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

Belgrade, Serbia
5th-7th march, 2020

Follow-up of MoU objectives: progress report of working groups. Scientific planning. - Scientific strategy (MoU objectives, GP Goals, WG tasks and deliverables).

WG1. D. Rako (Leader WG1). Duje commented on the great progress that has been made this year in the work of this working group. WG1 are working to prepare 3 manuscrits (Systematic Reviews). The first, concerning metal stents and their complications is about to be sent to a journal with impact factor. During GP4 the three publications will be sent and work will begin on the Hand- book of urinary stents that this WG1 has in its tasks. The WG is behind in its deliveries, but all this delay in GP4 is solved.

WG3. S. Stavridis WG3 Leader).In this WG, due to the movement of Dr. N. Buchholz to be the leader of WG6 at the previous MC meeting, there is a vacancy. WG3. Dr. W Kram, MC of Germany is the only person who shows up and because of his great activity and involvement in this WG3 is accepted by the attendees. It will be sent to an e-vote for quorum vote with all MC members.

WG4. Neither the leader nor the vice-leader attend, but as they are linked in many ways to WG5 the comments of this WG are made in conjunction with WG5.

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. In this GP3, the WG4-WG5 held a TS in Lublin (Poland), with a large participation and the conference proceedings were uploaded to our network intranet. The paper and the database are being finalized in conjunction with the WG4.

As in WG3, this WG5 also has a vacancy for the vide-leader position. Because Martin Järvelkülg has handed in his resignation due to his inability to combine this position with his work obligations. Prof. Dr. S. Tofail. (Ireland MC substitute), is the only candidate who has shown interest in filling this position. The AC informs that all MC members are offered in an email sent two weeks before the possibility of applying for this position.

WG6. Dr. N. Buchholz (WG6 leader) made a presentation of the objectives of this WG and the basic lines of development. As well as a detailed description of the manuscript in which they want to start working on GP4, as stated in MoU. On Saturday they will have the first joint activity with the group of members as they are the organizers of the Workshop.

Dissemination planning (Publications and outreach activities).

WG1. 3 open access scientific papers and one Handbook about Urinary stents.

WG2. Two open acess: Review: Currently known about the biomechanics of the urinary tract, both with and without stents. -Second paper: Review-Current state of the art of computational simulation of urinary tracts with or without stents, together with in vitro validation. -Whitepaper: Computational simulation in urinary stents.

WG3- *Whitepaper: Comprehensive Validation protocol on new stents designs.*

WG4-5. DataBase of materials used in Ustents. & Guidelines requeriments for biomaterials and coatings.-Paper: Future directions for ureteral stent technology. Biomaterials and DES.

WG6. Systematic Review of the different technologic tools suitable for Ustent development.

-Short Term Scientific Missions (STSM): review of completed reports and new applications"

The estimation for this 3GP is 9 STSM. With a total budget of 18.900 €. (eighty thousand and nine hundred euros). To the current date, we have financed 9 STSMs. The average costs as you can see is less than 1800€/STSM. And we foresee that we are left with funds for 1-2 more STMS during this period. Please note that stsms should be completed by 20th april to account for payment processing

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times. Mr Emile Talon, Prof Yannis F. Missirlis, Dr Biljana Todorovic Markovic, Ms Ivana Malagurski, Ms Elena Dragoni, Ms Nikita Greenidge, Ms Julia Estibaliz de la Cruz Conty.

Science Communication Manager. Update. AC comments that, we use the website mainly for two purposes, firstly to inform about the objectives of our network and secondly to disseminate the activities of the network. Both those that we are going to carry out and the reports of those that we have carried out. Anyway, whenever we update something on the website, AC usually send members an email to advertise it. Any member can send information about their projects, scientific papers, or disseminate their activities. The twitter is used directly to announce ENIUS events, to disseminate the events in real time, and to support the activities that the different groups that make up ENIUS are carrying out. I encourage you to follow it on the ENIUS twitter.

DISSEMINATION MEETINGS from Prof. Sarah Waters and Dr. Dario Carugo both from UK. They applied for Funds to attend: ***Incontinence: The Engineering Challenge XII. Institution of Mechanical Engineers. London (UK)*** *November 13th-14th, 2019.* The MC and the Science Officer of the Action should approve this request. AC sent for a vote on 9 october (e-Voted). On 16 october their request was approved by the MC. AC want to thank both Sarah and Dario for the great contribution they made, since they achieved that during this Congress there would be a forum to disseminate the activity of ENIUS. Therefore, for one hour, aspects related to ENIUS and WG2 were discussed. AC reminders to core group MC, there are always funds for this section: DISSEMINATION MEETINGS are high profile events or conferences not organised by the COST Action. Action MC members or their substitutes shall attend these meetings for the purpose of disseminating the Action's achievements.

