. 2. Radiograph in abdomen mode of (a) commercial ureteral stent (Biosoft® duo, Porges, Coloplast) and (b) biodegradable ureteral stent developed (formulation 2, 0.48 M).

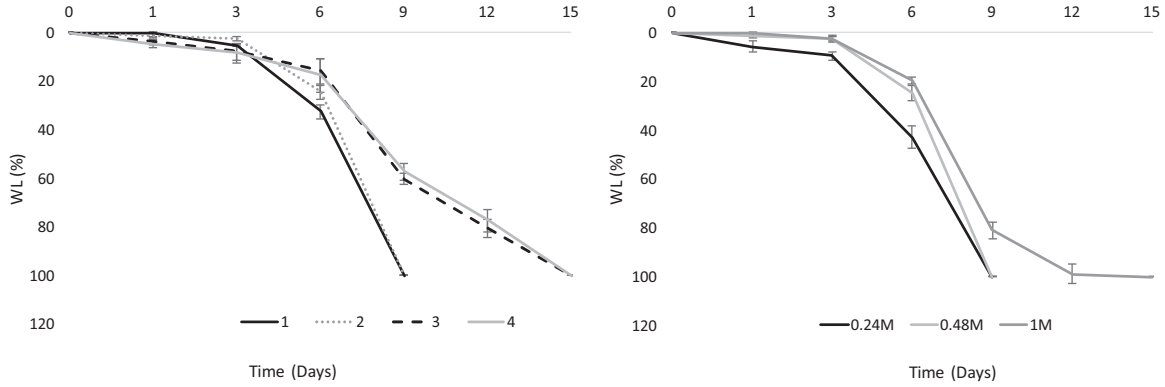

Fig. 3. Weight loss of developed biodegradable ureteral stents: (a) different formulations tests and (b) formulation 2 with different crosslinking concentration.

higher concentration of alginate (formulations 4 and 5, Fig. 3a) increases the degradation time. Comparing the different concentrations of crosslinking agent (Fig. 3b) the results show that stronger cross-linking lower is the degradation even though not statistically significant. This can be justified due to the presence of more calcium crosslinks with guluronic acid (G) blocks, increasing their covalently cross-linked network [25]. The divalent cations of the ionic crosslinking agent, bind exclusively to the G-blocks of the adjacent alginate chains, since the structure of the L-guluronate offers a greater flexibility than the D-mannuronate chains. By creating ionic inter-chain bridges, divalent ions replace the hydrogen bonds between the carboxyl group of D-mannuronate and the 2-OH and 3-OH groups of the subsequent L-guluronate, originating the gelation of aqueous alginate solutions [26,27]. The G-block length, concentration of polymer and molecular weight are thus critical factors affecting the physical properties of alginate and its resultant degradation. On the other hand, gelatin can form hydrogels by increasing and decreasing temperature, which is merely a physical crosslinking phenomena. The mechanism behind the crosslinking of gelatin molecules is a conformational change from a random coil to a triple helix. The degradation occurs then because the non-covalent associations are easily disrupted at temperatures higher than 30–35 °C, and therefore at body temperature [28]. This helps to understand that with higher amounts of gelatin in the formulation a faster degradation will take place. In our previous study, the alginate-based ureteral stents showed a slower degradation comparing with this work, for the same reason [16]. The polymer blend with the alginate is however unknown and hence a work correlation is difficult to establish compared with our formulation.

3.4. Gel permeation chromatography (GPC)

The polymeric leachables from the ureteral stent degradation at 1, 3, 6 and 9 days were first lyophilized and then dissolved in an appropriate eluent to be analyzed by GPC. As a control the raw materials alginate and gelatin was injected. GPC pattern of alginate and gelatin shows an overlap of the eluting peaks between 18 ml and 21 ml of retention volume and hence it is not easy to distinguish both. The leachables are composed essentially by the mixture of alginate and gelatin present in the biodegradable ureteral stent formulation. The overlap of the raw materials makes it difficult to identify separately the presence of the alginate and gelatin. However, it is possible to see an increasing intensity of the peaks on the elution curve with degradation time. Considering the retention volume of the peaks on the different elution curves, we observe a major contribution of gelatin instead of alginate. This was expected because this formulation (formulation 2) is composed of 65% gelatin and 30% alginate (Fig. 4).

