Management Committee Meeting of COST Action CA16217 “European network of multidisciplinary research to improve the urinary stents” REPORT

Porto, Portugal
1st-2nd February 2018

Scientifc advances in technical WGs including publications

. WG1. D. Rako (Vice-Leader WG1). The WG1 leader commented on the progress in preparing the database for the two scientific reviews that are being carried out in this WG1. It was also mentioned that Prof. Osther had decided to withdraw from ENIUS for personal reasons. It is proposed to Dr. H. Jung (Denmark MC member) as leader of WG1.

WG2. F. Clavica (Vice-leader WG2) was explaining the changes that WG2 members have made to the MoU to improve the objectives and make them more realistic. In addition to proposing a Workshop for September in Oxford (UK) within the activities of this WG2. The AC commented that for dissemination and to make travel expenses profitable, it was better to carry out for two-three days TS where trainees could have a practical part. WG3.

S. Stavridis (leader WG3), commented on the preparations being made to begin the work of WG3. Commenting on the need to request more support to bring together a multidisciplinary group due to the workload of this group.

WG4. A. Barros (leader WG4) presented the objectives of this WG. Commenting that the workload starts the third quarter of GP2. WG4 like the previous groups needs to increase the number of participants. It is foreseeable that the website will help us to increase the number of participants. WG5. G. Ciardelli (leader WG5) presented a brief review of the state of the art in this WG. Discussing the aims and milestones of this WG. The leader WG5 also proposed to hold a workshop in conjunction with the WG4 due to the points they have in common. It was proposed that due to the interest of this Workshop could be held the day after the MC meeting in 2019.

WG6. There was no presentation or comments in this WG as there was no presence of the leader or Vice-leader of this WG. No GP1 workload.

Science Communication Manager. Nuno Azevedo (Portugal) (Science Communication manager). N. Azevedo presented the ENIUS website (www.enius.org). As well as the ENIUS logo. We also talked about the opening of @enius.org on twitter and Facebook for the dissemination of ENIUS news and related topics. Future improvements to the website were discussed. Above all, the intranet and its usefulness as a forum for the activities of the WGs. Besides being the best tool for the dissemination and recruitment of new participants in the network. N. Azevedo comments the link to intranet: http://repository.enius.org. Members will receive a login and password in the next few weeks to access the website's intranet. The registration in the website (intranet) required the verification by AC. S. Tofaid (Irish MC Member) comments the inclusion of the ENIUS webpage in http://www.cost.eu/COST_Actions/ca/CA16217 to improve ENIUS visibility.

Short Term Scientific Missions (STSM): review of completed reports and new applications. Dario Carugo (STSM Coordinator).. Currently, the 4 applicants allocated for STSM in this GP1 are filled. A. Barros; A. Mosayebi, I. Cortese and M. Growochiz.

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Implementation of Cost polices on: Promotion of Gender balance. Gender Balance Coordinator. Valentina Cauda from Italy. Currently, only 30.7% of MC members are women and 27.3% of Core Group members. V. Cauda suggested some actions to facilitate the integration of women into ENIUS activities: Facilitate more trips during STSM, so you can visit the family. A kindergarten service available during ENIUS meetings. Specific awards for women for their contributions in poster or communications during the TS or workshops. It reaffirms the need to ask the Science officer the possibility of the aforementioned changes. And in the next invitation to ask the attendees the need for a childcare service included in the meeting.

Dissemination planning (Publications and outreach activities). During this GP1 only the WG1 members have a scientific manuscript as its milestone. About Dissemination planning. AC comments that dissemination has already begun among the Spanish urological community of ENIUS. Attending 2 Urology Congresses to disseminate ENIUS objectives. (XXVIII Spanish Meeting of Lithiasis, Endourology, Laparoscopy & Robotics Section of AEU. January 2018) and (XXXV Urology Residents Meeting from Valencia and Murcia Regions. October, 2017). The next Dissemination activities will be: 36th World Congress of Endourology and SWL, Paris, September 20-24, 2018. Endourological Society. And LXXXIII Spanish Congress of Urology. 13-16 June, 2018.

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SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

Action number: CA16217
STSM title: Composite organic/inorganic coatings for drug-eluting urinary stents
STSM start and end date: 15/04/2018 to 21/04/2018
Grantee name: Marta Grochowicz

PURPOSE OF THE STSM

The main purpose of the carried out STSM was to develop the method of synthesis of innovative antibacterial and drug-eluting stent coatings using porous zinc oxide (ZnO) nanomaterials developed by Politecnico di Torino and linear polymers obtained in Maria Curie Sklodowska University in Lublin. The antibacterial properties of ZnO and its regular porous structure make possible to use this material as a drug delivery carrier. However, the preliminary study showed that the release of the drug from the porous ZnO system is too fast and a burst release effect was observed (M. Laurenti, V. Cauda Materials, 2018 2018, 11(2) 314). The aim of this STSM was to slow down the release of drug by impregnation of the oxide/drug system with the solution of linear polymer having hydrogel character. After the evaporation of the solvent, the barrier layer of the polymer was formed on the surface of ZnO. The completed STMS is strongly aligned with the key aims of the ENIUS Action.

The additional purpose of the visit was to get familiar with procedures of obtaining the porous ZnO nanolayers and microparticles of ZnO, in the shape of desert roses, followed by characterizing them by means of FESEM technique. During my STSM the objectives planned in the application for the STSM were achieved. The mission was fully successful in training and getting practical skills as well as building the collaboration with Prof. Valentina Cauda and Dr Marco Laurenti from Politecnico di Torino.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

I started my STSM with the theoretical learning of the process of depositing porous ZnO coatings onto the flat hard substrate, i.e. silicon wafer as preliminary substrate. The process of ZnO thin film preparation is a two-step procedure: firstly the deposition of a porous Zn film by radio frequency magnetron sputtering at room temperature took place; secondly the oxidation/conversion of the Zn layer into ZnO was performed, by exposing the metallic thin film to water vapor.

In the next step, the adsorption process of model organic molecule was performed on earlier prepared silicon wafers covered by a thin layer of porous zinc oxide. As model molecule, the calcein dye was chosen. The adsorption was carried out from 2 mL of aqueous 1mM solution of calcein, during 2 h, at room temperature under orbital shaking (200 rpm). After adsorption, the samples were dried with the stream of compressed nitrogen and stored in darkness to prevent the UV degradation of calcein.

Subsequently, the porous ZnO samples with adsorbed calcein were covered by polymer solutions. Two different techniques were tried: vacuum impregnation and drop casting. The polyHEMA and poly(HEMA-co-acrylic acid) solutions in methanol in 10% and 20%wt concentrations were tested for infiltration of ZnO. The vacuum infiltration procedure was carried out in a round glass flask and started with evacuation of air for 10 minutes, then the polymeric solution was injected into the flask over the porous ZnO film and left for 30 minutes under vacuum pumping.

