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Original Article

Biodegradable ureteral stents: in vitro assessment of the degradation rates of braided synthetic polymers and copolymers

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Abstract: Objectives: The control and predictability of degradation rates and the absence of obstructive phenomena are two main challenges for research regarding biodegradable ureteral stents. The objectives are to assess the degradation performance and safety of braided combinations of three synthetic biodegradable polymers and copolymers; and to evaluate the interference of a heparin dip coating on degradation and bacterial colonization. Methods: The hydrolysis of polyglycolic acid (PGA), poly lactic-co-glycolic acid (PLGA) and Glycomer™ 631 is assessed in this in vitro study that comprises ten groups. Stent samples present a braided arrangement and are incubated in porcine urine that undergoes analysis and exchange every 48 h until degradation. Coating is carried out with sodium heparin via dip coating and determination of the heparin release is carried out by ELISA test. Variables of study are stent mass, mass fold change, degradation time, bacterial colonization and concentration of heparin released in artificial urine. Results: There is statistical significance in degradation times between all materials except between the Glycomer™ 631 alone and combined with PGA. Mass fold change analysis of the Glycomer™ 631 evidences an increasing trend of its mass during degradation. The combination of Glycomer™ 631 and PGA presents a progressive and gradual degradation, where PGA degrades at week 3 while Glycomer™ 631 remains intact until its fragmentation at the late stage of degradation. Heparin coating has no significant impact on mean degradation times and trends in any group, nor on bacteriuria rates; heparin concentration decreases significantly after 72 h. Products of degradation are released steadily with minimum dimensions. Conclusions: The combination of synthetic biodegradable polymers and copolymers with different degradation rates provides a gradual staged degradation. Heparin dip coating is a safe and feasible technique to coat biodegradable ureteral stents without interfering in degradation rates although it does not have a significant effect on the onset of bacterial colonization.

Keywords: Biodegradable ureteral stents, synthetic polymers, degradation, in vitro, dip coating, heparin coating, braided stent, heparin

Introduction

Double-J ureteral stents (DJS) are routine medical devices used in urological practice for a variety of indications. Their use is associated with the onset of stent-related adverse effects that cause a significant impact in the quality of life of stented patients [1]. Along with the adverse effects caused by DJS, the biostable nature of current DJS represents another major shortcoming. The need of a second procedure for device extraction entails anxiety, anesthetic risk for pediatric patients and health expenses [2, 3]. This economic burden increases by 6-fold when managing forgotten stents which, on the other hand, may lead to the loss of the kidney and even death [4, 5].

The development of biodegradable ureteral stents (BUS) represents one of the main milestones regarding current research for the improvement of ureteral stents [6]. Despite the inexistence nowadays of a commercially available BUS, several advances have been made at a preclinical level with promising results [3]. The main drawbacks that prevent the clinical valida-

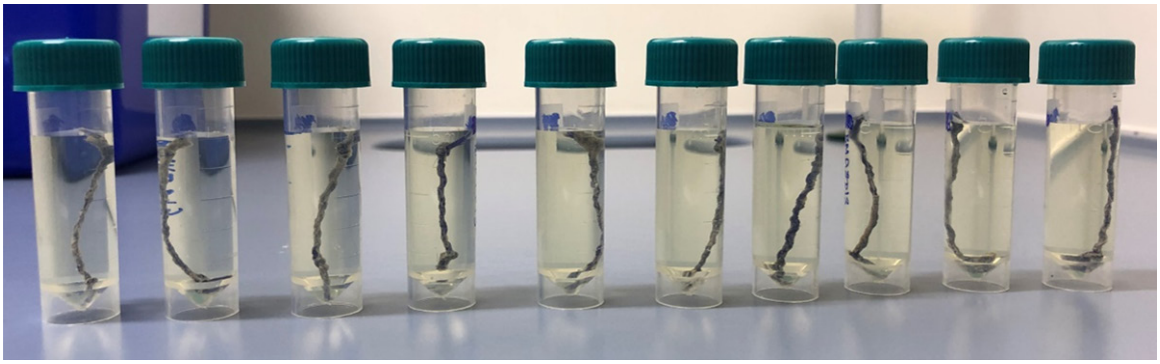

Figure 1. Disposition of the ten braided samples of coated Glycomer™ 631 and PGA in test tubes for incubation.

tion of these current BUS are the lack of control and predictability of degradation rates, the challenge of maintaining mechanical strength and the likelihood of obstruction due to degradation products [3]. Surface coatings and drug-eluting stents constitute another significant approach to improve the performance of current DJS. These technological developments focus mainly on the prevention of biofilm formation and encrustation, the treatment of patient discomfort and the local delivery of chemotherapeutic drugs [2, 6, 7].

Our group has developed a biodegradable braided intraureteral stent known as the BraidStent®. This design and its heparin-coated counterpart, the BraidStent®-H, have been assessed in the porcine model showing a safe and predictable degradation of 3-6 weeks, in agreement with the indications for these designs [8-11]. The rationale for the design and the composition of BraidStent® lies in the combination of biodegradable synthetic polymers and copolymers in a braided design for a controlled, predictive and non-obstructive degradation [8, 9].

Hereby we present the *in vitro* study of coated and uncoated braided biodegradable ureteral stents, made of synthetic polymers, in order to investigate their potential for the control of degradation. Based on the aforementioned, the objectives of this study are, firstly to assess the degradation performance in urine of different braided combinations of three synthetic polymers and copolymers. The second objective is to evaluate how a heparin dip coating can affect both the degradation of these biomaterials, and the occurrence of bacterial colonization.

