Tailor made degradable ureteral stents from natural origin polysaccharides

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INTRODUCTION

A urinary 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. Silicone, latex, polyvinylchloride and polyurethanes are the most widely used materials for the preparation of stents. Nonetheless, severe clinical complications may result from the use of these materials such as fracture, encrustation and infection. In some of the cases, the ureteral stents are temporary and it is often required a second surgery to remove the stent. The main complications with ureteral stents are dislocation, infection, and blockage by encrustation [1]. Recently, a tendency has been noticed favouring less invasive approaches (e.g. pharmacological or catheterization) in treating patients who exhibit symptoms or signs of urinary retention [2]. 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 [1]. Different types of temporary and permanent stents have been introduced into urological practice to relieve obstructions [3]. Particular attention should be devoted to polymers as they represent a highly versatile class of materials. Despite the fact that silicon continues to be the gold standard material for urological stents, there have been fast developments in manufacturing processes, as well as the introduction of new biodegradable materials in order to overcome the drawbacks of the available products. Polyurethane continues to be the most widely used material for polymeric stents; however it frequently promotes biofilm formation and bacterial adhesion leading to severe infections [2]. The concerns regarding existing stents are the motivation to design new biodegradable urological stent systems based on natural polymers, specifically polysaccharides, which present inherent biocompatibility, anti-bacterial properties and that can be tailor-made into a custom suitable stent for a particular patient.

KEYWORDS: ureteral stent, kidney stone, biodegradable polymers, aerogel/hydrogel natural polysaccharide, supercritical fluid technology

MATERIALS AND METHODS

Gelzan CM, k-carrageenan, alginic acid sodium salt, urea, ethanol, potassium chloride and calcium chloride were purchased from Sigma-Aldrich (Germany). The potassium dihydrogen ortho-phosphate (99.5%), magnesium chloride hexahydrate (99%) were obtained from Riedel-de Haën. All reagents were used as received.

Hollow tube formulation

Polymers were dissolved into hot distilled water at different concentrations (6 wt% for alginate, 4 wt% for gellan gum and 5 wt% for K-carrageenan. Their blends with gelatin were in proportion of 40% of gelatin. The solutions stirred for 1 hour. A template of appropriate geometry (compact glass tubes of 1 mm of diameter) was immersed into the polymeric solution for 2h. The polymer-coated template was transferred into a reticulation bath ($CaCl_2$ (0,24 M) or KCl (0,60M) solution) kept at room temperature under constant magnetical stirring. This later step allowed the removal of the template without damaging the polymeric tubular structure.

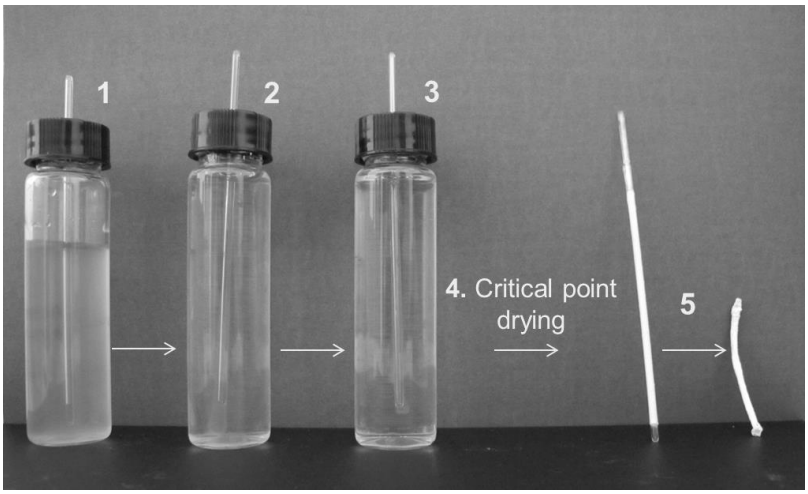


Figure 1 *Technology used to obtain aerogel hollow tubes for biodegradable ureteral stents: 1 - immersion in polymer solution; 2 - gelification of this solution surrounding the template; 3 - solvent exchange; 4 - supercritical point drying with carbon dioxide and 5 - tubular aerogel structure after template removal.*