The calcein release study were then carried out to evaluate the influence of polymer impregnation on the

kinetics of its release, in comparison to the pristine ZnO materials. Moreover, since both polyHEMA and poly(HEMA-AAc) have a swelling behavior depending on the water-based solution pH, the release of calcein was assessed at two different pH. In particular, two calibration curves were prepared in buffered saline solutions at pH 5 and 7. The calibration points were determined for 1mM, 100 μM, 10 μM and 1 μM concentrations of calcein. The calcein was then released into 2 mL of buffered solution of pH 7 for 2 h, then the plates were put into a citric acid buffer solution of pH 5 and the release experiments were continued until 24 h at 37℃ under orbital shaking (200 rpm).
Moreover, we performed the characterization of obtained porous ZnO films covered with polymer. The chemical structure of the prepared materials was proved by means of infrared spectroscopy. I also learned the techniques for morphology and chemical composition investigation including, Field Emission Scanning Electron Microscopy (FESEM) and Energy Dispersive Spectroscopy (EDS). This experience allowed me to expand the knowledge of material requirements for medical device applications.
At the end of my STSM, I had also the chance to visit the laboratory of Prof. Gianluca Ciardelli. Prof. Ciardelli is the member of the Action CA 16217, in particular he is the leader of WG5, involved with development of drug eluting stents based on the polymers, mainly polyurethanes and biopolymers.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Firstly, following the learned process of depositing porous ZnO coatings onto flat hard substrates by sputtering technique, we preformed the adsorption of calcein on the ZnO plates. Calcein was chosen as a model molecule due to its availability and the easy detection by UV absorption spectra with the use of multiplate reader.

In the following step, the infiltration experiments of polymeric solutions into ZnO – calcein films were performed. We intended to use such polymers, which are of the nature of hydrogels swelling in water conditions. For this reason, we used poly(2-hydroxyethyl methacrylate) – polyHEMA, and poly(2-hydroxyethyl methacrylate-co-acrylic acid) – poly(HEMA-AAc) solutions in methanol with concentration of 10%wt and 20%wt. Starting from 1 mL of polymeric solutions, we prepared four types of ZnO-polymer composite films with adsorbed calcein using the vacuum infiltration approach (Table 1). However, at the beginning we eliminated the poly(HEMA-AAc) 20% due to the crush of final layer of polymer. To evaluate the results of the impregnation we performed FESEM analysis. Exemplary images are presented in Fig 1. The microscope analysis showed the success of the impregnation processes. The thickness of polymer layers measured in cross-section mode was in the range from 26 μm to 136 μm. In contrast, whereas the thickness of ZnO layer is about 12 μm, meaning that the thickness of the polymer layer had to be reduced. For the further studies, we choose the 10%wt solution of polyHEMA and performed the infiltration in two ways: vacuum impregnation and drop casting, using the amounts of polymer solution given in Table 1. The FESEM analysis indicated, that the polymer layer thickness was significantly reduced by using smaller amount of polymeric solutions. The cross-section analysis will be made in the near future to assess the quality of the polymeric impregnation inside the porous ZnO structure. Moreover, the FTIR analysis of ZnO-polymer composite films was performed. The comparison of spectra of ZnO-polymer composite and pure polymer did not show any differences in the structure of polymer after infiltration process.

Table 1. Parameters of the infiltration process

Sample	Polymer amount	Thickness of polymer layer
Vacuum infiltration		
ZnO-polyHEMA_10	1 mL	30 μm
ZnO-polyHEMA_20	1 mL	136 μm
ZnO-poly(HEMA-AAc)_10	1 mL	26 μm
ZnO-poly(HEMA-AAc)_20	1 mL	Crushed layer
ZnO-polyHEMA_10	100 μL	To be measured
ZnO-polyHEMA_10	50 μL	To be measured
Drop casting		
ZnO-polyHEMA_10	100 μL	To be measured
ZnO-polyHEMA_10	50 μL	To be measured

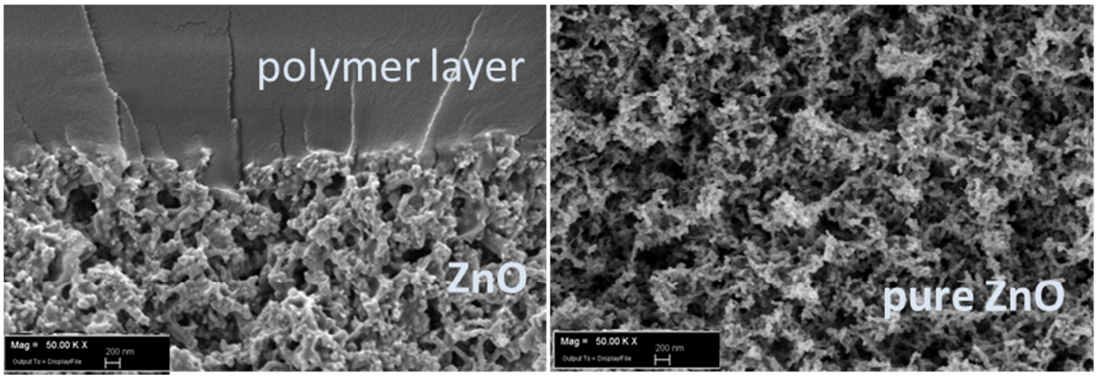

Fig. 1. FESEM images of pure ZnO (right) and ZnO-polyHEMA_10 (left)

In parallel, we carried out the preliminary release experiment of calcein from ZnO- polyHEMA_10, ZnO-polyHEMA_20 and ZnO- poly(HEMA-AAc)_10 plates, to evaluate the influence of polymer impregnation on the kinetics of its release, in comparison to the pristine ZnO material. However, this part of the project could unfortunately not be completed within the STSM, we will continue the release experiment to determine the exact amount of adsorbed and released calcein. At this moment, it can be only stated that the copolymer poly(HEMA-AAc) do not prevent the fast release of calcein, which was completely desorbed in two hours.

Additionally, I had the opportunity to synthesize the ZnO in the form of flower like structure, called desert roses, with an hydrothermal synthetic approach. During this synthesis a precursor of zinc, zinc nitrate hexahydrate, was combined with a base, potassium hydroxide, under vigorous stirring in water solution and then thermally treated at 70 ℃ for four hours. The obtained white powder was then filtered and dried, then characterized by X-Ray diffraction and FESEM techniques.

FUTURE COLLABORATIONS

Over the course of this STSM we started the release experiments from porous zinc oxide covered with the barrier layer of polymer. We are planning to extend the research with the use of nonsteroidal anti-inflammatory drugs such as ibuprofen and diclofenac. Moreover, characterization of the swelling properties and the thermal behaviour of the obtained polymer/porous ZnO material containing drug molecules will be made. As a result of our collaboration we are planning to publish at least two scientific papers in which ENIUS will be acknowledged and propose new future STSM or Master student exchange among the involved institution.

SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

The STSM applicant submits this report for approval to the STSM coordinator

Action number: CA16217
STSM title: Peptide based coating applied to natural-origin based ureteral stents
STSM start and end date: 05/02/2018 to 19/02/2018
Grantee name: Alexandre Barros

PURPOSE OF THE STSM/

The aim and motivation for this STSM are to combine the knowledge of antifouling peptides that have been developed in last years from at Prof. Meital Reches research group and apply as an antimicrobial coating onto the natural-origin based stents developed by the 3Bs research group. Currently, nearly 100% of the people who have a urological stent are likely to develop a bacterial infection within 30 days, which increases morbidity threefold. Different coatings have been applied to the conventional stents the ideal stent has not been designed yet. The two weeks spent in Prof. Reches lab will allow learning how we can obtain these antifouling peptides and how we can incorporate them as a functional stent coating that resists bacterial and biofilm formation. Using this peptide-based coating we would be able to prevent protein adsorption and interrupt stent biofilm formation. During the two weeks at the Hebrew University, the peptide sequence that I will use has three elements that enable (i) its self-assembly into a coating, (ii) its adsorption onto any substrate and (iii) its antifouling activity. The element that direct the assembly of the peptide comprises two adjacent fluorinated phenylalanine residues. The amino acid 3,4-dihydroxy-L-phenylalanine (DOPA) is the main constituent of mussel adhesive proteins (MAPs), the glue proteins of marine mussels. DOPA itself can adhere to various surfaces and this amino acid will be the glue of the peptide on the stent. To coat the ureteral natural-based origin stents with the peptides, we will simply dip the stents in the ethanol-peptide based solution.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

Preparation of biodegradable ureteral stents

Biodegradable ureteral stents (BUS) were developed according to the procedure described by Barros et al. (Barros et al., 2015a). The stents were composed of 30% alginic acid sodium salt, 65% of gelatin and 5% of bismuth (III) carbonate. The cross-linking solution was calcium chloride ($CaCl_2$) at 0.48 M. Briefly, polymers were dissolved in hot distilled water (70 °C). The solution was 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. BUS were then transferred into a crosslinking solution of calcium chloride ($CaCl_2$), at room temperature. After crosslinking, BUS were relocated in an alcoholic solution (100% ethanol) to obtain an alcohol gel. BUS were dried using a high-pressure vessel with supercritical carbon dioxide ($scCO_2$) at 40°C and 100 bar for 90 min, in continuous mode.

Figure 1 – Biodegradable ureteral stents from natural origin polymers prepared.

Property - Peptide Synthesis

The peptide sequence, which we designed, contains three elements that enable (i) its self-assembly into a coating, (ii) its adsorption onto any substrate and (iii) its antifouling activity. The element that we chose to direct the assembly of the peptide comprises two adjacent fluorinated phenylalanine residues. Due to aromatic interactions, the dipeptide diphenylalanine and its fluorinated analogs can self-assemble into stents surface.

The peptide that we used to coating the stent was: DOPA-Phe(4F)-Phe(4F)

Peptide- Coating of the stents

The coating of the stents was performed by immersion the biodegradable ureteral stents in a 0.5, 1 and 2 mg/ml peptide dissolved in pure ethanol overnight. Stents were washed three times with absolute ethanol to remove the uncoated peptide.

Figure 2 – Biodegradable Ureteral Stents immersed in peptide solution.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Due to the short period of this STSM, we only performed few analysis of the coated stents performed at Prof. Reches lab. A full physic-chemical characterization will continue at the 3Bs Research Group.

Contact Angle and XPS were characterized to the coated stents during the STSM.

To determine if the peptide indeed generated a "Teflon-like" coating on the surface of the stents we measured their contact angle. As we expected, the peptide coating stents exhibited an increase in the contact angle, indicating an increase in the surfaces' hydrophobicity. This result was the first screening to confirm the presence of this peptide indeed coated the biodegradable ureteral stents.

Using X-ray Photoelectron Spectroscopy (XPS) analysis, we characterized the peptide-coated biodegradable ureteral stents. When compared with non-coated stents, the signals resulting from the stents coated indicated the presence of fluorine, confirming the presence of the coating.

Comparing the three different concentrations of peptide solutions we concluded the best concentration for coating the stents is 0.5 mg/ml.

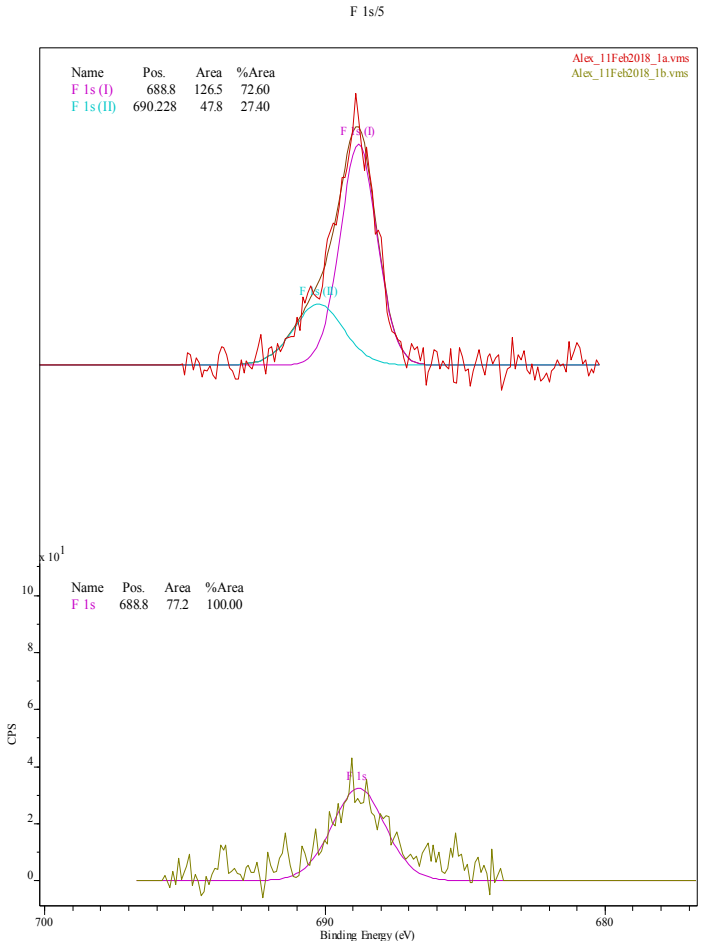

Figure 3 –XPS analysis of a stent coated with the peptide. The result proves the presence of fluorine .

FUTURE COLLABORATIONS

This STSM objective was achieved, as we expected to exchange knowledge between the two researcher groups and investigators. Due to the excellent preliminary results as output one scientific publication in an international scientific journal is expected. In this sense, the collaboration will continue with the full characterization of the coated stents at the 3Bs Research Group. The peptide will be synthesized and deliverd to 3Bs by the Reaches lab . With the knowledge acquired during this STSM, 3Bs researchers will

be able to perform the coating with this peptide.

During this STSM a new exploring ideas were discussed and will be tested during the coming months with exchange researchers between both labs.

Fabrication of stents with conical side-holes: first prototypes

1.1 Outline

The present report describes the research conducted during the Short Term Scientific Mission of Ali Mosayyebi (University of Southampton, UK) at Artorg Center (University of Bern, Switzerland) presents the development and characterisation of a first methodological approach towards the manufacturing of ureteric stents containing conical side-holes. Holes' fabrication was performed on hole-free ureteric stents (obtained from a commercial stent manufacturer), using a milling method developed in-house.

