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Bioresorbable ureteral stents from natural origin polymers

Alexandre A. Barros,[1,2] Ana Rita, C. Duarte,[1,2] Ricardo A. Pires,[1,2] Belém Sampaio-Marques,[2,3] Paula Ludovico,[2,3] Estevão Lima,[2,3] João F. Mano,[1,2] Rui L. Reis[1,2]

[1]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, 4806-909 Taipas, Guimarães, Portugal
[2]ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal
[3]Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal

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Abstract: In this work, stents were produced from natural origin polysaccharides. Alginate, gellan gum, and a blend of these with gelatin were used to produce hollow tube (stents) following a combination of templated gelation and critical point carbon dioxide drying. Morphological analysis of the surface of the stents was carried out by scanning electron microscopy. Indwelling time, encrustation, and stability of the stents in artificial urine solution was carried out up to 60 days of immersion. *In vitro* studies carried out with simulated urine demonstrated that the tubes present a high fluid uptake ability, about 1000%. Despite this, the materials are able to maintain their shape and do not present an extensive swelling behavior. The bioresorption profile was observed to be highly dependent on the composition of the stent and it can be tuned. Complete dissolution of the materials may occur between 14 and 60 days. Additionally, no encrustation was observed within the tested timeframe. The ability to resist bacterial adherence was evaluated with Gram-positive *Staphylococcus aureus* and two Gram-negatives *Escherichia coli* DH5 alpha and *Klebsiella oxytoca*. For *K. oxytoca*, no differences were observed in comparison with a commercial stent (Biosoft® duo, Porges), although, for *S. aureus* all tested compositions had a higher inhibition of bacterial adhesion compared to the commercial stents. In case of *E. coli*, the addition of gelatin to the formulations reduced the bacterial adhesion in a highly significant manner compared to the commercial stents. The stents produced by the developed technology fulfill the requirements for ureteral stents and will contribute in the development of biocompatible and bioresorbable urinary stents. © 2014 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 00B: 000–000, 2014.

Key Words: aerogel/hydrogel, natural polymers, bioresorbable polymers, kidney stones, supercritical fluid technology, ureteral stents

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INTRODUCTION

Stents have a wide range of applications in urology. Stent-based strategies are usually applied in the ureter to ensure its patency, which may be compromised, for example, by a kidney stone. This method is sometimes used as a temporary measure to prevent damage to a blocked kidney until a procedure to remove the stone is performed. Indwelling times for these cases are typically from 15 up to 60 days. In the case of tumors, stents are indicated to hold open ureters, which have been compressed in the area of the tumor or by the tumor itself. Stents used to guarantee drainage of urine flow through the ureter should have, in these cases, indwelling times of 12 months or longer.[1] The main complications with ureteral stents are dislocation, infection, and blockage by encrustation.[2,3] Currently, nearly 100% of the people who have an urological stent are likely to develop a bacterial infection within 30 days, which increases morbidity threefold.[4] Different types of temporary and permanent stents have been introduced into urological practice to relieve obstructions.[5,6] In terms of ureteral stents composition materials, the gold standard are polymeric compounds from different families, including silicone, polyurethane Siliteck, among others.[7] However in some cases, polymeric stents demonstrate certain limits in their ability to resist external compression forces and in certain cases metallic materials have been introduced as they are more resistant stents.[8]

Correspondence to: A. R. C. Duarte (e-mail: aduarte@dep.uminho.pt) or R. A. Pires (e-mail: rpires@dep.uminho.pt)
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Despite the fact that stent designs has improved over the years, they present one major key disadvantage, which is the fact that they have to be removed by second intervention. Avoiding a secondary procedure to remove the ureteral stents is highly desirable. The development of biodegradable ureteral stents has been pursued previously. However, regardless of early positive results with various models,[9–12] these attempts were abandoned due to biocompatibility issues in porcine ureters[13] or because they degraded in inhomogeneous, premature, or delayed fashion.[14,15] Hydrogels can be applied as a coating surface modification to ureteral stents. Hydrogel-coated stents have advantages such as improved material biocompatibility, hydrophilization, and lubrication.[16,17] Hydrogels are polymeric networks which may present relevant mechanical properties, appropriate degradation rates, reduced biofilm formation,[18–20] and constitute *per se*, an interesting alternative to conventional urological stents. An ideal stent for the lower urinary tract would provide adequate support to the duct wall, like the urethra or ureter, keeping the lumen open during and after the healing process, and then biodegrade totally from the body.[2,21] The material needs to fulfill the biocompatibility demands according to the guidelines of tissue biocompatibility analysis and risk assessment of new medical devices. The rigidity of the material has to be suited to the place of application; the degradation products should be biocompatible; and the rate of degradation should be adequate to allow healing. The devices also need to be easily sterilized without change in the morphological and mechanical properties.[22] It has been reported in the literature that the coating of polymeric stents with hydrogels is able to improve the properties of the stent, like anti-bacterial properties[2,16]; however, to our knowledge, simple hydrogel stents have not been reported. The concerns regarding existing stents are the motivation to design new bioresorbable urological stent systems based on natural polymers, which present inherent biocompatibility, anti-bacterial properties, and can be tailor-made into a custom suitable stent for a particular patient. The characteristics of an ideal temporary stent include easy placement under local anesthesia, minimal local side-effects, such as tissue hyperplasia or encrustation, and a low risk of migration. The device must also be easily removable or, preferably, bioresorbable to reduce the necessity of further surgical intervention.