Material and methods

This study comprised two trials, the *in vitro* comparative study of the degradation of the polymers and the *in vitro* assessment of the release of the heparin coating.

In vitro comparative study of the degradation of the polymers

The polymers and copolymers used in this study correspond to absorbable synthetic materials, whose biocompatibility and tissue absorption have been proven and described by the manufacturers. The three materials are polyglycolic acid (PGA), poly lactic-co-glycolic acid (PLGA), and Glycomer™ 631. They were chosen according to their reported degradation velocity in tissues, being the PGA and PLGA the ones presenting faster rates (60-90 days) and the Glycomer™ longer absorption time (90-110 days). The coating was carried out by using heparin sodium 25000 UI/5 ml Hospira (Pfizer, USA).

For this evaluation, ten groups were established with 10 samples each: group 1, heparin coated PGA; group 2, PGA; group 3, heparin coated PLGA; group 4, PLGA; group 5, heparin coated Glycomer™; group 6, Glycomer™; group 7, heparin coated Glycomer™ and PGA braided together; group 8, Glycomer™ and PGA braided together; group 9, 3-cm of a 7 Fr polyurethane DJS as positive control group and group 10, urine without samples as negative control group.

These polymers and copolymers were arranged in stent samples of 3 cm with a braided architecture with 4 threads (**Figure 1**), following the

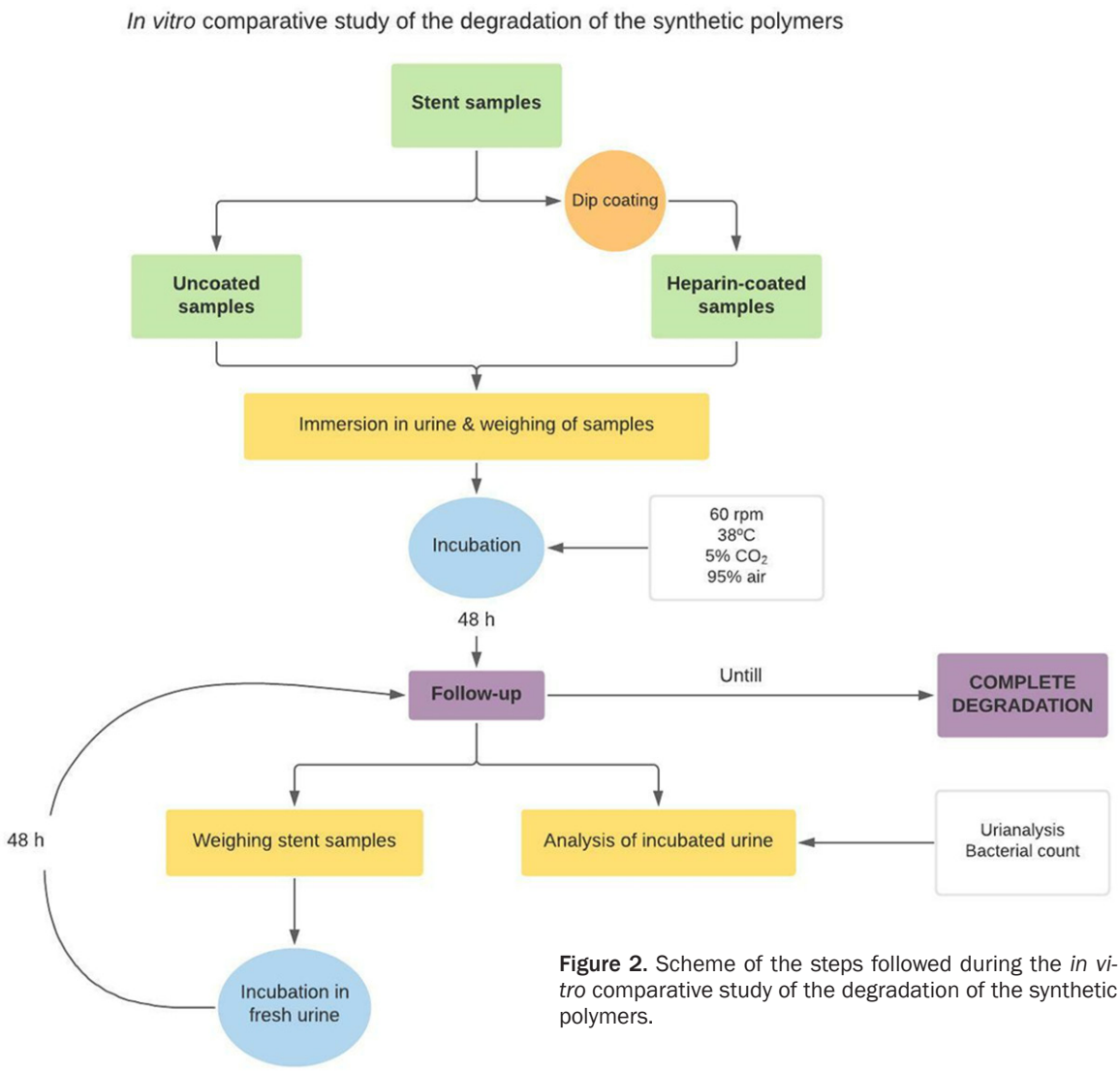

Figure 2. Scheme of the steps followed during the *in vitro* comparative study of the degradation of the synthetic polymers.

design of the ureteral stent BraidStent® developed by our research group [8]. The heparin-coated samples were subjected to the dip coating technique [12]. These stent samples were washed in isopropyl alcohol for 5 minutes. They were then completely immersed in heparin and dried in an oven at 60°C for 2 hours. This 2-hour period has been established by means of pilot tests prior to this study to determine the time required for the complete drying of the heparin. All the samples, including the DJS sections, once prepared and coated, were sterilised individually.