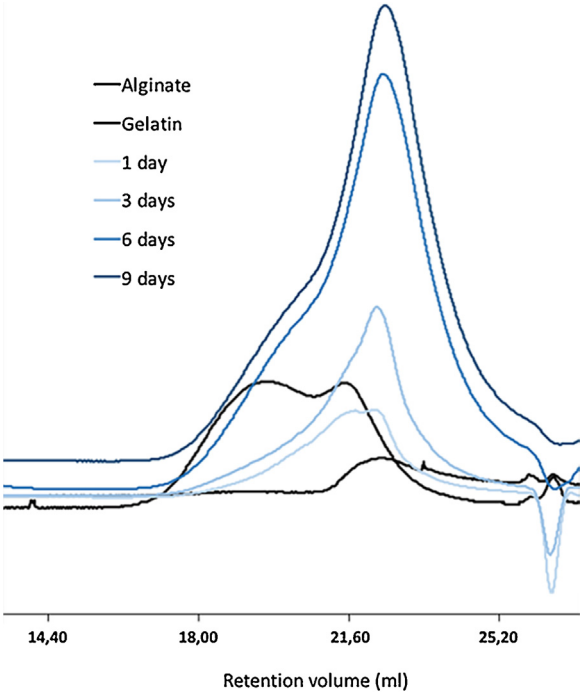

Fig. 4. GPC chromatograms of stent raw materials (alginate and gelatin) and the leachables at 1, 3, 6 and 9 days' time points.

3.5. Inductively couple plasma (ICP)

The ICP analysis of bismuth concentration in the immersions solutions from different time points from formulation 2 is presented in Table 4. The results show a gradual release of bismuth during the degradation process from the stent to the artificial urine solution. According to the degradation profile (Fig. 3a) of ureteral stents, formulation 2, and the bismuth measured in the immersion solutions we can see that the release of bismuth is associated with the degradation and it does not occur due to swelling of the stent or diffusion from the stent to the AUS. To support this observation

Table 4
Concentration of bismuth obtained by ICP, in immersion solution (AUS) during the degradation.

Days	Bismuth (g/L)	Std	Release (%)
1	0.0570	0.0058	~1%
3	0.271	0.0496	~5%
6	1.285	0.06	~20%
9	5.953	0.1912	100%

and considering a homogenous distribution of bismuth in the stent, it would be expected to have a correlation between the degradation profile and the amount of bismuth in solution. On day 3 the ureteral stent with formulation 2 presents a degradation around 5%, corresponding the value of bismuth in solution is 0.271 g/L, that is approximately 5% of the total bismuth present in the stent. The same is observed at time point day 6 in which the value 1.285 g/L is 20% of the total bismuth and again is close to the value of the degradation observed in Fig. 3a.

3.6. Leachables cytotoxicity

The cytotoxicity of the leachables obtained from stent degradation was evaluated in accordance with the protocol described in ISO/EN 10.993 [20]. The viability of the cells cultured in a tissue culture plate, in the presence of the leachables, was determined as a function of the cells cultured in Dulbecco's modified Eagle medium (DMEM) culture medium. Fig. 5 presents the cell viability after 72 h in contact with the material dissolved in the culture medium. Significant differences were observed for the cell viability in the presence of the leachables in comparison with the latex, which was used as a positive control. The results demonstrate that there is no toxic interaction between the leachables from day 1 to day 9 and L929 cells.

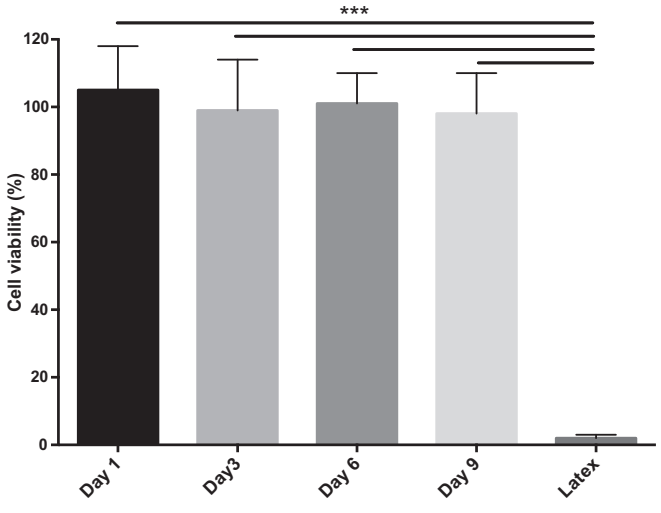

Fig. 5. Cytotoxicity study by cell viability measured after 72 h.