The compositions herein tested were based on polysaccharides of natural-origin, in particular alginate, gellan gum, and their blends with gelatin. These polysaccharides have advantages over other polymers currently used to produce stents. As a main advantage, they are bioresorbable and its use does not require a second surgery to remove the stent. The mechanical properties of these materials suggest that they can be used for this purpose, since they present elasticity and, at the same time, they allow urine flow through the obstructed region. With the properties of the proposed stents it is expected a reduction of the pain experienced by patients when compared to conventional stents as the stents prepared are softer. Furthermore, it is expected that the stent implantation is easier, due to the lubricity properties of the hydrogels, being hereafter more comfortable for the patient. Conventional stents coated with hydrogels have been reported to provide an improvement to the resistance to bacterial adhesion, and biofilm formation.[23] Furthermore, the developed stents also exhibited adequate resistance to encrustation. The development of these stents with the above-mentioned properties anti-bacterial, bioresorbable, and appropriate geometry will be pursued in this work.

TABLE I. Polymer and Crosslinking Agent Concentrations Used to Prepare the Stents

Polymers	Polymer Conc. (wt %)	Crosslinking Agent	Crosslinking Agent Conc. (*M*)
Alginate	6	$CaCl_2$	0.24
Alginate:gelatin (60:40)			
Gellan gum	4		
Gellan gum:gelatin (60:40)			

MATERIAL AND METHODS

Materials

Gelzan CM (gellan gum), alginic acid sodium salt, gelatin, urea, potassium chloride, calcium chloride, and ethanol were purchased from Sigma-Aldrich (Germany). Potassium dihydrogen ortho-phosphate (99.5%) and magnesium chloride hexahydrate (99%) were obtained from Riedel-de Haën (Germany). Carbon dioxide (99.998 mol %) was supplied by Air Liquide (Portugal). All reagents were used as received.

Preparation of polymer solutions

Polymers were dissolved in hot distilled water (90°C) at different concentrations, and stirred for 1 h. The polymeric solution was injected in a template of appropriate geometry and immersed at room temperature in a stirred $CaCl_2$ or KCl crosslinking solution. This step allows the gelification of the polymer. In Table I, the concentrations of polymers and crosslinking agents used to prepare the aerogel-based stents are summarized.

Sample drying

Supercritical fluid drying with CO_2 is an alternative process to the conventional drying techniques, which preserves the properties of the wet gel in the dry form. The stents were dried in a high-pressure vessel with carbon dioxide at 40°C and 100 bar for 2 h, in a continuous mode, with a CO_2 flow rate of 15 g/min. The conditions were chosen in order to ensure complete miscibility between the CO_2 and ethanol. Different processing times were tested and 2 h was established as the necessary drying timeframe.

Characterization

Scanning electron microscopy. The morphology of the stents was analyzed on a Leica Cambridge S360 scanning electron microscopy (SEM). The samples were fixed with mutual conductive adhesive tape on aluminum stubs and covered with gold/palladium using a sputter coater.

TABLE II. Composition of the Artificial Urine Solution (AUS)

	Component	% wt/vol
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		0.125

After immersion in artificial urine solution (AUS) the inner section of the stents was also analyzed to monitor the deposition of crystals.

Artificial urine uptake. AUS was prepared as described by Khandwekar and Doble,[23] with the composition presented in Table II.

The AUS uptake capability of the samples was measured for a period up to 60 days by their immersion in AUS. Pre-weighted stents were immersed in 10 mL of AUS and placed in a water bath at 37°C, 60 rpm for 1, 7, 14, 28, and 60 days. All the experiments were executed in triplicate. At the predetermined time periods, the samples were weighted in order to determine the uptake capability of the stents. AUS uptake was determined using the following equation:

$$\%\ \text{AUS uptake} = (w_w - w_f)/w_f \times 100 \quad (1)$$

where w_w is the weight of the wet sample and w_f is the final weight (dried after immersion). The presented data is the average of at least three measurements.

Swelling. Images of dry and wet samples were taken using a Stereo Microscope + Lamp (Schott KL 200), stemi 1000 model (ZEISS), with a magnification ×2 and the swelling of the matrix was evaluated by the measurement of the thickness of the wall using Image J software. The presented results are an average of at least five measurements (±SD) of each sample.

Indwelling time. The indwelling time was measured as function of the weight loss of the samples. Samples immersed in AUS were dried and weighted to determine the weight loss, which was calculated according to the following equation:

$$\%\ \text{weight loss} = (w_f - w_i)/w_i \times 100 \quad (2)$$

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.

Tensile mechanical analysis

Tensile mechanical analysis of the materials produced (A—alginate; AG—alginate:gelatine; GG—gellan gum; GGG—gellan gum:gelatine) was measured using an INSTRON 5540 (Instron Int. Ltd, High Wycombe, UK) universal testing machine with a load cell of 1 kN. The samples were hydrated before testing in simulated urine for 30 min. 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.

Encrustation development

The evaluation of the deposition of crystals on the surface of stents was performed following the procedure described by Tunney et al.[24] Samples of the different stents were immersed in AUS for predetermined time periods. They were removed and rinsed gently with distilled water to remove any salts (which might be only deposited on the surface). Energy dispersive X-ray spectroscopy (EDS) was performed together with SEM in a Link Exl-II spectroscope (Oxford Instruments, United Kingdom) for elemental analysis, with an energy of 15.0 keV. Samples were fixed as described for SEM analysis and carbon coated using high vacuum carbon deposition.