The study was developed by incubating on day 0, each sample of each group, in 4 ml of porcine urine in sterile test tubes. Prior to incubation, the samples were weighted twice on a precision scale. Given that stent are weighed along follow-ups with the aim of analyzing mass loss, baseline weight was also taken after hydrating the samples by immersion in urine from their respective tubes. Protocol is depicted in **Figure 2**.

The incubation medium chosen for this study was swine urine obtained via ultrasound-guided cystocentesis. This urine, previously to filling the tubes, is subjected to urinalysis and bacterial quantitative count. The cutoff point for the diagnosis of bacterial colonization was established at $\geq 10^5$ colony forming units (CFU)/ml [13]. The urine is obtained from a single animal.

The tubes are placed horizontally on a rack and subjected to continuous agitation at 60 rpm on

a Heidolph® Unimax 1010 shaker (Heidolph, Germany), at 38°C in an atmosphere of 5% CO_2 and 95% air [14]. The horizontal position and the continuous movement are intended to create incubating conditions mimicking the flow dynamics of urine.

Urine refills and follow-ups take place every 48 hours from day 0, until complete degradation of the samples. Samples were considered as degraded when they lost their braided conformation and it was not possible to manipulate them for weighing. Urine, which is exchanged by fresh medium, undergoes urinalysis and bacterial count on a Neubauer cell counting chamber. All biodegradable stent samples are weighed on the precision scale during medium replacement. In order to avoid contamination, during all phases of the study, material and urine handling, refills and weighing are carried out under sterile conditions under a Thermo Scientific™ MSC-Advantage™ (Thermo Fisher Scientific, USA) Class-II biosafety cabinet.

Degradation performance is comparatively analysed among groups 1 to 8, in order to evaluate how materials and braided conformation may affect these variables. Degradation performance is defined as degradation time and changes of the mass of samples through weighing along follow-ups. Degradation time is considered to be the incubation time until the stents lose their braided conformation and their handling is no longer possible. Bacterial colonization and urine pH are also compared, using groups 9, double-j stent, and 10, urine, as positive and negative control groups, respectively.

In addition, a macroscopic qualitative assessment of degradation of the polymers and copolymers is performed by direct observation. Observation indicators are defined as coloration, polymer morphology, texture and morphology of degradation products, degradation onset time and degradation duration. The evaluation of polymer morphology is carried out by assessing changes in the external appearance of the samples and hydration of the materials. The degradation products are categorized as either suspended particles or as fragments. Fragments are regarded as those degradation products that maintain similar characteristics to those of the original polymers or copolymers.

In vitro assessment of the release of the heparin coating

An additional study group is established, to assess the duration of the release of the heparin coating. Ten 3-cm samples of braided Glycomer™ 631 and PGA, with the same design shown in **Figure 1**, are coated with heparin via the aforementioned dip coating technique. Similar to the *in vitro* study of material degradation and bacterial colonization, the stent samples, placed in their respective sterile test tubes, are incubated with 4 ml of artificial urine (BioIVT, USA). A 500 µl sample is extracted immediately after immersion of the fragments in the solution, to evaluate the possible washout effect of heparin upon contact with an aqueous medium. The 500 µl extracted are replenished again with artificial urine.

The tubes are then placed in a rack, positioned horizontally in the Heidolph® shaker, under the conditions of 38°C temperature, 5% CO_2 atmosphere and continuous movement of 60 rpm [14].

Follow-ups consist of successive urine exchanges at 3, 6, 12, 24, 48, 72, 96 and 120 hours, in which, in addition to extracting 500 µl of each sample for storage in a 2 ml Eppendorf tubes, urine and test tubes are replaced. Samples are frozen at -80°C until further processing. The presence and concentration of heparin are analyzed using the ELISA Kit: Human Heparin Sodium (HS) ELISA Kit MBS3802043 MyBioSource® (MyBiosource, USA). The protocol of this trial is depicted in **Figure 3**.

Statistical analysis

The variables of study are the mass of samples along follow-ups, degradation time, urine pH, onset of bacterial colonization and heparin concentration expressed in ng/ml. For the pH analysis, data from samples with positive nitrites urine test were eliminated.

Additionally, to analyze the degradation rate and control the confounding factor of initial stent sample weight and its difference between groups, the ratio of mass loss over the follow-ups is calculated. It is represented as mass fold change [14].