3.7. Tensile mechanical tests

The tensile mechanical properties like maximum load (N), maximum tensile strain (%) and Young's modulus (MPa) of

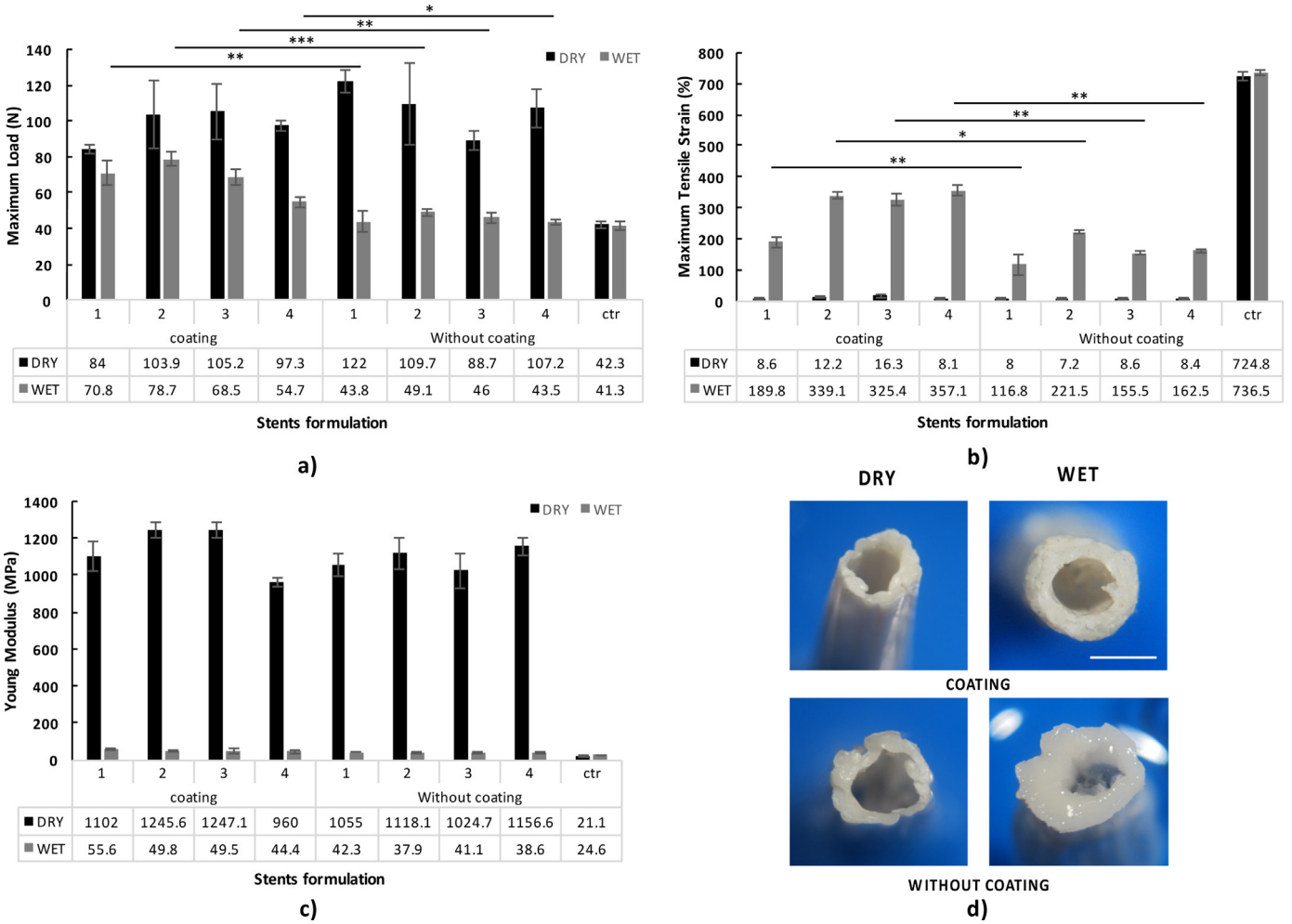

Fig. 6. Mechanical properties of the biodegradable stents (0.48 M crosslinking concentration) before and after PCL coating in terms of (a) maximum load (N), (b) maximum tensile strain (%) and (c) young modulus (MPa). (d) Images of biodegradable stents before and after coating in dry state and in wet state immersion in AUS (scale bar 2 mm). ctr – (Biosoft® duo, Porges, Coloplast). Values are represented as average ± SD, $n = 3$. Statistical differences ($^{*}p < 0.05$, $^{**}p < 0.01$) using one way-ANOVA followed by a Tukey post-test.