Bacterial adhesion studies

Bacterial adhesion studies were performed according to Khandwekar and Doble.[23] A quantitative short-term adhesion (4 h) study was performed with *Staphylococcus aureus* (NCIM 5021), a Gram-positive organism, and two Gram-negative organisms *Escherichia coli* DH5 alpha and *Klebsiella oxytoca*; 100 mL of lysogeny broth (LB) medium (1% bacto tryptone, 5% bacto yeast extract, and 1% sodium chloride) was inoculated with a single colony of bacteria from a LB agar stock plate. Cells were grown at 37°C and 200 rpm, overnight. Cells were then split between two falcon tubes, centrifuged at 3500 rpm for 20 min and resuspended in phosphate buffered saline (PBS). Cell suspension was washed twice with PBS and resuspended at a concentration of 1×10^8 cells/mL. Three tubes (3 mm length) of each formulation were placed in a 24-well plate and were incubated with 1 mL of the cell suspension for 4 h at 37°C with shaking. The bacterial cells were eluted from the surfaces into 2 mL sterile PBS. The procedure involved 4 min sonication followed by 1 min mild vortexing (repeated three times) using an ultrasonic cleaner (Bandelin Sonorex Digitec) with an ultrasonic frequency of 35 kHz. A known volume of the sample was inoculated into LB agar and incubated at 37°C for 24 h. The colony forming units (CFUs) were counted indicating the total number of bacteria retained on the surface.

Cytotoxicity and cell adhesion studies

Cell culture. An immortalized mouse lung fibroblasts cell line (L929) purchased from the European Collection of Cell Cultures, was maintained in basal culture medium DMEM (Dulbecco's modified Eagle's medium; Sigma–Aldrich,

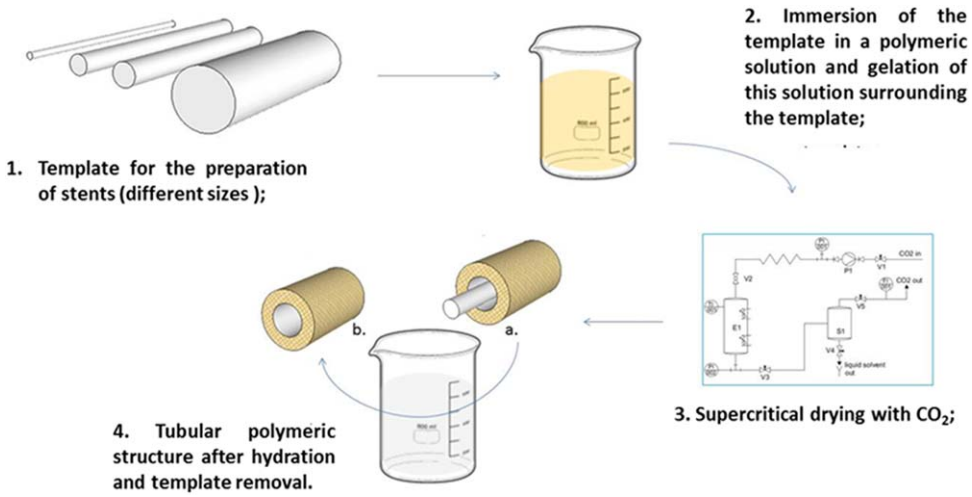

FIGURE 1. Methodology used to generate the different stents. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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.

Indirect cytotoxicity studies. The cytotoxicity of the stents developed was assessed using an immortalized mouse lung fibroblasts cell line (L929) purchased from the European Collection of Cell Cultures. The effect of the leachables released from the stents (during 24 h) on the cellular metabolism was performed using a standard MTS (Cell Titer 96® Aqueous Solution Cell Proliferation Assay, Promega, USA) viability test, in accordance with ISO/EN 10993 guidelines. A latex rubber extract was used as positive control for cell death; the extracts from a commercial stent (Biosoft® duo, Porges) was used as a reference material; while cell culture medium was used as negative control representing the ideal situation for cell proliferation. Cell viability was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphofenyl)-2*H*-tetrazolium (MTS) assay after 72 h. This assay is based on the bioreduction of a tetrazolium compound MTS into a water-soluble purple formazan product. 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 were tested using 12 replicates.

Direct contact studies. Confluent L929 cells were harvested and seeded in the stents as follows: stents were distributed in a 48-well cell culture plate (BD Biosciences, USA); samples were initially immersed in sterile PBS in order to swell; afterwards, PBS was removed and a drop (20 μL) of a cell suspension with a concentration of 1×10^5 cells/mL was added to each material. Cell seeding on the commercial stent was also carried out as control. The cells-stents constructs were statically cultured for 1, 3, and 7 days under the culture conditions previously described. Cell adhesion to the surface of the materials was determined after the predetermined culture times by the MTS assay described above. The cell-stents were transferred to a new culture plate in order to evaluate the presence of viable cells only on the surface of the developed materials. Optical density (wavelength of 490 nm) was determined for each time point and compared to polystyrene tissue culture plate, used as a positive control. All cytotoxicity screening tests were performed using three replicates.

Statistical analysis

Statistical analysis was performed to compare the results obtained using GraphPad Prism 5. Shapiro–Wilk test was used to verify the normality of the data obtained. Normally distributed data were analyzed by *t*-student test comparing each tested stent with the commercial stent, in case of bacterial adhesion study. Non-parametric tests were performed in the case of cytotoxicity and cell adhesion samples with deviation of the data from the normal distribution. Mann–Whitney test was performed to pairs of independent samples in order to compare the medians of the results. Statistical significant differences were considered when $p < 0.05$.