The aim of this study was to evaluate the accuracy and robustness of the method in creating inclined/conical side-holes through a ureteric stent. Although a vertex angle of 45° provided the best performance against encrustation in the microfluidic-based models an angle of 90° was selected in the present study, due to the thickness of the stent being greater than 0.3 mm. A greater vertex angle was thus required to increase the resistance of the vertex tip of the triangle, against deformation during milling. Dimensional and morphological characterisation of the manufactured side-holes was performed, in order to assess the fabrication accuracy and repeatability.

This preliminary study may open new avenues in the manufacturing of stents and catheters containing microscale features, which could potentially expand to include other medical devices with architectural features of comparable shape and dimensions.

1.2 Introduction

There are different manufacturing processes and technologies that have the potential for fabricating medical devices containing micrometre and/or millimetre sized features [1]. Ureteric stents are currently fabricated following a two-step method: an hollow polymeric tube is first produced *via* extrusion [2], and side-holes are subsequently punched at pre-defined locations along the tube [3]. Manual or automated punching however, does not offer the level of control and the resolution required to manufacture sub-millimetre, conical side-holes in stents.

This study was supported by the COST Action "European network of multidisciplinary research to improve the urinary stents" (CA16217), and conducted in collaboration with the University of Bern.

There is an IP associated to this work as follow: *A. Mosayyebi, X. Zhang, C. Manes, D. Carugo; UK priority patent | application GB1714337.1 | filed 6th September 2017 | Patent Title: Stent with streamlined side-holes*

A manufacturing method for this specific application should fulfil the following requirements:

a. Ability to create small holes, within the range 500 μm - 800 μm in diameter (as in commercial stents [4]), with fine control over the obtained hole shape;
b. Suitability for usage on thin and flexible tubes, made of polymeric materials (specifically silicone and polyurethane);
c. Should not irreversibly alter the chemical or physical properties of the bulk material of the stent [5];
d. Cost-effectiveness, scalability, and compliance with regulatory requirements [6].

The above criteria limit the range of potentially suitable manufacturing techniques to micro-milling, laser cutting, and water-jet cutting [7]. These are described below, to facilitate the identification of the most appropriate fabrication method.

1.2.1 Micro-Milling

Micro-milling is defined as the mechanical interaction of tools with micron-size cutting grooved tips with a substrate material, resulting in subtraction of a defined amount of material along pre-defined paths [8]. It is largely employed in the fabrication of biomedical microdevices, at relatively low cost and with potential for automation and scaling up [7, 9, 10]. Moreover, it has demonstrated compatibility with different materials, encompassing metals, ceramics, and polymers [11]. A potential challenge associated with milling of flexible elastomers - and of tubular shapes in particular - is the deformation of the substrate during milling. It could however be overcome by a careful selection of the milling tools, spindle speed (especially for smaller features), and supporting material [12].

1.2.2 Laser Cutting

Laser cutting relies on the focusing of thermal energy to a small spot (or around 0.2 mm) on a substrate, in order to vaporise or melt the substrate material with little mechanical stress. There are different types of lasers that differ based on the active medium used for light intensity amplification [13]. Different mechanisms of laser cutting include inert gas melt shearing, reactive gas melt shearing, vaporization, chemical degradation, and scribing [13]. Except for scribing, these methods could be employed on polymeric substrates (including polymeric elastomers). A challenge associated with the use of lasers on polymeric materials is the deformation of the cut zone and the potential

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melting of material outside the cut zone. These could however be addressed by using a high speed laser, such as a picosecond laser, to reduce unnecessary laser beam interaction with the material [13]. High-speed lasers would however significantly impact on the overall fabrication costs, limiting the scalability of this method.

1.2.3 Water-Jet Cutting

Water jetting uses a high-pressure jet of water in order to cut a substrate along a pre-defined path [14]. It utilises pure water or water containing abrasive particles (to assist cutting of harder materials such as steel) [14, 15]. Compared to other techniques, water-jet cutting is associated with minimal generation of hazardous by-products or fumes [16]. However, cutting holes with diameter smaller than the substrate thickness is challenging; it would require an initial drilling step prior to water jetting, in order prevent the substrate from breaking. This would significantly increase the overall fabrication times and costs.

1.2.4 Selection of the most suitable manufacturing method

Based on the above considerations relating to each candidate fabrication method, and taking into account aspects relating to regulatory approval, access to instrumentation, and ability to customise the experimental setup and vary its functional parameters, micro-milling has been selected as the most suitable manufacturing method.

Therefore, the following sections will describe the development and characterisation of a micro-milling apparatus, which has been customised to generate conical side-holes through a commercial ureteric stent lumen. It is herein hypothesised that a careful selection of the milling tools and operational parameters, together with control over the alignment between milling tool and stent, would provide a platform for creating inclined and conical side-holes of controlled shape.

1.3 Materials and methods

The main aim of the current study is to establish a methodology framework for generating conical side-holes; future adaptations will focus on improving the experimental apparatus towards the manufacturing of stents with different dimensions of their side-holes and lumen.

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1.3.1 Milling setup

Hole-free ureteric silicone stents were used as a substrate in this study, and were purchased from a stent manufacturing company (Ningbo GreatCare Trading Co., Ltd., Ningbo, China). A stent with outer diameter of 7 Fr (~2.3 mm), thickness of 0.4 mm (which was the minimum stent thickness available from this manufacturer), and length of 26 mm was employed for conducting this investigation.

The milling machine used in this study was a Picomax-20 (Fehlmann® AG, Maschinenfabrik, Seon, Switzerland), with a milling tool of 0.65 mm in diameter (Magafor©, France). Upon activating the milling machine and waiting until the milling spindle had reached its maximum speed of 10,000 rpm, the milling tool was displaced towards the sample (using a manual handle) until it came into contact with the outer surface of the stent. The tool then started to mill through the stent wall and a complete cut was assessed upon observation of swarf.

The spindle speed in the machine was set to its maximum achievable value (10,000 rpm), in order to obtain the best milling finish [17]. Moreover, the indexing head rotation was opposite to the rotation direction of the milling tool, which would also contribute to improve the milling finish [18]. Alignment was carried out to ensure that the tip of the milling tool touched the centre line of the stent lumen surface.

Different milling styles were investigated to achieve a single cut:

a) A single 360° rotation (full round),

b) Two individual 180° rotation (half round)

c) Four individual 90° rotation (quarter rounds).

1.3.2 Procedures of analysis

As outlined at the beginning of this report, the aim of this study is to evaluate the accuracy and robustness of a method for manufacturing inclined/conical side-holes through a ureteric stent. Micromilling was identified as a suitable manufacturing technique for this application to manufacture a 90° vertex angle.

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There are few parameters that need to be defined for milling holes with conical shape on a tubular substrate. These include the diameter of the stent, size of the milling tools, milling style (as defined above), and the alignment between milling tool and stent. Each of these parameters could affect the outcome of the fabrication process thus, it is important to establish a procedure for evaluating them.

The following paragraphs describe the procedures that were followed in order to determine the cutting and vertex angles. These comprised optical imaging and quantification of geometrical features of the stent and its side-holes.