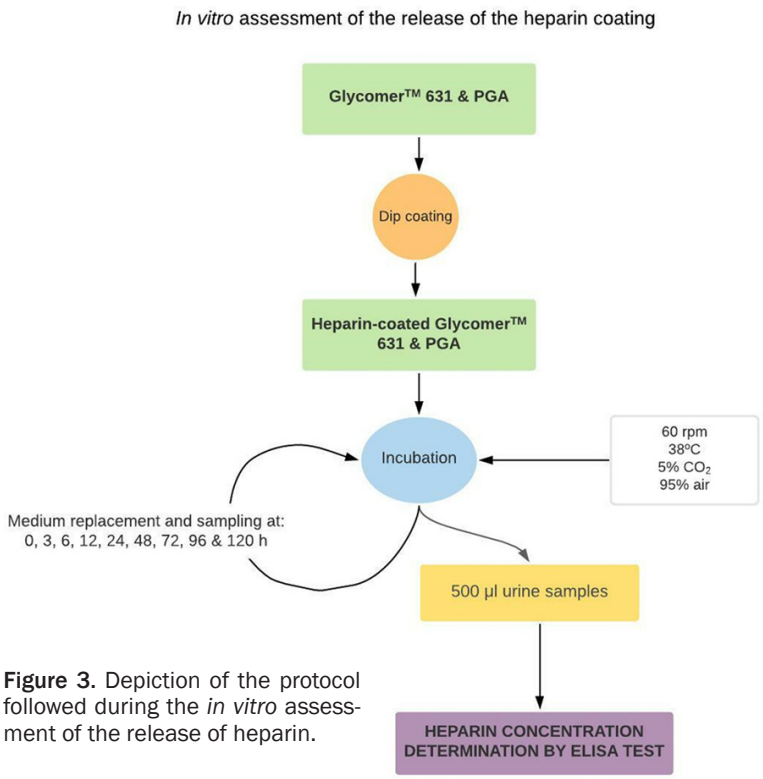

Figure 3. Depiction of the protocol followed during the *in vitro* assessment of the release of heparin.

Table 1. Mean degradation times in each group

Group	Days (mean ± standard deviation)
1. PGA-heparin	15.20±0.63*
2. PGA	16.20±1.03*
3. PLGA-heparin	45.70±1.89*
4. PLGA	41±5.91*
5. Glycomer™ 631-heparin	69.10±3.46
6. Glycomer™ 631	69.80±1.03
7. Glycomer™ 631 + PGA-heparin	65.83±6.83
8. Glycomer™ 631 + PGA	68.75±4.14

*Statistical significance. Significance level is set at P<0.05.

Statistical analysis was performed with the SPSS 25.0 program for Windows (IBM, USA). The variables pH, degradation time, weight and bacterial colonization were defined as the mean ± standard deviation, and the normality study of these variables was carried out using the Shapiro-Wilks test. Except for the data relating to sample weight, the variables that fit a normal distribution are compared using Student's t-test for independent samples, while the contrast of hypotheses between non-parametric variables is performed using the Mann-Whitney U test. The evolution of sample mass and mass fold change throughout the study is evaluated using a general linear model for repeated measures. The variable heparin concentration in ng/ml is subjected to a descriptive study of its trend throughout time. Moreover, the observation indicators corresponding to the qualitative assessment of degradation are not subjected to statistical analysis, but to a descriptive evaluation.

Results

Degradation of polymers and copolymers

The mean degradation times for each group are shown in **Table 1**. Comparison among groups shows significant differences in degradation times between all materials except between the Glycomer™ 631 group and the combination of Glycomer™ 631 and PGA. The heparin coating does not significantly impact these mean degradation times in any group. It is noted that PGA has significantly the shortest degradation times, in contrast to the longest shown by Glycomer™ 631 and the combination of Glycomer™ 631 with PGA.

The analysis of the general linear model for repeated measures evidences that the trend of degradation is significantly different between the 4 groups of materials. However, the trend is similar between each material and its coated counterpart. Unlike the Glycomer™ 631 coated and uncoated, the rest of the groups show a decreasing trend curve, the most steep being the two PGA groups. In the two Glycomer™ 631 groups, an increase in mass is observed until day 41, which does not start to decrease until day 61.

The degradation curves represented by the evolution of the sample weights and the mass fold change throughout the follow-ups are

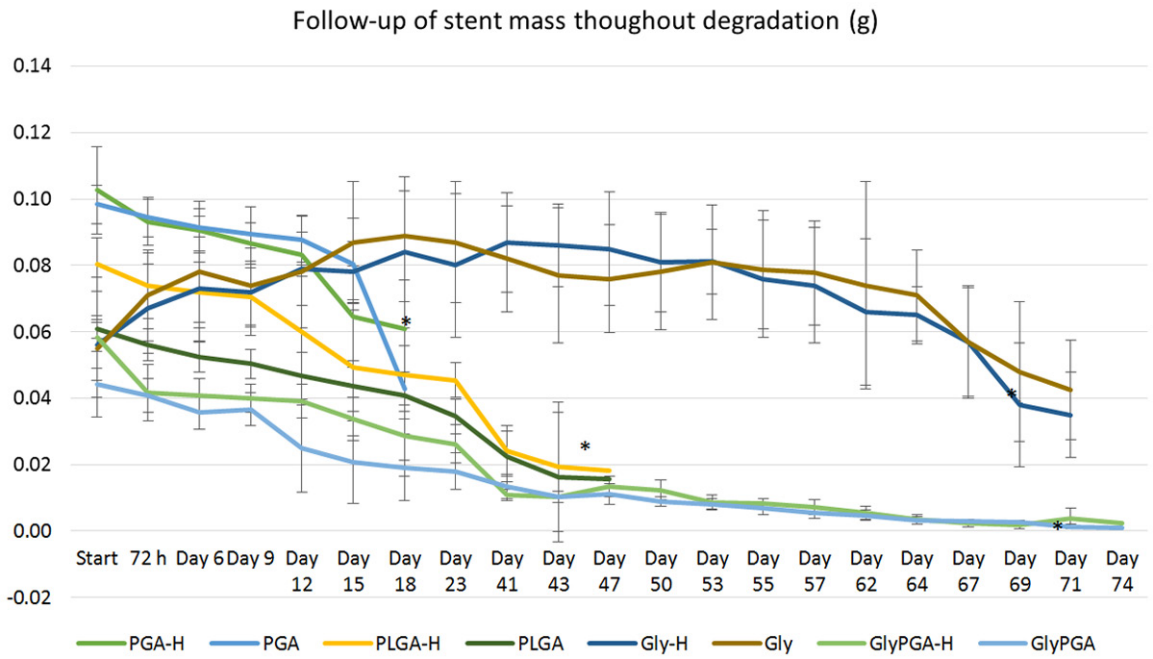

Figure 4. Degradation curves represented by the evolution of sample mass over time. Where PGA-H: heparin-coated PGA; PLGA-H: heparin-coated PLGA; Gly-H: heparin-coated Glycomer™ 631; Gly: Glycomer™ 631; GlyPGA-H: heparin-coated Glycomer™ 631 and PGA; GlyPGA: Glycomer™ 631 and PGA. All curves show significant differences between the different materials, but not between their heparin-coated counterparts ($P<0.05$).

shown in **Figures 4** and **5**, respectively. **Table 2** shows the initial and final weight of each group, and the percentage that the latter represents with respect to the initial weight.