RESULTS

Stents from natural origin polymers

Stents were prepared following the processing steps represented in Figure 1. The tube is formed from an initial aqueous solution of biopolymer from which gelation is induced. Gelation in the case of alginate tubes was promoted by ionic crosslinking with a $CaCl_2$ solution. In the case of gellan gum lowering the solution temperature induces gelation, which is a physical crosslinking method. In order to confer stability to the tubes, and avoid their premature dissolution in aqueous solutions, gellan gum was also ionically crosslinked with KCl and $CaCl_2$ solution, respectively. The hydrogels were, then, dehydrated and subject to a solvent exchange step where an alcohol gel was formed. In this process, ethanol replaced water and the material was further dried using supercritical carbon dioxide.[25]

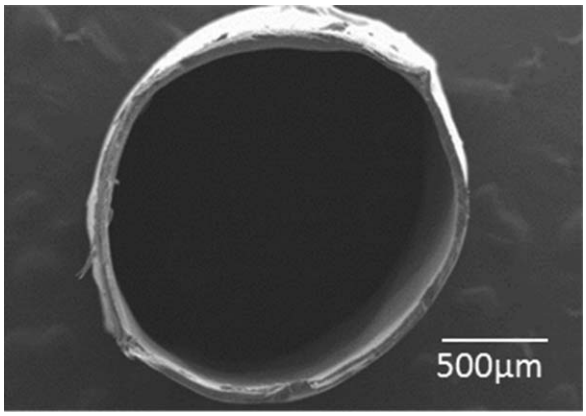

FIGURE 2. SEM micrographs of the gellan gum: gelatin stent (60:40% wt/wt).

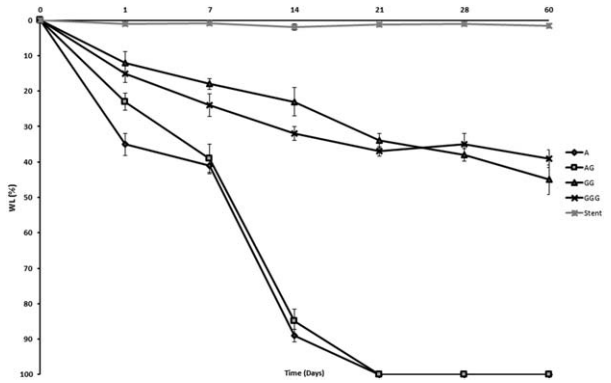

FIGURE 4. Weight loss of the developed stents (A—alginate; AG—alginate:gelatine, GG—gellan gum; GGG—gellan gum:gelatine) during a time frame of 60 days (indwelling time). Biosoft® duo, Porges Stent was used as a control.

Scanning electron microscopy. Figure 2 presents a SEM image of the hollow tubes after drying. This procedure allowed the preparation of a stent with a diameter of 1 mm in dry sate, which did not show any dependence on: the type of biopolymer used; on the polymer concentration in solution; nor on the type of crosslinking.

Characterization

Artificial urine uptake and indwelling time. The micrographs in Figure 3 show that, upon hydration, the inner diameter of the stents increased from 1.0 (SD ±0.3) to 1.8 (SD ±0.2) mm. These stents are able to maintain their shape and integrity upon immersion in simulated body fluids, as observed for periods up to 60 days of immersion.

In clinical practice, the indwelling time is defined as the time ranging from the implantation of the stent until its removal, which is dependent on the clinical treatment defined for each patient.[26] Therefore, it would be desirable to tune the degradation of the stents to accommodate the devices for a given indwelling time. The weight loss, measured as the percentage of mass lost when immersed in AUS for a predetermined time period, was assessed for the different formulations of stents developed (Figure 4).

Using the processing methodology, herein detailed, it is possible to tune the degradation rate of the stents by selecting the materials used to produce them. Furthermore, the extent of crosslinking can confer different properties to the stents. *In vitro* performance demonstrates that the indwelling time of the proposed materials in solution can be tuned from 14 up to 60 days. The stents based on alginate were the ones that presented a faster dissolution and after 21 days of immersion the materials was completely dissolved in the solution.

Mechanical tests. The mechanical properties of the stents prepared were evaluated in tensile mode. Table III presents the results obtained for the different formulations tested. The results presented were determined in the wet state, in AUS, mimicking the real application conditions.

Encrustation development. The deposition of salts was evaluated by SEM and EDS. Figure 5 presents a micrograph