Characterization

Artificial urine solution (AUS) was prepared as described by Khandwekar *et al* [4]. The morphology and the inner section of the aerogel tubes (after immersion in AUS) were analysed on a Leica Cambridge S360 Scanning Electron Microscope (SEM). The fluid uptake capability and the indwelling time (as a function of the weight loss) were measured for a period up to 60 days by the immersion of the samples in 10 ml of AUS at 37ºC and 60 rpm. The development of encrustation was evaluated following the procedure described by Tunney *et al* [5, 6]. Bacterial adhesion studies were performed according to *Khandwekar et al* [4], using gram-positive *Staphylococcus aureus.* Finally, the cytotoxicity and cell adhesion studies were also executed to compare the developed hollow tubes with a commercial stent. Confluent SaOS-2-cells, with a concentration $5x10^5$ cells/ml were harvested and seeded on samples of the hollow tubes and of a commercial stent (Biosoft Duo, Sedinger 0.035” 150cm).

RESULTS AND DISCUSSION

In this work, the possibility to prepare hollow tubes from natural origin polysaccharides, *i.e.* alginate, k-carrageenan, gellan gum and a blend of these with gelatin was evaluated. Hollow tubes, with a diameter of 2 mm were prepared and characterized in terms of surface morphology by SEM (figure 2).

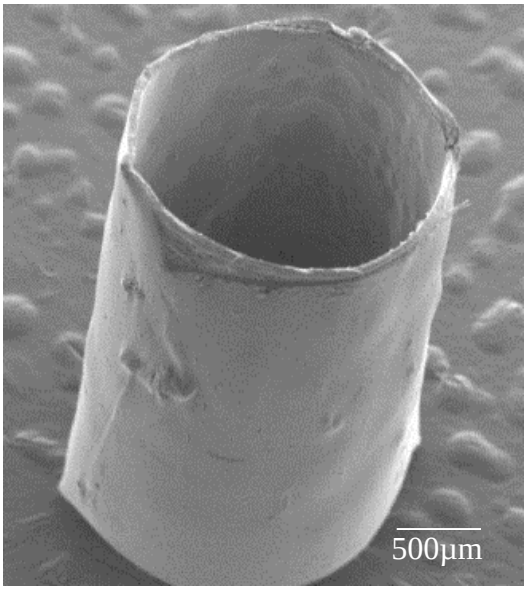


Figure 2 *SEM micrographs of the cross-section of gellan gum : gelatin stent (60:40 % w/w).*

Water uptake and polymer degradation were evaluated using an AUS as immersion medium. Figure 3 shows a cross-section of a hollow tube in the wet and dry state.

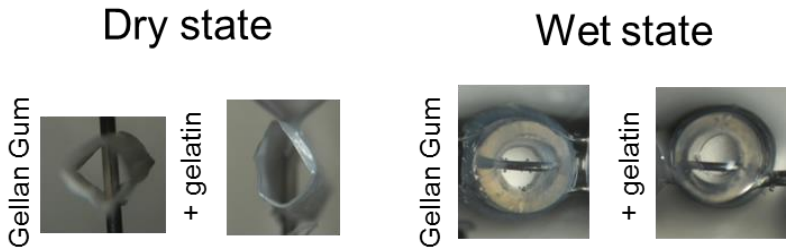
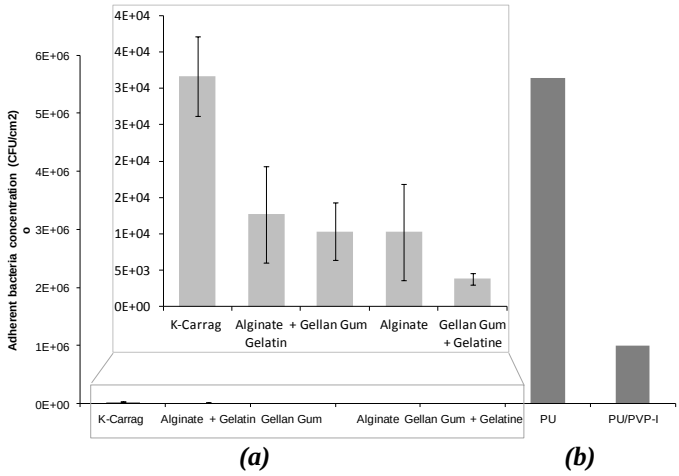