The qualitative assessment of the effect of urine on the materials reveals that the degradation of PGA and PLGA is characterised by the fact that the materials increase in volume and lose their multifilament configuration as they hydrate, and degradation products are gradually released. These degradation products consist of suspended particles that provide the urine with a cloudy appearance. PLGA suffers discolouration after one week of incubation before starting its degradation. In contrast, Glycomer 631 is degraded by fragmentation and its degradation products maintain a similar appearance to their initial conformation. Concerning the combinations of Glycomer™ 631 with PGA, both coated and uncoated, show how PGA starts to degrade and release its products by week 3, while Glycomer™ 631 remains intact until the end of the whole degradation period, when it undergoes fragmentation. Aspect of degrading stent fragments and products of degradation is shown in **Figure 6**.

Bacterial counts and urine pH

As for bacterial colonization analysis, bacterial counts above 10^5 CFU/ml were obtained from the first follow-up at 48 hours. All materials, including the biostable DJS, show significantly higher bacterial counts compared to the control group of swine urine at all follow-ups. At two weeks, the biodegradable materials show a significantly lower bacterial colonization rate than the DJS, with no significant differences between them. Heparin-coated materials exhibit lower bacterial counts compared to their corresponding uncoated groups. However, these differences are not statistically significant (**Table 3**).

Urinary pH fluctuations remain similar in all groups during all follow-ups; there is not a significant variation along time within each group. Heparin does not cause pH changes compared to uncoated materials. Mean pH value for uncoated materials is 6.88±1.53, while coated samples exhibit 6.49±0.87 pH values.

Release of heparin

The results for the mean concentrations of heparin analyzed by the ELISA test are shown in

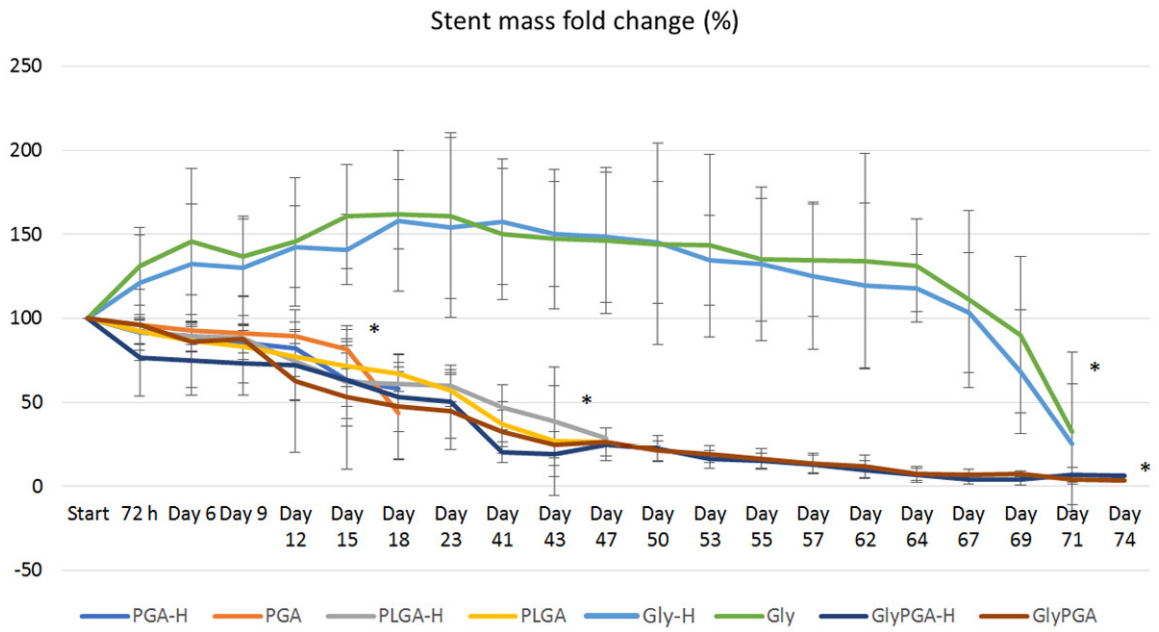

Figure 5. Degradation curves represented by mass fold change over time. Where PGA-H: heparin-coated PGA; PLGA-H: heparin-coated PLGA; Gly-H: heparin-coated Glycomer™ 631; Gly: Glycomer™ 631; GlyPGA-H: heparin-coated Glycomer™ 631 and PGA; GlyPGA: Glycomer™ 631 and PGA. All curves of different materials show significant differences between them, but not with respect to their heparin-coated counterparts ($P<0.05$).