1.3.2.1 Microscope imaging of side-holes

An axioplan-2 upright fluorescence microscope (Zeiss®, Germany) was employed to measure the vertex angle of the milled conical side-holes. Prior to imaging, the milled segment of the stent was cut using a scalpel and placed on a glass slide. The sample was positioned in such a way that the side-hole would face the lens of the microscope objective (with 5× magnification). Images were captured using the AxioCam HRc (Zeiss®, Germany) to measure the shape of the hole at different depths. Individual images were also combined using the 3D viewer in Fiji (NIH, USA) to reconstruct a 3D architecture of the side-hole. The same protocol was also employed to measure the stent wall thickness.

Overall, measurements and calculations were performed with the aim of assessing the general shape and finish of the hole (qualitative), major and minor axes of holes (quantitative), and stent vertex angle (quantitative) (see Figure 5-1). These steps are outlined in the following paragraph.

1.3.2.2 Dimensional characterisation of side-holes

To determine the wall thickness, the stent was cut into 11 pieces. Then, internal and external radii (i.e., *IR* and *ER* respectively) of the stent were measured using the microscope built-in software (Axiovision, Zeiss®, Germany). The wall thickness (*T*) was then calculated as (see Figure 5-1):

$$T = ER - IR \qquad \text{(Eq 5-1)}$$

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The major axes of the elliptical cross-section of the side-hole, taken at different depths (d_1, d_2 and d_3; as illustrated in Figure 5-1d) were measured from optical microscope images. The following trigonometrical relations were then employed to calculate the cutting angle (α) and the vertex angle (θ)(see Figure 5-1).

$$\alpha = \mathrm{ATan}\left(\mathrm{T} - \left(\frac{d_1 - d_2}{2}\right)\right) / \left(\frac{d_3 - d_2}{2}\right) \quad \text{(Eq 5-2)}$$

$$\theta = 2 \times \alpha \quad \text{(Eq 5-3)}$$

Thus, in order to obtain a vertex angle (θ) of 90° in the side-hole, the cutting angle (α) was set to a nominal value of 45° (see Figure 5-1).

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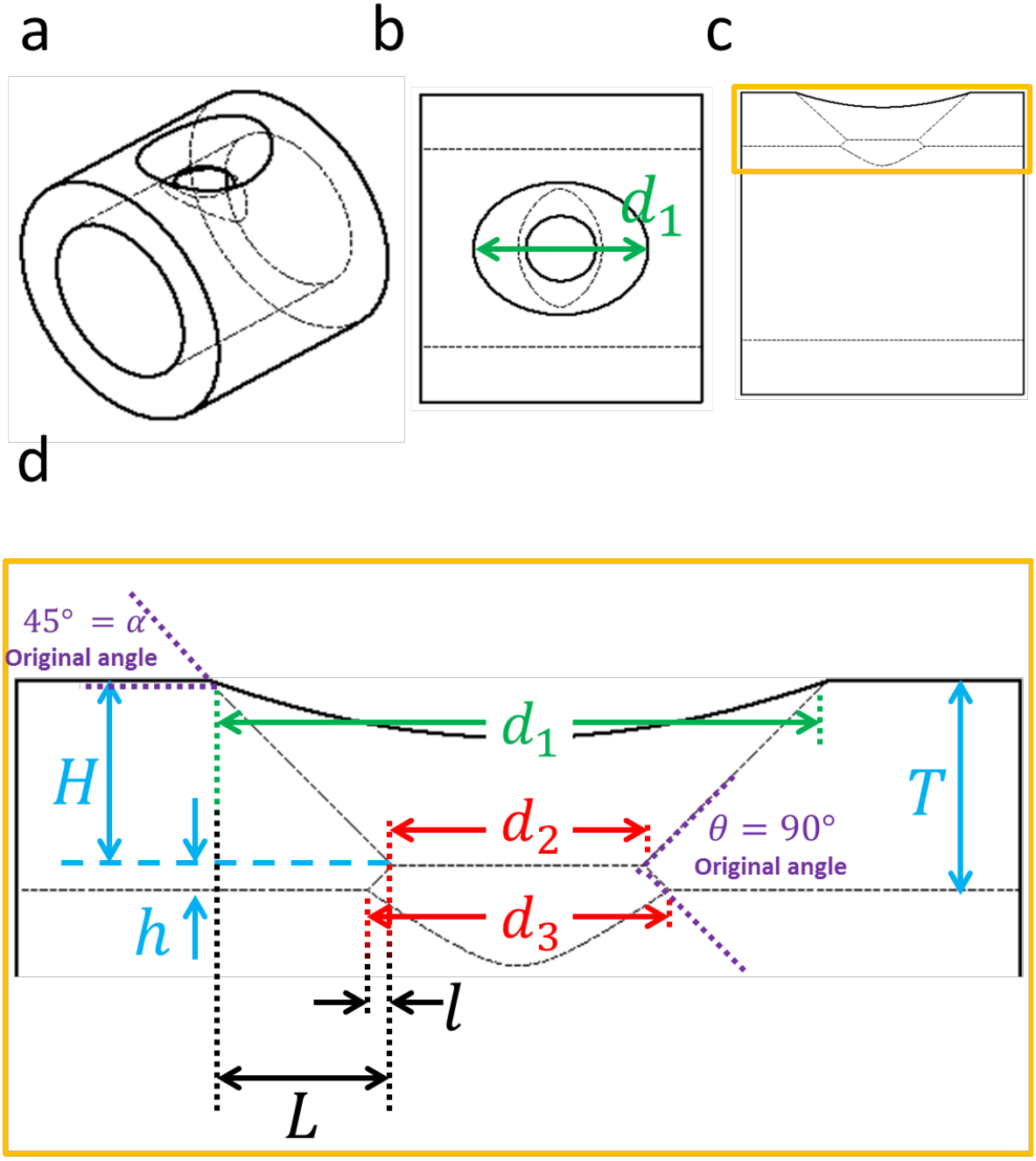

Figure 5-1 (a) Asymmetric view of the CAD drawing of a segment of the stent containing the generated side-hole shape, created using Inventor® Pro 2018 (Autodesk®, USA). (b) A top view of the fabricated side-hole, with an elliptical outlet, and with the major axis of the ellipses labelled in green. (c) Side view of the side-hole, with the corresponding zoomed-in view shown in (d). Labels indicate both inner and outer portions of the hole, and their corresponding thickness resulting from the cutting (*H* and *h*), the total wall thickness (*T*), and the axes of the elliptical major arms (d_1, d_2, d_3) taken at different

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depths along the side-hole. Both cutting (α) and vertex (θ) angles are also reported, together with their nominal values.

1.4 Results and Discussion

Figure 5-2a shows experimental values of the external radius (*ER*), internal radius (*IR*), and wall thickness (*T*) of the stent, taken at different positions along the stent. Mean values are equal to 1,278.4 ± 2.6 µm (*ER*), 755.3 ± 1.3 µm (*IR*), and 423.2 ± 1.8 µm (*T*). Figure 5-2b&c also shows two representative images of cross-sectional cuts of the stent (i.e., Cut 4 "C4" and Cut 11 "C11"). Variations in the experimental data taken along the stent (as shown in Figure 5-2a), and some localised geometrical alterations (as shown in Figure 5-2b&c), suggest that commercial stent lumens may present some defects or inaccuracies that need to be considered when milling conical side-holes.