Figure 3 *Swelling of developed gellan gum (and its blends with gelatin) aerogel hollow tubes during a timeframe of 60 days, with internal diameter of 1mm.*

In vitro performance demonstrated that all tubes have high water uptake ability, although being able to maintain their shape and integrity upon immersion in AUS. The degradation in solution can be tuned between 14 and 60 days. Furthermore, *in vitro* assessment of possible encrustation, *i.e.* the deposition of magnesium and/or calcium salts on the developed stents was also carried out using SEM coupled with energy dispersive x-ray spectroscopy (EDS). No encrustation was observed up to 28 days. The ability to resist to bacterial adhesion was evaluated with gram-positive *Staphylococcus aureus* (figure 4). It was observed a drastic reduction in bacterial adhesion in comparison with commercial stents



(a) ***(b)***

Figure 4 *Bacterial adhesion on materials incubated with approximately 1 x 10^8 S. aureus bacterias during 4 h. (a) Developed aerogel hollow tubes. Values represent means ± standard deviation from a single experiment performed in triplicate, which was representative of three independent experiments. (b) Commercial stent, data from Khandwekar et al [4].*

Cytotoxicity and cell adhesion studies were also evaluated comparing the developed hollow tubes with a commercial stent. Figure 5 shows the cytotoxicity results obtained for all materials tested with cell line SaOS-2. Cytotoxicity tests demonstrated that the material prepared does not have a detrimental effect on cell growth being comparable to the commercially available stent.

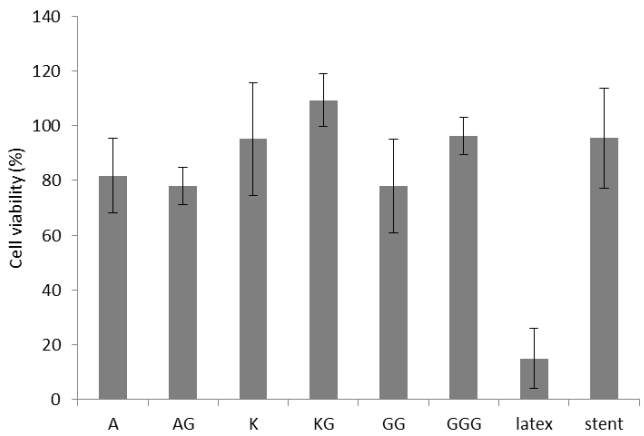


Figure 5 *Cytotoxicity tests with cell line SaOS-2 in tubes made by alginate (A), alginate : gelatin (AG), K-carrageenan (K), K-carrageenan : gelatin (KG), gelan gum (GG), gelan gam: gelatin (GGG) and the commercial stent.*

The results revealed that the proposed materials are major breakthroughs in the development of biocompatible and biodegradable urinary stents.

CONCLUSIONS

In this work we demonstrated that it is possible to use natural origin, biodegradable polymers (with inherent anti-bacterial properties) to prepare urinary stents with adequate morphology. The developed devices do not present encrustation, avoiding the need for a second surgery. All the reported characteristics are foreseen as major breakthroughs in the development of biocompatible a degradable urinary stents.

We propose the creation of hollow tubes from natural origin polysaccharides. This methodology generates biodegradable stents that are envisaged to compete with the

commercially available ones. The products herein evaluated present additional advantages such as adequate degradation rates, no encrustation and inherent anti-bacterial properties. Hollow tubes, prepared using the reported methodology, when in contact with a body physiological medium forms hydrogels, which are hydrophilic polymeric networks, biocompatible and non-cytotoxic. The presence of high equilibrium water content renders softness, lubricious and flexible characteristics similar to natural tissue. The obtained results demonstrate the feasibility to develop biodegradable stents from natural origin polysaccharides.

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