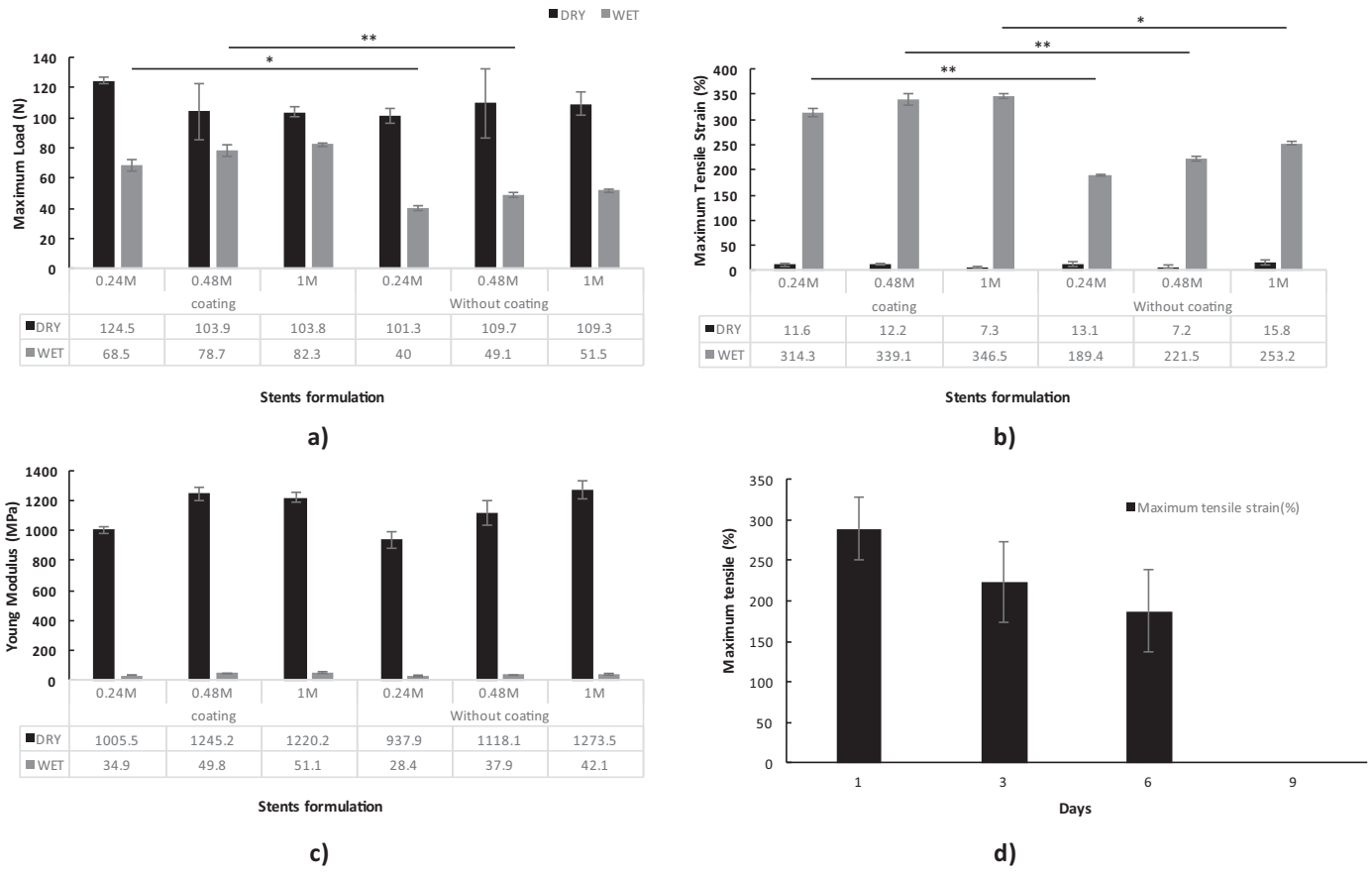

Fig. 7. Mechanical properties of the biodegradable stents prepared with the formulation 2 with different concentrations of crosslinking agent before and after PCL coating in terms of (a) maximum load (N), (b) maximum tensile strain (%), (c) young modulus (MPa) and (d) maximum tensile strain (%) of ureteral stent formulation 2 during the degradation time. Values are represented as average ± SD, $n = 3$. Statistical differences ($^{*}p < 0.05$, $^{**}p < 0.01$) using one way-ANOVA followed by a Tukey post-test.

the biodegradable ureteral stents developed are presented in Figs. 6 and 7. Fig. 6 presents the results in dry and wet state of the four different formulations of stents when using a concentration of 0.48 M crosslinking agent. As a control the tensile results for the commercial stent (Biosoft® duo, Porges, Coloplast) are also presented. Comparing all studied formulations, significant differences were observed before and after hydration and with and without coating, in terms their mechanical properties. In all formulations and as expected of hydration in AUS (Fig. 6d) the values of tensile properties decrease in terms of Young modulus but increase in terms of maximum tensile strain. Furthermore, the ureteral stents after hydration become more elastic than in dry state. The results for the hydrated samples are far more important for the clinical purpose, i.e. if complications occur it may be necessary to pull out the stent. Regarding the highest values, the maximum load was 78.7 N and in terms of Young's modulus it was 49.8 MPa after hydration for the coated stents of formulation 2, compared with 41.2 N and 24.6 MPa, respectively, for the commercial stent. On the other hand, in terms of maximum tensile strain (%) or elongation at break the control presents values around 736.5% compared with 339.1% obtained for formulation 2. In general, the contribution of gelatin seems to increase the mechanical properties