Table 2. Initial and final weight of each group and ratio of remaining stent mass before complete degradation, expressed as a percentage

Group	Initial weight (mg)	Final weight (mg)	Final weight/initial weight (%)
PGA-heparin	102.60±13.03	66.10±15.27	56.42±27.08
PGA	98.38±5.71	42.73±26.25	61.75±14.00
PLGA-heparin	80.28±8.02	18.27±,19.51	41.64±17.51
PLGA	60.83±2.89	15.80±21.71	56.93±28.90
Glycomer™ 631-heparin	56.00±6.99	35.00±12.91	59.29±22.42
Glycomer™ 631	55.00±9.72	42.50±15.00	72.57±30.10
Glycomer™ 631 + PGA-heparin	58.33±18.01	2.40±3.11	8.07±6.14*
Glycomer™ 631 + PGA	44.17±9.96	1.10±0.67	5.12±2.34*

*Statistical significance. Significance level is set at $P<0.05$.

Table 4. Heparin is released steadily for the first 24 hours, then begins to decrease and after 72 hours it drops by 76.76% with respect to the peak concentration at 24 hours.

Discussion

Biodegradable ureteral stents represent one of the major lines of research concerning ureteral stents. The uncertainty of degradation rates and the onset of obstructive phenomena arisen from degradation fragments are issues that preclude the availability of BUS for clinical use [3]. The dynamics of the urinary system and the composition of urine represent a hostile environment for the development of biomaterials and designs that provide a safe and controlled degradation along with the maintenance of the functionality of the devices [15]. Three main approaches have been proposed according to the choice of biomaterials, which are natural origin polymers, synthetic polymers and metallic alloys [3, 16, 17]. In the present study, synthetic polymers and copolymers are chosen over natural origin polymers and metallic alloys. Synthetic polymers used for the man-

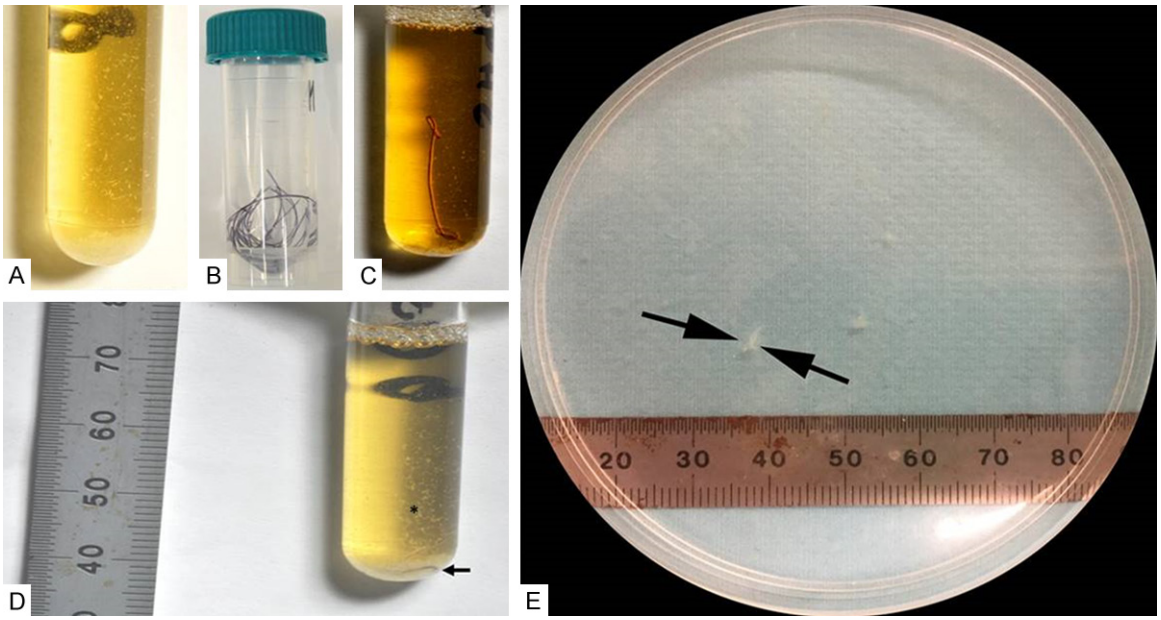

Figure 6. Degradation of the polymers and copolymers of study. A. Degradation products from group 3, coated PLGA. B. Fragments of uncoated Glycomer™ 631, group 6. C. Staged degradation of group 8, uncoated Glycomer™ 631 and PGA. The PGA has already degraded and provides cloudy appearance of the urine while Glycomer™ 631 remains unfragmented. D. Final degradation of group 8, coated Glycomer™ 631 and PGA. Star: suspended particles of PGA; arrow: fragment of Glycomer™ 631. E. Degradation product of PGA (group 1), the texture hinders manipulation for measurement, dimensions are below 4 mm.

Table 3. Bacteriuria expressed as CFU/ml in each study group at 48 hours and at weeks 1, 2 and 3