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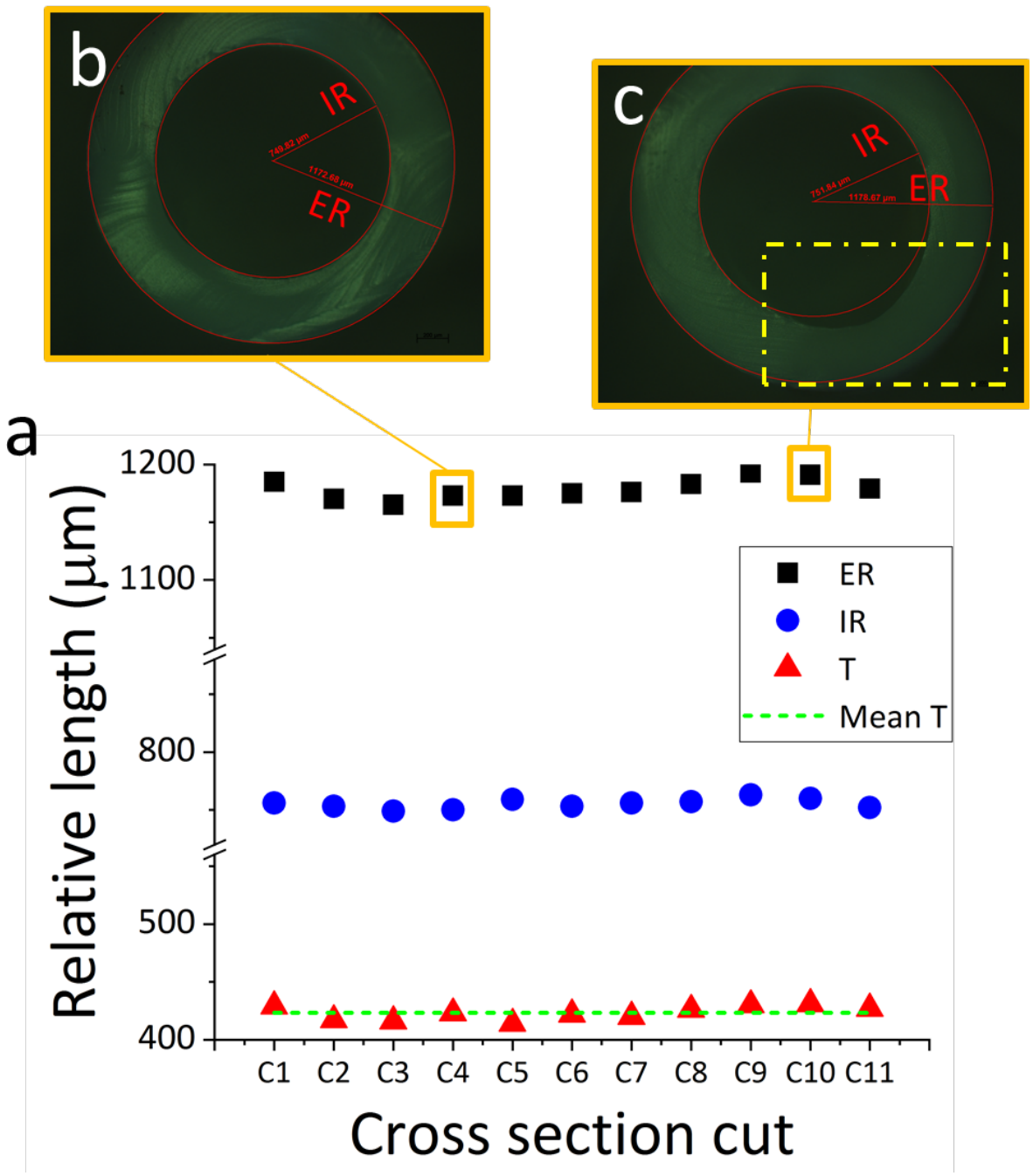

Figure 5-2 (a) shows the values of external radius (*ER*), internal radius (*IR*), wall thickness (*T*) and the mean wall thickness (*Mean T*) taken from 11 cuts along the stent length. (b) The cross sectional view of 'cut 4'. (c) The cross sectional view of 'cut 11', and a localised deformation of the stent wall shown within a yellow dot-dashed rectangular box.

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Table 5-1 shows the values of the major axes of the elliptical cross-section of the side-hole taken at different depths (d_1, d_2 and d_3), and measured from four samples. These values, together with the measured value of mean wall thickness reported above, were used to determine the cutting and vertex angles plotted in Figure 5-6..

Table 5-1 length of the major axes of the elliptical sections of the side-hole (d_1, d_2, and d_3) measured experimentally, as well as the corresponding milling style used (for a 7 Fr stent).

Sample number	d_1 *(µm)*	d_2 *(µm)*	d_3 *(µm)*	*Milling Style*
Sample 1	1,873	1,175	1,179	Single Round
Sample 2	2,005	1,343	1,352	Single Round
Sample 3	2,035	1,218	1,215	2 X Half Rounds
Sample 4	1,913	1,120	1,106	4 X Quarter Rounds

Figure 5-3a&b show the calculated values of cutting angle (α) and vertex angle (θ), respectively, and the green dashed line represents the nominal angles. Note that an experimental cutting angle of 45° was set in all cases, and measured using a protractor. The vertex angles were equal to 97.7°, 102.3°, 90.6° and 91.2° for samples 1, 2, 3 and 4, respectively. Sample 1 and 2 were manufactured using the same 'Single Round' milling style, whilst samples 3 and 4 using the '2 X Half Rounds' and '4 X Quarter Rounds' milling styles, respectively. The results show that vertex angles in samples 3 and 4 were very close to the original (nominal) angle. This demonstrates that milling conical side-holes with multiple half- or quarter-rounds results in a more accurate fabrication process, as opposed to using a single-round cutting method.

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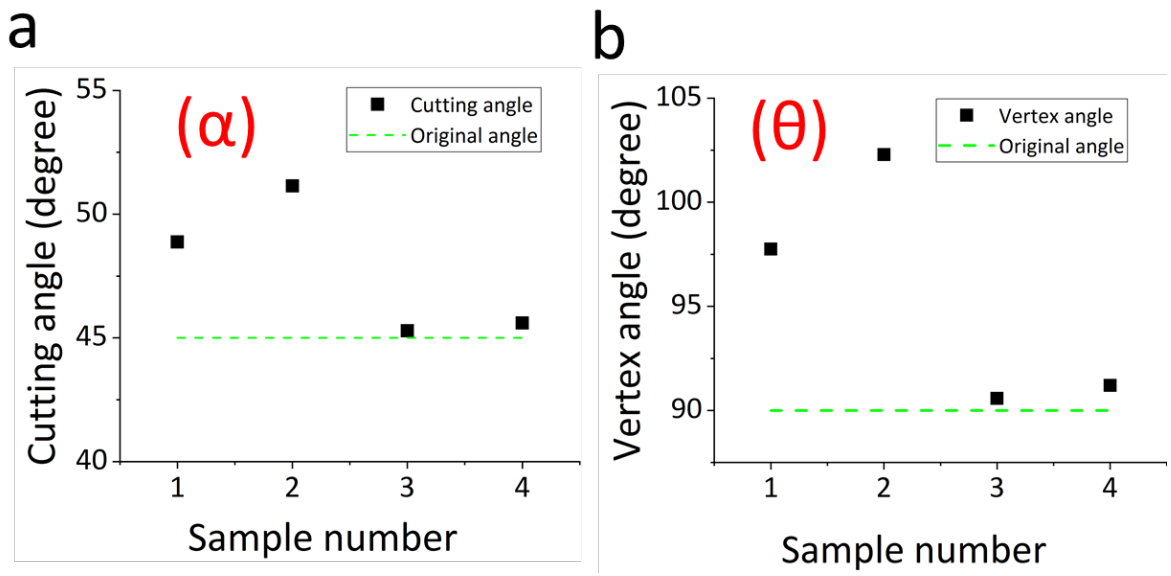

Figure 5-3 (a) values of cutting angle plotted for each test, calculated *via* trigonometrical calculations (described in the Methods section). The dashed green line represents the nominal cutting angle. (b) Values of vertex angle plotted for each test, calculated *via* trigonometrical calculations (described in the Methods section). The dashed green line represents the nominal cutting angle.