of the biodegradable stents. The hydration of the stents further contributes to increase the elasticity of the material. Analyzing the effect of the PCL coating it also contributes to increase the elasticity and the ductility of the stents with significant differences. With the objective to study the influence of calcium ions concentration as crosslinking agent three different concentrations were tested with the formulation 2. This formulation was selected according to the results obtained, due to the balance in terms of ductility and elasticity of biodegradable ureteral stent.

Fig. 7 shows herein the results for the ureteral biodegradable stent with formulation 2, using different concentrations of crosslinking agent, namely 0.24 M, 0.48 M and 1 M. Comparing the different concentrations, the results suggest that the crosslinking concentration does not have a great impact in the final mechanical properties of the biodegradable ureteral stent, although, a slight increase is observed in the calcium ions concentration. These results have been previously reported in the literature. The presence of calcium ions enhances the crosslink of alginate matrix. Nonetheless, in this work, we did not observed in this work significant changes in the mechanical properties changing the calcium concentration [29].

The maximum tensile strain (%) during the degradation process was measured (Fig. 7d) and the results show decrease of the mechanical properties during time of degradation. Nonetheless on day 6, before the complete degradation, the ureteral stent (formulation 2) shows an average maximum tensile near 200%. Although the mechanical properties decrease during the degradation process the properties seem to be enough to maintain the function of the ureteral stent before the total degradation. These observations are extremely important in case there is the clinical need to remove the stents without compromising the obstruction of the ureter by possible fragments left.