Group	Bacteriuria 48 h (CFU/ml)	Bacteriuria 1 week (CFU/ml)	Bacteriuria 2 week (CFU/ml)	Bacteriuria 3 week (CFU/ml)
Control	$2.13{\cdot}10^{6}{\pm}3.68{\cdot}10^{6*}$	$5.68{\cdot}10^{6}{\pm}1.11{\cdot}10^{6*}$	$2.00{\cdot}10^{4}{\pm}4.47{\cdot}10^{7*}$	$5.38{\cdot}10^{6}{\pm}1.57{\cdot}10^{6*}$
DJS	$1.61{\cdot}10^{8}{\pm}3.67{\cdot}10^{7*}$	$6.64{\cdot}10^{8}{\pm}2.20{\cdot}10^{8*}$	$9.19{\cdot}10^{7}{\pm}1.62{\cdot}10^{7*}$	$2.47{\cdot}10^{8}{\pm}5.13{\cdot}10^{8*}$
PGA-heparin	$4.34{\cdot}10^{7}{\pm}0.89{\cdot}10^{7}$	$6.43{\cdot}10^{7}{\pm}2.44{\cdot}10^{7}$	$4.94{\cdot}10^{7}{\pm}2.10{\cdot}10^{7}$	n.a.
PGA	$5.29{\cdot}10^{7}{\pm}3.11{\cdot}10^{7}$	$7.49{\cdot}10^{7}{\pm}4.21{\cdot}10^{7}$	$5.61{\cdot}10^{7}{\pm}2.33{\cdot}10^{7}$	n.a.
PLGA-heparin	$7.44{\cdot}10^{7}{\pm}6.32{\cdot}10^{6}$	$9.12{\cdot}10^{7}{\pm}3.60{\cdot}10^{7}$	$4.33{\cdot}10^{7}{\pm}8.81{\cdot}10^{7}$	$8.95{\cdot}10^{7}{\pm}0.43{\cdot}10^{7}$
PLGA	$7.90{\cdot}10^{7}{\pm}1.17{\cdot}10^{7}$	$1.45{\cdot}10^{8}{\pm}8.93{\cdot}10^{7*}$	$5.08{\cdot}10^{7}{\pm}3.12{\cdot}10^{7}$	$9.50{\cdot}10^{7}{\pm}2.42{\cdot}10^{7}$
Glycomer™ 631-heparin	$3.43{\cdot}10^{7}{\pm}1.86{\cdot}10^{7}$	$5.57{\cdot}10^{7}{\pm}9.73{\cdot}10^{6}$	$1.09{\cdot}10^{7}{\pm}1.90{\cdot}10^{7}$	$4.28{\cdot}10^{7}{\pm}6.57{\cdot}10^{6}$
Glycomer™ 631	$4.09{\cdot}10^{7}{\pm}2.90{\cdot}10^{7}$	$6.61{\cdot}10^{7}{\pm}1.45{\cdot}10^{7}$	$6.67{\cdot}10^{7}{\pm}2.60{\cdot}10^{7}$	$4.48{\cdot}10^{7}{\pm}2.16{\cdot}10^{7}$
Glycomer™ 631 + PGA-heparin	$3.86{\cdot}10^{7}{\pm}2.14{\cdot}10^{7}$	$4.90{\cdot}10^{7}{\pm}1.64{\cdot}10^{7}$	$7.23{\cdot}10^{6}{\pm}3.16{\cdot}10^{6*}$	$1.25{\cdot}10^{7}{\pm}1.99{\cdot}10^{7}$
Glycomer™ 631 + PGA	$5.11{\cdot}10^{7}{\pm}1.61{\cdot}10^{7}$	$5.09{\cdot}10^{7}{\pm}1.77{\cdot}10^{7}$	$1.20{\cdot}10^{7}{\pm}1.97{\cdot}10^{7}$	$1.43{\cdot}10^{7}{\pm}1.24{\cdot}10^{7}$

*Statistical significance. Significance level is set at *p* values below 0.05. n.a. Not applicable. DJS: double-j stent.

ufacturing of BUS have proven biocompatibility, are degraded mainly by hydrolysis, are free of corrosion issues and lack immunogenicity, carcinogenicity, teratogenicity and toxicity [18-20]. Their degradation properties can be controlled by variations in the length of molecular chains, chemical modifications and by the mixture of various polymers in different proportions [3].

As for the degradation rates of the polymers and copolymers of the present study, degradation in urine is remarkably faster than their reported absorption times in tissue [21, 22]. There is, however, a correspondence between short-, mid- and long-term rates, presenting the Glycomer™ 631 and its combination with PGA the longest degradation time, PLGA an intermediate rate and PGA the fastest degradation. Regarding the interpretation of these results, the poor correlation between *in vitro* and *in vivo* BUS degradation rates reported in the literature should be considered [23]. The

Table 4. Heparin concentration released in urine by coated Glycomer™ 631 combined with PGA, expressed in ng/ml (determined by ELISA test)

Hours	*Heparin (ng/ml)*
0	21.81±3.88
3	19.85±3.23
6	20.74±5.67
12	17.94±3.68
24	21.39±2.95
48	11.62±2.42
72	4.97±0.70
96	4.72±0.71
120	4.56±0.50

PCL-coated alginate BUS developed by Barros et al. [23] shows an *in vitro* degradation of 14-60 days, while *in vivo* it degrades at a significantly faster rate of 10 days. Similarly, the BraidStent®-H, made by the same combination of Glycomer™ 631 and PGA used in this study, degrades *in vivo* at 35-42 days, in contrast to the 65.83-68.75 days shown *in vitro* [11]. This indicates that *in vivo* urinary tract conditions are very likely to shorten the degradations times observed in this *in vitro* study, owing to the difficulty of reproducing in a laboratory setting all the conditions involved in the degradation of BUS. In addition to the materials and their behavior in urine, the urodynamic repercussions of the designs, the possible presence of vesicoureteral reflux, ureteral peristalsis, variations in urine composition and pH fluctuations have also to be considered [20]. In view of these limitations, the interpretation of the results obtained *in vitro* represents a critical point for the translation to the *in vivo* environment [23]. Moreover, dynamic models may be of use to study degradation times more reliably. Unlike the static model presented hereby where urine is subjected to stirring and exchanges, dynamic models provide an inflow and outflow, which is closer to the urine flow in the ureter *in vivo* [24]. Nevertheless, most of *in vitro* BUS studies performed so far have used static models [16, 25, 26].