Figure 5-4 shows images of the top view of conical side-holes, which as shown in Figure 5-1 look elliptical. As shown in Figure 5-4a&b, images of sample 1 and 2 associated with more debris (red dashed rectangular box) and fracture/bumps (yellow dashed rectangular box) compared to Figure 5-4c&d representing images related to sample 3 and 4. This further demonstrates that cutting side-holes by Half and Quarter Rounds are more favourable in terms of having better finish than the case with a Single Round. Interestingly, there is a fracture/bump in the left corner of holes, which happens to be the first point of contact between milling tools and the stent surface at the beginning of cutting and it's almost identical in terms of the shape, size and location.

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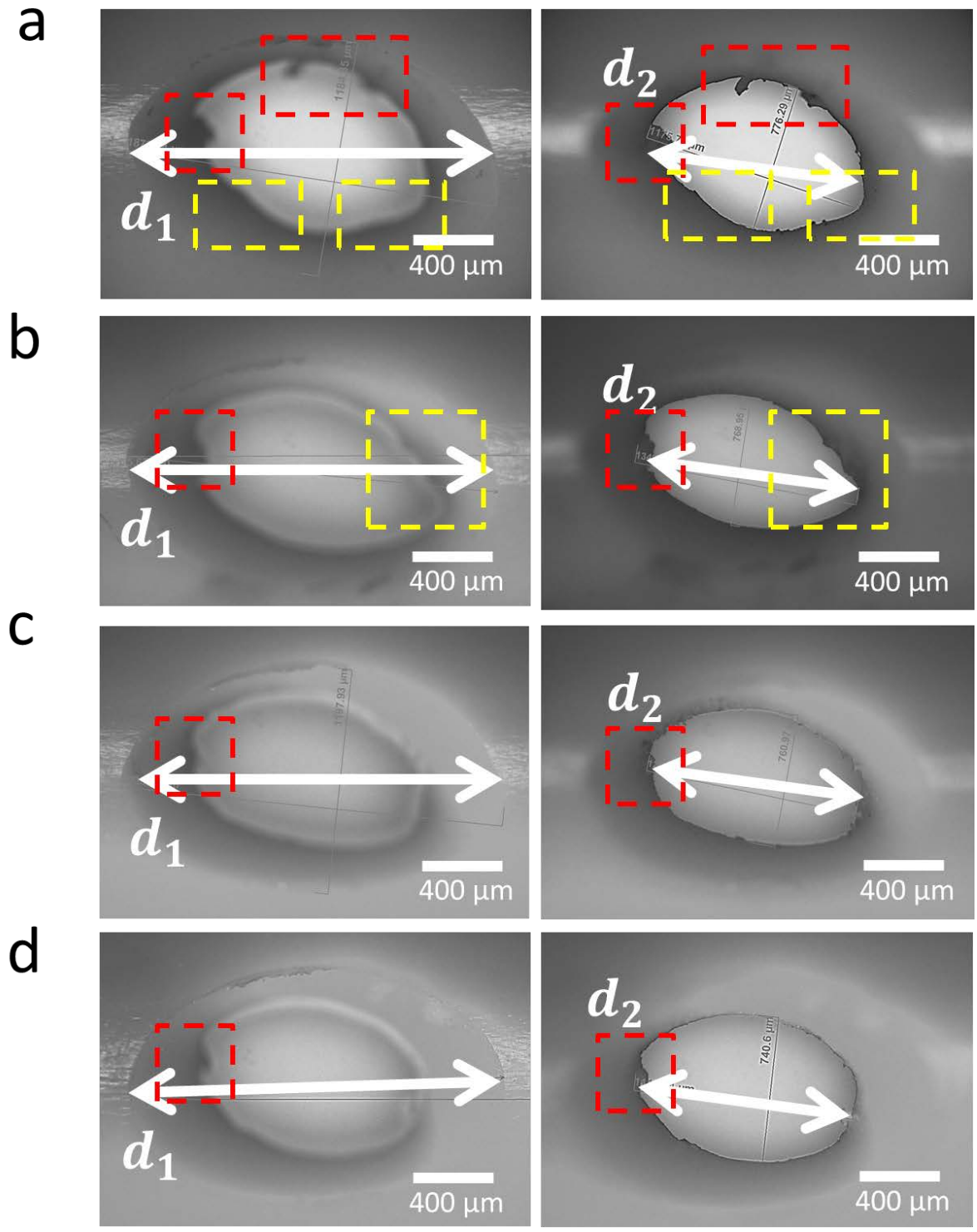

Figure 5-4 Top view of a conical side-hole manufactured on a 7 Fr silicone stent, focusing on the external surface (left) of the stent and the vertex tip plane (right) of the stent, for sample number (a) 1, (b) 2, (c) 3, and (d) 4. Red dashed rectangular boxes in all pictures indicate the position of debris, and yellow dashed rectangular boxes highlight edges with fractures/bumps.

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1.5 Conclusion

We streamlined side-hole with conical shape that has potential for significantly reducing accumulation of encrusting deposits in ureteric stents, and particularly within inactive side-holes. There is currently no established technique for producing this specific side-hole architecture on a ureteric stent. In this study, preliminary results are presented demonstrating the usage of a micromilling-based manufacturing method to fabricate side-holes of arbitrary shape. The method employed a commonly used micro-milling technology and an indexing head, and combined them with an in-house built stent holder in order to cut conical side-holes through tubular stents.

The evidence provided in this report indicates that this method has potential for generating conical side-holes (with elliptical cross-section) through the wall of a ureteric stent. Results also demonstrated that milling using multiple half- and/or quarter-rounds, rather than a single-round, provided a more accurate reproduction of the vertex angle and a superior milling finish.

In this method, there are different parameters that play important role on the quality of the end-product and the fabrication robustness, including the relative size of milling tool and stent, milling style, and direction of the milling tool rotation relative to the rotation of the indexing head. The following steps could be performed in order to improve the developed manufacturing method: 1) employ an automated system to assist the alignment of the stent, in order to achieve the correct cutting angle and a repeatable offset position; 2) investigate different milling styles, such as a combination of the ones used in this study (e.g. two individual half rounds, followed by a full round); 3) utilise stents with a more uniform thickness, as it likely influences the vertex angle; 4) increase the number of samples, to obtain statistically significant data; and 5) identify a suitable cleaning procedure for removing debris after manufacturing.