In the first generation of biodegradable ureteral stents made by natural polymer the values obtained were three times lower compared with the second generation [16]. Clearly, increasing the gelatin concentration, the modification of the fabrication process

and an incorporation of a new biodegradable coating allow the preparation of a biodegradable ureteral stent capable to be used *in vivo* following conventional ureteroscopy an ideal ureteral stent is expected to have adequate performance in terms of mechanical properties.

Comparing our maximum tensile strain results with a resorbable ureteral stents made from PGA and PLGA [15,30] the natural origin materials here used present higher elongation comparing with the synthetic materials. In terms of global mechanical performance obtained in this study demonstrated to be similar or better than commercial stent available, Biosoft® duo, Porges, Coloplast.

3.8. In vivo *placement technique validation*

The validation of the stent placement *in vivo* was performed in different female domestic pigs. Conventional ureteroscopy was employed to implant the developed stents. The first stent tested *in vivo* was the first generation of biodegradable ureteral stents based on natural origin polymers reported by Barros et al. [16]. The first generation demonstrated upon surgical procedure the stents slipped perfectly into the cystoscope and the hydrophilic guidewire into the bladder through the urethra. The ureteral stent developed remains intact throughout the procedure and is not fragmented and removal proved to be easy if necessary. However, it was not ductile enough in order to be able to be positioned correctly in the ureter. On the contrary, this new second-generation of biodegradable ureteral stents was successfully implanted *in vivo*. The new formulation together with the PCL 787 coating and the poor interfacial bonding between the polymers is an important feature for the success of the in *vivo* studies. The PCL 787 layer provides a hydrophobic layer that delay the hydration of the material upon implantation and after implantation it will be delaminated from the surface of the stent. The thin layer delaminated is eliminated in the early stages of implantation and after 3 days we did not observe