Preclinical evaluations of BUS developed so far have defined 28-42 days as satisfactory degradation rates, although indications of these devices often remain unmentioned [26-29]. Stenting time is determined according to the clinical indications for which the stent is placed and thus will differ depending on the condition suffered and treatment undergone by the patient. Therefore, BUS degradation rates should also be based on the indications these devices will be used for, which is likely to lead to the existence of BUS categorized in different degradation ranges [10]. To this end, the availability of polymers and copolymers with different degradation rates, as in the present study, should enable the modulation of the degradation. According to our results, for short-term stenting, PLGA may be a suitable material; whereas combinations of Glycomer™ 631 will provide longer degradation times which, as seen in previous animal model studies of the BraidStent®, correspond to an *in vivo* stenting time within 3-6 weeks [8]. Concerning PGA, the degradation curve depicted on **Figure 4** suggests that this biomaterial in the present braided conformation may provide an abrupt degradation very likely to be excessively short at its translation to an *in vivo* context.

The combination of different polymers and copolymers has been described by several authors as an strategy to control the duration of degradation [17, 29, 30]. The BUS made by the combination of PGA and PLGA by Zou et al. [30] has shown *in vitro* that crystallisation of the PGA and the variation of the ratio of PGA and PLGA allows longer degradation rates and the control of mechanical properties. The group of Wang et al. [17] have developed by electrospinning a BUS composed of polycaprolactone (PCL) and PLGA, where increases in PCL proportions provide longer degradation times and higher mechanical strength. In addition to the accuracy of degradation rates, manufacturing BUS with polymers with different degradation velocities promotes safe and gradual degradation [25, 26, 29]. The use of a core material forming a membrane or a mesh which is covered afterwards by polymers that degrade at a higher speed generate a two-phase staged degradation. The breakdown of the materials that cover and fill the BUS structure occurs firstly, without compromising the device's integrity, and once the membrane is exposed to urine, complete degradation takes place [25]. The braided stents of the present study follow this same degradation principle, since PGA and PLGA degrade and release their products gradually, while Glycomer™ 631 fragments only

at the later stages. Therefore, the behavior shown by PGA and PLGA suggest that they may be of use as integrated biomaterials, whereas Glycomer™ 631 has demonstrated potential as core material for a braided BUS. Moreover, qualitative assessment of degradation revealed that the braided conformation resulted in minimal fragments and particles in all eight biodegradable groups in this study, suggesting that this arrangement may provide safe degradation.

As shown on **Figures 4** and **5**, when combining PGA and Glycomer™ 631, the latter leads the overall degradation rate since there are no significant differences with the Glycomer™ 631 by itself. The addition of PGA provides however, a progressively decreasing trend of degradation in contrast to the curves shown by both coated and uncoated Glycomer™ 631 incubated independently. The ascending trend observed in these latter two groups suggests that the hydrolysis process of Glycomer™ 631 entails a hydration, which increases its mass. A sudden drop is observed after 64 days, which is consistent with the fragmentation of this copolymer and indicates the final stage of degradation. This rise in mass can be associated with an increase in volume, which represents a potential risk of obstruction. When both materials are incubated braided together, the PGA may act mitigating Glycomer™ 631's hydration process.

The percentages of remaining stent mass prior to complete degradation shown in **Table 2** are significantly different in the combined Glycomer™ 631 and PGA groups. The Glycomer™ 631 with and without coating degrades when it reaches up to 72.57% of its mass, while the combined Glycomer™ 631 and PGA groups maintain stent integrity until their mass represents 5.12-8.07% of their initial weight. High values of this ratio represent an increased likeliness of obstructive phenomena, as they indicate that the stent will disintegrate when its mass is closer to initial values. Besides the aforementioned compensation of the hydration of the copolymer, the groups of Glycomer™ 631 braided with PGA may provide greater safety during degradation, since prior to its complete degradation its mass represented less than 9% of the initial BUS. Based on this, it is also likely that the loss of mechanical properties will be lesser in this group in comparison to single material stents, although mechanical tests are necessary to prove it.

Concerning the heparin coating of the stents, dip coating is included among the main techniques for ureteral stent coating, representing the simplest and most innocuous approach to coat the samples of the present study. It has shown to be safe and does not significantly modify degradation time and trend of the materials. However, the heparin coating has not shown to be effective against urine colonization. This non-significant effect of the heparin may be due to duration of the coating itself, whose concentrations decrease notably after 72 h of incubation, or to the drug chosen for the coating. Despite having demonstrated an anti-encrustation effect both *in vivo* and *in vitro*, there is no consensus on the antibacterial effect of heparin-coated ureteral stents and the results of the present study do not significantly favor its *in vitro* effectiveness [11, 31, 32].

Given that achieving a good balance between controlling an appropriate degradation rate and maintaining sufficient mechanical strength of the device is a key challenge un BUS development, the fact that no mechanical tests were performed in this study, represents a limitation [3, 16]. Another limitation of the present investigation is the non-dynamic conditions used for incubation, which may explain the gap between degradation times obtained *in vitro* and those reported previously *in vivo* [8-11].

The combination of the synthetic polymers and copolymers used in the present study for the manufacturing of BUS provides a gradual staged degradation owed to the different degradation rates of each biomaterial. The polymer with longest degradation rate comprises the core material of the stent and its arrangement in a braided design with secondary polymers enhances the safety of the release of degradation products. The heparin dip coating does not cause interferences with the degradation of the polymers that constitute the device. However, this coating has not provided a significant decrease in bacteriuria levels.

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Disclosure of conflict of interest

None.

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