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SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

The STSM applicant submits this report for approval to the STSM coordinator

Action number: CA16217
STSM title: European network of multidisciplinary research to improve urinary stents
STSM start and end date: 05/03/2018 to 16/03/2018
Grantee name:

PURPOSE OF THE STSM

This STSM was designed to strongly contribute to the prime objective of the ENUIS COST Action dedicated to address inherent problems in urinary stents. The STSM began a collaborative study between the Athlone Institute of Technology (AIT) (Ireland), acting as home institution for the STSM applicant and 3Bs Research Group, a research Group at the University of Minho (Portugal), acting as Host Institution (HI). This work was carried out in conjunction with Teleflex, a global provider of urology products and urinary stents. The focus of the STSM was the development of pioneering biodegradable urinary stents through strengthening scientific/technical and market interactions, exchange of expertise and access to essential facilities between the HI and AIT.

Biodegradable urological stents produced from natural origin polymers, which are designed to meet patient needs and avoid second surgeries for stent removal, are urgently required. To this end, 3Bs research Group is developing proprietary biodegradable stent prototypes (HydrUStent) using tailored polymer formulations with anti-adhesion coatings to prevent bacterial colonisation of the stent. The HydrUStent biodegradable prototype requires extensive *in vivo* and *in vitro* characterisation to optimise the stent formulation and processing in order to achieve the best clinical performance characteristics. The University of Minho has *in vivo* model facilities in which stents were tested via implantation in pigs and their resultant performance analysed. AIT has microbiological testing facilities, which have been made available to HydrUStent after the STSM period for *in vitro* testing. The aim of this STMS was to provide shared access to models, prototypes, facilities and expertise between AIT and 3Bs Research Group.

This STMS directly addressed scientific objective 3 of the ENIUS Action, which aims to assess the “opportunities for improved stents related to the evaluation of new biomaterials, nanotechnology applications, new coating, eluting drugs stents and biodegradable stent materials”. In particular, the development and characterisation of biodegradable urinary stents was carried out with shared access to a breadth of important highly specific facilities and expertise. This STSM has strongly contributed to the training of young researcher objectives of the ENIUS Action, from the substantial exchange and sharing of both knowledge, facilities, experimental and operational procedures. Additionally the STMS targeted the capacity-building Action objectives 7, 8

and 9, dedicated to training young researchers, stimulating the rise and growth of novel scientific idea in the field, and linking the scientific research with industrial.

The relationship formed between AIT, the University of Minho, and 3Bs research group during the STSM will provide opportunities for prospective interdisciplinary collaboration and exchange or other young researchers.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

During the STSM the young researcher worked alongside HI representative Dr Alexandre Barros and together they produced several batches of biodegradable stents at the 3Bs research facility. The stent production protocol is outlined in detail in the paper by Barros et al., (2016). The protocol involved first preparing the hydrogel solution by combining gelatine, sodium alginate, bismuth, and water over heat to dissolve.

Figure 1 Ureteral stent mould (top) and packaged biodegradable ureteral stents (bottom).

Bismuth was used to visualise the stent with x-ray imaging as it is radiopaque. After the hydrogel was in solution genipin was added which interacts with the gelatine in the solution and increases the cross-linkage in the hydrogel. The solution was then injected by hand into a stent mould and placed in a 4°C room to solidify see Figure 1. Once the stents had solidified they were submerged in ethanol to dehydrate the hydrogel before being submerged in a calcium chloride solution. Calcium chloride creates cross linkages within the sodium alginate in the hydrogel. The stents were then left overnight in the ethanol to ensure that no water was present within the stent forming an alcohol gel.

Figure 2 Supercritical reaction chamber

The stents were dried the following day using supercritical carbon dioxide in a high-pressure vessel at a controlled temperature and pressure see Figure 2. The supercritical drying process works by bringing carbon dioxide to its supercritical point where it is a liquid briefly, but long enough to mix with the ethanol inside the stent. Once the ethanol and carbon dioxide are mixed high-pressure forces the solution out of the chamber with the carbon dioxide transitioning back into a gas phase and the ethanol expelled in a liquid phase.

Once the stents were dried they were polished to remove rough edges before being coated. The stents were immersed into the coating with contained a solvent, a copolymer, and an anti-adherent compound. The anti-adherent coating is intended to prevent bacterial cells adhering to the stent and thus prevent biofilm formation. Biofilms are an ongoing problem with indwelling urinary medical devices causing conditions such as urinary tract infections, pyelonephritis, and kidney/bladder stones.

The *in vivo* testing was carried out in the University of Minho school of medicine. *In vivo* testing was observed on two different days with two different animal species. On the first day a biodegradable ureteral stent produced in the lab was inserted into an anesthetised rabbit. The stent was then visualised *in situ* with ultrasound imaging before the urinary tract of the rabbit was excised and examined.

Figure 3 *In vivo* insertion of biodegradable ureteral stent into a pig model by Dr Alexandre Barros

The second day of *in vivo* testing again included biodegradable ureteral stent insertion, however a pig was used as the test subject. Unlike the rabbits the urinary tract was not removed from the pigs and they were not euthanised. The ureteral stents were left in place, in the future the team at the HI will excise the urinary tract and take histological samples to observe how the stent biodegrades over time, visualise any tissue damage, and evaluate the effectiveness of the new anti-adhesion coating.

Barros, A.A., Oliveira, C., Lima, E., Duarte, A.R.C., Reis, R.L., 2016. Gelatin-based biodegradable ureteral stents with enhanced mechanical properties. Appl. Mater. Today 5, 9–18.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

From completion of the STSM, the STSM applicant received training in polymer processing, specifically hydrogel formation and supercritical drying. The knowledge gained during the STSM was disseminated to colleagues in AIT and will aid in the newly formed connection between AIT and 3Bs/The University of Minho.

The results of the *in vivo* testing will not be available for this report as the stents will be left in place within the pig's ureters until they fully break down. Although no established *in vivo* results are available at present the experience of *in vivo* testing was invaluable to the STSM applicant as AIT does not currently have any *in vivo* testing facilities on site and it was the STSM applicant's first *in vivo* experience. Observing the environment, equipment, and methodology of *in vivo* testing will help the STSM applicant in the future of their own postgraduate studies of novel urological medical devices.

The microbiology testing of the biodegradable stent and in particular to the anti-adhesion coating are ongoing in AIT. The STSM applicant will assess the efficacy of the coating with respect to biofilm prevention by investigating both static and dynamic flow biofilm models with gram positive and gram negative bacteria.

FUTURE COLLABORATIONS (if applicable)

The work produced by the STSM applicant along with the *in vivo* experimentation will form part of a joint publication by 3Bs and AIT.

One of the main focuses of this STSM was to establish a new connection with the 3Bs research facility, the University of Minho and AIT. Through this newfound connection, there will be an ongoing collaboration between the STSM applicant and Dr Alexandre Barros.

AIT and the HI are interested in future STSM student exchanges and are actively looking for mechanisms to fund collaborative projects such as Horizon 2020 applications.