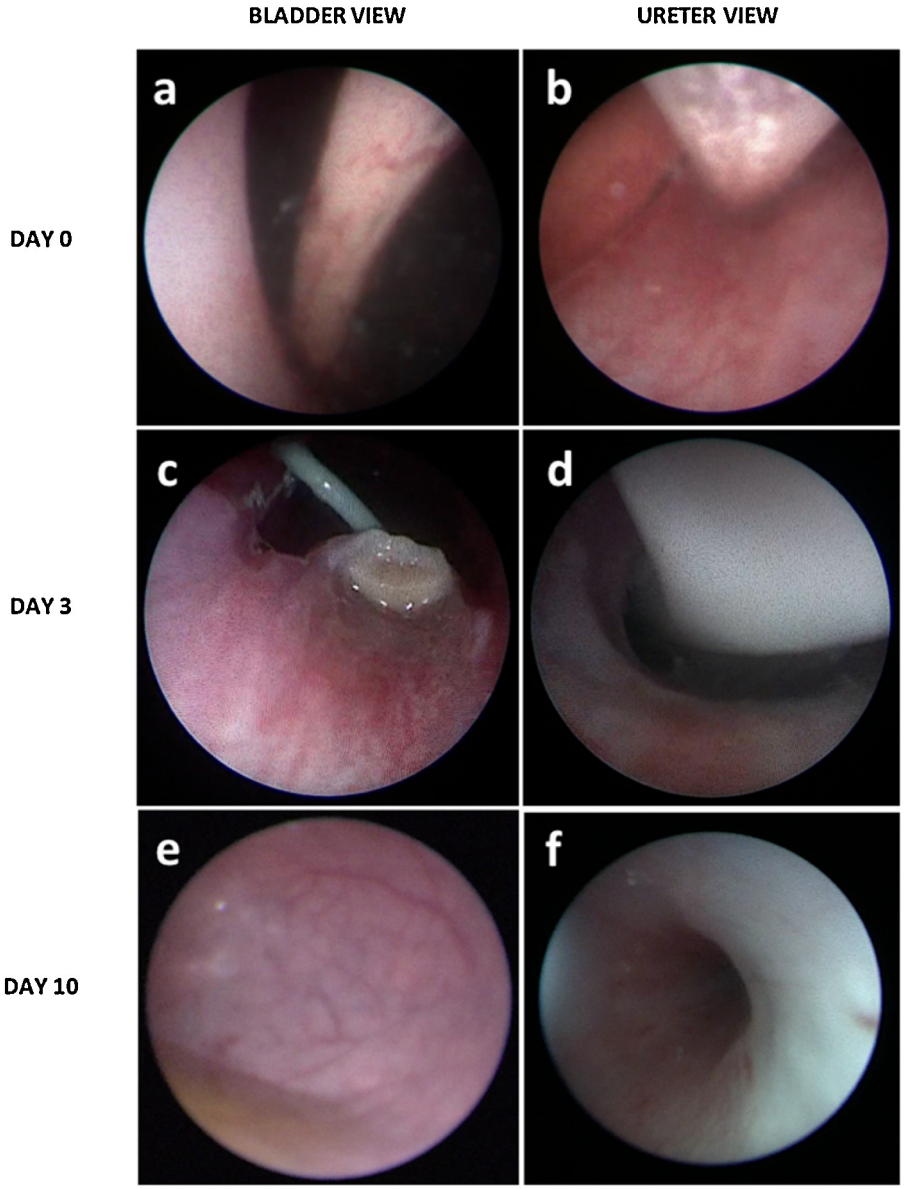

Fig. 8. Conventional ureteroscopy of the stented ureter *in vivo* in a pig model: (a) biodegradable ureteral stent placement, (b) biodegradable ureteral stent inside the right ostium pig ureter at placement time, (c) biodegradable ureteral stent after 3 days at the entrance of the right ostium pig ureter, (d) after 3 days with the biodegradable ureteral stent (image taken in the middle of the stent), (e) after 10 days of the biodegradable ureteral stent and (f) right ostium pig ureter after the degradation at day 10.

any fragments of PCL in the bladder or ureter. The biodegradable ureteral stents of this second-generation at formulation 2 were placed in the right ureters without any complication and as a control a commercial stent (Biosoft® duo, Porges, Coloplast) was placed in the left ureter, following the conventional surgical procedure. In Fig. 8 it is possible to see the second generation of biodegradable stent placed in the ureters of the pig model. During the experiments all the animals remained asymptomatic and with a normal urine flow. After 3 days, an ureteroscopy was performed to evaluate the morphology of the ureters and the stents. The biodegradable stent remains intact and maintain its stability (Fig. 8c). Furthermore, no undesired side effects were observed in ureters (Fig. 8d). On day 10, we performed again an ureteroscopy. At this time point the stents had completely degraded and no signs of fragments of the biodegradable ureteral stents were found. The morphology of the ureters remains normal with no major signs of inflammation or adverse reactions (Fig. 8e and f) at least at macroscale level. These biodegradable ureteral stents prepared from formulation 2 were demonstrated to be intact during the first 3 days and after 10 days they are completely degraded and no stent residues were observed in the urinary tract. Three independent experiments were carried out and all procedures lead to the same observation. In comparison with the first generation of stents reported in our previous work we herein demonstrate the improvement of the mechanical properties of the biodegradable stent allowing its placement in the ureter and validation of its degradability within 10 days. However, an extensive *in vivo* study needs to be performed to be able to validate clinically the material produced.

4. Conclusions

The results obtained from the experiments performed demonstrate that different mixtures of alginate and gelatin and different concentrations of crosslinking agent can be used to obtain a biodegradable ureteral stent from natural origin polymers which may be used for the treatment of urological disorders. In this work we show that this second-generation of stents, presents radiopaque properties even in the wet state. Furthermore, we demonstrate that in *vitro* a higher concentration of gelatin in the biodegradable stent resulted in higher mechanical properties, and a higher concentration of alginate slows the degradation in *vitro*. The leachables and the degradation products have shown to be non-cytotoxic and the degradation of the stent has shown to be homogenous as the degradation occurs by erosion of the material. The second-generation of biodegradable ureteral stents herein developed could be implanted following the conventional surgical procedure performed daily in the clinical practice. The ureteral stent remains intact during the first 3 days and starting to degrade after that. Full degradation is achieved after 10 days, without any presence of stent remaining inside the ureter. The stents developed was demonstrated to be safe and fulfilled the function of keeping the flow of urine from kidney to bladder while implanted in the ureter.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.apmt.2016.07.006.

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