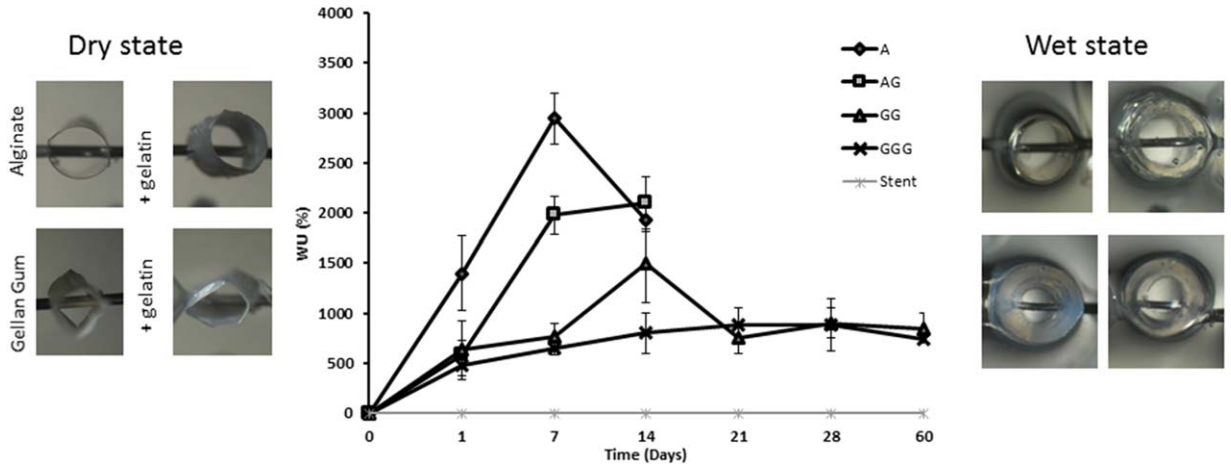

FIGURE 3. AUS uptake by the developed stents (A—alginate; AG—alginate:gelatine, GG—gellan gum; GGG—gellan gum:gelatine) during a timeframe of 60 days, and swelling images using a magnifying lens (2×), showing a change in the internal diameter from 1.0 mm to 1.8 mm. Biosoft® duo, Porges Stent was used as a control. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE III. Mechanical Properties of the Stents Prepared

	Maximum Load (N)	Maximum Tensile Strain (%)	Young Modulus (Mpa)
A	0.73 ± 0.01	11.75 ± 0.60	18.17 ± 1.04
AG	0.56 ± 0.02	15.84 ± 0.58	13.19 ± 0.82
GG	1.11 ± 0.04	20.93 ± 0.78	28.25 ± 1.47
GGG	0.96 ± 0.04	29.68 ± 0.54	26.62 ± 1.24

of the inner surface of the stents after immersion and the corresponding EDS spectra. The results point out that no encrustation was detected in any of the materials herein proposed.

Bacterial adhesion studies. Bacterial adhesion is a serious concern related with the formation of biofilm on the surface of the stents. The ability of bacteria to adhere and proliferate on the surface of the materials was studied for three different bacteria: *S. aureus* (Gram-positive); and *E. coli* DH5 alpha and *K. oxytoca* (Gram-negative) and the obtained results are presented in Figure 6. Statistical analysis indicated a significant reduction in adhesion of both *S. aureus* and *E. coli* DH5 *alpha* to alginate gelatin and gellan gum + gelatin stents in comparison with the commercial stent (∼×12, ∼×41, and ∼×2, respectively). The extent of reduction was found to be greater for *S. aureus* when compared to *E. coli* DH5 *alpha* (Figure 6). Relative to *K. oxytoca*, no alteration on the adhesion profile was observed for any of the tested stents. Moreover, it is relevant to notice that the stents prepared with gelatin present significantly lower bacterial adhesion, suggesting that these materials may be the ones that have higher potential in the development of bioresorbable ureteral stents.

Cytotoxicity and cell adhesion studies. The cytotoxicity of the six developed stents was evaluated in accordance with the protocol described in ISO/EN 10,993.[27] As a control, the commercial stent Biosoft® duo (Porges) was used. The viability of the cells cultured in a tissue culture plate, in the presence of the stents, was determined as a function of the cells cultured in Dulbecco's modified Eagle medium (DMEM) culture medium. Figure 7(a) presents the cell viability after 72 h in contact with the material. The obtained results were compared to cell growth on tissue culture plate.

No significant differences were observed for the cell viability in the presence of the developed stents in comparison with the one obtained for the commercial stent, which was used as a negative control.

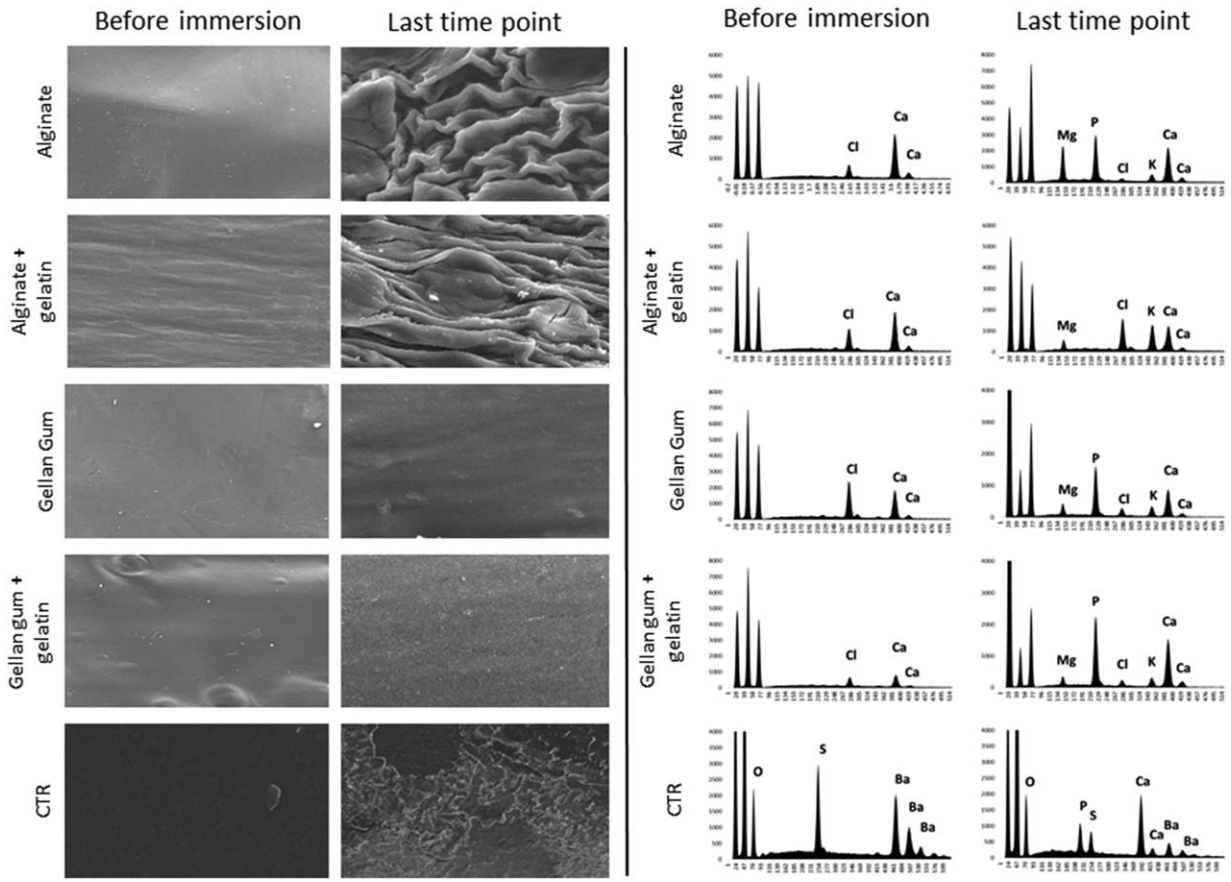

FIGURE 5. SEM micrographs and EDS spectra of the surface of the stents prepared with the different polymers before and after immersion in AUS—last time point corresponds to 14 days for alginate-based stents and 60 days for gellan gum-based ones. Biosoft® duo, Porges Stent was used as a control.

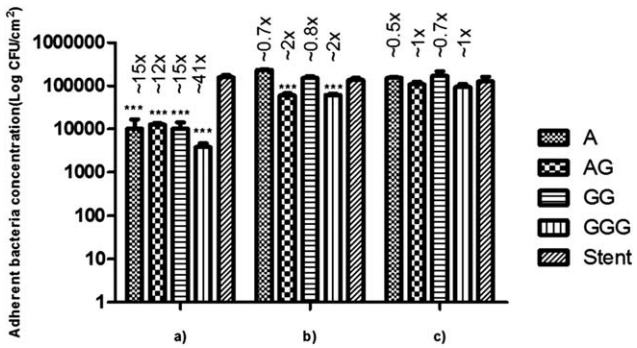

FIGURE 6. Bacterial adhesion on the stents incubated with approximately 1×10^8 (a) *S. aureus* bacterias (Gram+), (b) *E. coli DH5 alpha* (Gram−), and (c) *K. oxytoca* (Gram−) for 4 h. Values indicate mean ± standard deviation from a single experiment performed in triplicate, which was representative of three independent experiments. Fold adhesion reduction between each tested stents and the commercial stent is indicated on top of each bar. Significance of the values between each tested stents and the commercial stent was determined by the *t*-student test (***$p < 0.001$). A—alginate; AG—alginate:gelatine, GG—gellan gum; GGG—gellan gum:gelatine.

In this context, cells were seeded directly on the surface of the stents and the adhesion was studied after 1 and 3 days of culture by MTS viability assay. Cells seeded on tissue culture plate were used as control [Figure 7(b)].

MTS analysis revealed that no cells are present in the surface of the materials after 1 or 3 day of culture. As it can be observed from Figure 7(b), the developed stents present a behavior similar to the commercial stent. Clear differences between cell growth on the tissue plate and on the stent surfaces are observed. This observation would be expected due to the hydrophilic nature of the tested biopolymers. Nonetheless, there are reports in the literature which indicate that cells are able to adhere and grow on the tested substrates.[28–30]

DISCUSSION

An urological stent is defined as a thin tube, which is inserted in the ureter to prevent or treat the obstruction of urine flow from the kidney to bladder. Bioresorbable natural-origin hydrogels present characteristics, which confer them several advantages, such as, biocompatibility, interface lubricity, as well as resistance to biofilm formation and encrustation.[31] The main objective of the present research work is the preparation of hollow tubes from natural origin polymers, namely, alginate, gellan gum, and their blends with gelatin and to evaluate suitability, to be used as ureteral stents. Different blends of natural polysaccharides with gelatin have been reported in the literature.[31–33] The variability of gelatin chemical composition confers this biopolymer different molecular weight and polydispersity, which *per se* have shown to substantially influence mechanical and thermal properties of physical gels.[34] The combination of polysaccharides with gelatin is expected, to induce changes in the water uptake, degradation profile, and the *in vitro* biological performance of the ureteral stents. These changes were particularly noticeable in the bacteria adhesion studies, which have demonstrated that the presence of gelatin in the blend lower the number of adhered bacteria.

The technology developed for the preparation of the hollow tubes, although not new, has not been applied for the purpose reported in this work (Figure 1). One of the main advantages of the process is the use of supercritical carbon dioxide as drying agent. This has demonstrated several advantages over conventional drying methods, such as freeze-drying or vacuum drying.[28] Both freeze-drying and vacuum drying involve a phase transition, solid–vapor and liquid–vapor, respectively. The phase transition is responsible for an interfacial tension, which results in the shrinkage and deformation of the produced matrices. On another hand, supercritical drying with carbon dioxide is a process which the matrices do not undergo any phase transition and therefore the integrity of the structures is not compromised.[33] In the supercritical drying process, carbon dioxide replaces the solvent molecules within the polymeric matrix and removes the organic solvent due to their miscibility at the drying conditions. The production of hollow tubes by the proposed technology (Figure 2) foresee its versatility as the change of the template allows the preparation of materials with different shapes, making it possible to control the thickness of the wall, the inner diameter, and the mechanical properties. It is also possible to create a material with different layers by dipping the template, sequentially, in different polymeric solutions. Such bottom up approach to obtain multilayered hydrogels was validated before on spherical objects.[25]

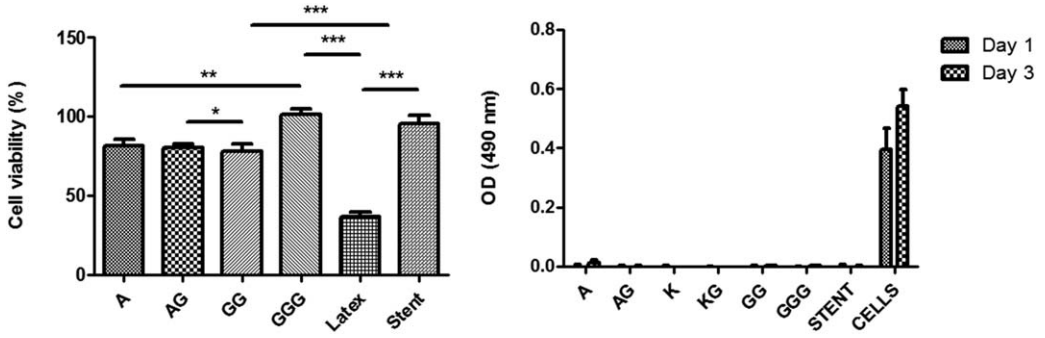

FIGURE 7. Cytotoxicity and cell adhesion studies: (a) cell viability measured after 72 h and (b) cell adhesion on the surface of the different stents. Biosoft® duo, Porges Stent was used as a control. Statistical significant differences were considered as ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

The biopolymers used in the preparation of the stents are highly hydrophilic. An inherent characteristic of these stents is the fact that, in the presence of water, a hydrogel is formed.[35] This behavior is usually coupled with a high swelling ability, which may disrupt the structure of the matrices. The prepared stents have high water uptake ability, up to ~1000%, after 1 day of immersion (Figure 3). However, the devices do not present an extensive swelling behavior as observed by a magnifying lens. Augst et al. refer that ionic crosslinked alginates dissolve upon losing the divalent cations responsible for the crosslinking.[33] The observed dissolution rate might also be due to the ionic change between Ca^{2+} ions by monovalent ions, which weaken the structure.[36] Hydrogels of alginate occurs as a result of the formation of ionic crosslinks between carboxyl groups in guluronic acid residues within the polysaccharide. Each calcium ion is then chelated by two alginate molecules, forming crosslinks, thus resulting in gelation. When immersed in distilled water alginate the mechanical properties will be conserved, however, in the presence of monovalent ions (K+ or Na+), in our work in AUS, ion exchange will occur with the crosslinking Ca^{2+} resulting in a rapid reduction in mechanical properties.[33,36–38]

In our experiments, the variation of the ratio of polysaccharide:gelatin did not show a significant effect on the bioresorption profile of the samples, although it has been reported that it may tune the resorption kinetics of hydrogels for a wide range of applications and conditions.[32,39] In this perspective, it is possible to design polymeric stents with the dissolution timeframe that best fits the treatment strategy (Figure 4). Consequently, the need for a second surgery to remove the stent can be avoided using the proposed natural-based stents.

The mechanical properties of urinary stents are an important parameter in order to access the feasibility of the hydrogel tubes to withstand the forces applied when the stent is inserted in the patient, assuring that the material does not break obstructing the ureter and preventing the flow of urine. Results reported in the literature for a polyurethane double J stents refer an ultimate tensile strength between 18 and 35 MPa, an elongation at break between 104 and 509% and a Young modulus between 38 and 41 MPa.[40] As expected these values are significantly higher than the ones reported in our work for the hydrogels prepared. The tensile properties of the stents produced change in the presence of gelatin and the results indicate that the presence of gelatin increases the maximum tensile strain while decreasing on the other hand the maximum load and the Young modulus. Few reports in the literature provide comparable data to the one presented in this work. Nandakumar et al. report the development of hydrophilic high glycolic acid–poly(DL-lactic-*co*-glycolic gcid)/polycaprolactam/polyvinyl alcohol blends as uretheral materials and tensile tests carried out refer tensile stresses at maximum load in the range of 0.66–8.82 MPa, depending on the formulation.[41] Nonetheless, the data reported was measured in thin films and not in hollow tubes. Furthermore, there is no indication that the results were determined in the wet state. Another work by Jones et al., who evaluated the possibility to prepare stents from poly(ε-caprolactone) and poly(ε-caprolactone)-polyvinylpyrrolidone-iodine blends, refer also the tensile mechanical properties of the films prepared which, disregarding possible geometrical effects, is in the same order of magnitude of the polyurethane commercial stents.[42] A straightforward comparison of the results should not, hereafter, be carried out. The alginate and gellan gum-based stents prepared in this work reveal good mechanical properties and provide the stability and strength necessary for manipulation during the placement process and its function in ureter.

A major concern in urology is the development of encrustation on urological stents. This phenomenon is related with the deposition of salts (present in the urine) on the surface of the stent. When encrustation occurs urine flow is blocked, causing distress and pain to the patients. Particularly relevant are magnesium salts in the form of struvite and or calcium salts in the form of hydroxyapatite. Alginate and gellan gum stents, as well as the ones prepared with their mixture with gelatin, were immersed in AUS for different time periods. In order to study this effect, SEM micrographs (Figure 5) show a smooth inner surface of the stents at the initial time points. The micrograph for the last data point shows some rugosity, which can be explained by the polymeric dissolution. However these results do not indicate any evidence of deposited crystals and demonstrate that no encrustation was developed during the lifetime of the stent when in contact with AUS. Furthermore, these observations are consistent with EDS analysis presented in Figure 5. The EDS spectra clearly indicated the absence of ions that could suggest struvite or hydroxyapatite formation. The Ca^{2+} and Cl^{-} ions detected in the spectra of all polymers are from the crosslinking agent, as they appear only in the spectra of the materials before immersion and disappear after immersion in AUS. The hydrophilicity of the tested biopolymers can explain these observations, as the high hydration capacity of hydrogels prevents the deposition of soluble salts.

Concerning biofilm formation and bacterial adhesion, reports in the literature suggest that coating commercial stents with hydrogel may lead to a significant decrease in bacterial adhesion.[23,35] In the study of Khandwekar and Dobel[23] commercial stents (Tecoflex®) were compared to stents made of polyurethane (Tecoflex®) modified vinylpyrrolidone–iodine (PVP-I) complex. In their work, the PVP-I modified stent was highly hydrophilic and more lubricious than the control polyurethane. Adherence of both Gram positive *S. aureus* (by 1×10^6 CFU/cm^2) and Gram-negative *Pseudomonas aeruginosa* (by 2×10^6 CFU/cm^2) was significantly reduced on the modified surfaces. Our results obtained for the commercial stent (Biosoft® duo, Porges), following the same experimental procedure, indicated the presence of $\sim 1.8 \times 10^5$ CFU/cm^2 for the three tested bacteria's (*S. aureus*, *E. coli DH5 alpha* and *K. oxytoca*). The materials tested demonstrated a lower bacterial adherence, particularly for the Gram-positive bacteria *S. aureus*, whereas a reduction of about ×12 to ×41 of bacterial

adhesion was observed. For the Gram-negative bacteria, the tested materials demonstrated a behavior similar to the commercial stent, although with *E. coli* DH5 *alpha*, it was observed an adhesion reduction of about $\times 2$ with two of the used biomaterials (alginate gelatin and gellan gum + gelatin). These results can be explained by the bacterial cell surface structure, since cell-surface hydrophobicity is an important factor in the adherence and proliferation of microorganisms on solid surfaces.[23,24] Consistently, it was reported that *S. aureus* has higher hydrophobicity ability in comparison with *E. coli* (cell surface hydrophobicity (CSH) of *E. coli*, *S. aureus*, and Aspergillus niger and the biodegradation of diethyl phthalate (DEP) via microcalorimetry.[43,44] Considering the implications of cell surface hydrophobicity on cell adhesion, our results suggest that including gelatin on the formulations will be a promising approach in the reduction of bacterial adhesion as demonstrated by the data described in Figure 6.

Another crucial concern, in products for medical use and human consumption, is the evaluation of the cytotoxicity of the developed materials. Reports in the literature have described the non-cytotoxicity of the polysaccharides used in this study, although, it is not straightforward the extrapolation for the developed stents.[45] Different processing techniques may influence the characteristics of the materials and may induce an undesirable toxic response. The experiments carried out following an ISO[27] guideline have shown that neither the materials nor its leachables are toxic as cell viability in the experiments with the developed stents is comparable to the ones in tissue cultured plates. In addition, ureteral stents should not induce cell adhesion because they could promote an abnormal cell growth which may compress the stent, and consequently constrain the normal urine flow.[1] In fact, these materials have been reported for other tissue engineering applications in which it is possible to promote cell adhesion.[46] It is also well documented that cells respond to particular morphological and topological cues.[24] Our findings suggest that the stents prepared did not induce cell adhesion at the surface. The observed differences may be related to the processing methodology which results in a smoother surface, not favorable to cell adhesion.

CONCLUSION

The present work constitutes an important step toward the development of bioresorbable urological stents. Here, we described a methodology to prepare stents using natural-origin polymers templated gelation and drying, using critical point carbon dioxide, generated stents able to compete with the commercially available ones. Biopolymers, such as, alginate and gellan gum, as well as their blends with gelatin, present different advantages compared to the commercial products, such as: adequate bioresorption rates; no development of encrustation; and anti-bacterial properties. Stents prepared from alginate present the fastest bioresorption rate, corresponding to an indwelling time of 21 days. Longer indwelling periods may be achieved with the use of gellan gum. The addition of gelatin to the blends decreases the bacterial adhesion demonstrating to be a promising strategy to reduce the bacterial adhesion. Furthermore, the technology proposed is highly versatile, allowing a wide range of stent designs. The described stents, when in contact with a physiological medium, become hydrogels, exhibiting biocompatible and non-cytotoxic characteristics. The presence of a high equilibrium water content, provides soft, lubricious, and flexible characteristics to the devices, similar to natural tissue. The obtained results demonstrate the feasibility to develop bioresorbable stents from natural origin polysaccharides.

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