Implemention of Cost polices on: Promotion of Gender balance. **mplemention of Cost polices on: Promotion of Gender balance**. Trainers gender balance in Workshops. Currently, only 35.8% of MC members are women and 27.3% of Core Group members. About Training activities (TS & Workshops); 41.7% women (Trainers) and 74.9% women (Trainees) and 71.5% women in STSM.
ECI. There are 58 ECIs involved in ENIUS activities in this GP3. Regarding Trainers, we have 20% in Bern-TS; 33% in Lublin-TS and on Saturday Workshop we will have 28% ECI. AC comment, it's to be satisfied (an average of 25%). Regarding Trainees. The majority of attendees are ECIs:100% in Bern-TS; 80% in Lublin-TS and 100% in Belgrade-Workshop. 88% of STSM applicants are ECIs. 37% of Core-Groups members are ECIs.
Inclusiveness. The percentage related to activities carried out in these countries should be improved. Leaderships roles ITC countries 23%. Location Action meetings. GP3: Serbia and Poland. (50% of 3GP activities). Promoting STSMs in ITC countries. 78%.

COST Action CA16217 ***European Network of Multidisciplinary Research to improve the Urinary Stents*** **– STSM Report**

COST STSM Reference Number: COST-STSM-CA16217-45392

Synthesis and characterization of electrospun medium chain PHA-based biomaterials for biomedical applications

Dr. Ivana Malagurski from the Faculty of Technology and Metallurgy (University of Belgrade, Serbia) has conducted a short term scientific mission (STSM) in the laboratory of Prof. Dr. Ramesh P. Babu (AMBER Center, Trinity College Dublin, College Green, Dublin 2, Ireland) during the period from 16th to 30th September, 2019. STSM was related to the work group 4 (WG4).

Description: The aim of this STSM was to develop novel fibrillary polyhydroxyoctanoate (PHO)-based biomaterials as potential components in biodegradable urinary stent design and to characterize the obtained biomaterials regarding their structural and functional properties.

Application of biodegradable ureteral stents in treatment of compromised kidney drainage offers certain advantages: (1) there is no need for second, removal procedure; (2) limited insertion period minimizes risks associated with biofilm formation and/or encrustation and (3) stents can serve as matrices for localized drug delivery [1,2].

Medium chain polyhydroxyalkanoates (mcl-PHAs) are biopolymers with big potential for biomedical application due to their biocompatibility [3], controllable surface biodegradability with non-toxic degradation products [4,5] and the fact that they can be obtained using variety of carbon sources including waste streams [6]. However, their properties have to be adjusted to ensure improved processability and therefore wider application. The easiest way to do so is to blend them with other polyesters with superior mechanical properties like polylactic acid (PLA), or to incorporate a nano-phase within biopolymer to obtain nanocomposites.

Electrospinning is a simple method for production of fibrillary, porous materials. Electrospun biomaterials have high specific surface area, tunable porosity and mechanical properties. Porosity improves ureteral stent characteristics in terms of local drug delivery and by reducing the risk of reflux thus enhancing the urine flow [7]. From our previous work, mcl-PHA, including PHO, solutions were found not suitable for electrospinning.

The aim of this study is to test the hypothesis whether addition of PLA-SiO_2 and/or TiO_2 into PHO will enable electrospinning process and result in electrospun biomaterials with improved and tunable properties.

1. Fabrication of PHO-based biomaterials

Spinning solutions were prepared by dissolving PHO in $CHCl_3$/DMF (80/20 vol%). In order to obtain fibrillary mats by electrospinning, different process parameters (*i.e.* applied voltage, needle diameter,

polymer concentration and flow rate) were varied, however neither neat PHO nor PHO/TiO_2 could be electrospun because of the low viscosity of the spinning solution due to the weak intermolecular interactions among polymer chains. Taking into account that PHO, as well as PHO solutions containing various amounts of TiO_2 was unsuitable for electrospinning, solvent casting method was employed to produce PHO/TiO_2 nanocomposites.

Film solutions, obtained by combining biopolymer solution (PHO or PHO/PLA-SiO_2) with TiO_2 suspension, were casted in glass Petri dishes and dried in a fume hood at room temperature for seven days. Uniform and homogenous nanocomposite films were produced by combining PHO and TiO2 (**Fig. 1**), while samples with PLA-SiO_2 exhibited phase separation (**Fig. 1**), probably due to the different crystallization rates of the two polymers. This has indicated that PHO and PLA-SiO_2 are not compatible and PHO/PLA-SiO_2/TiO_2 films were not further analyzed.

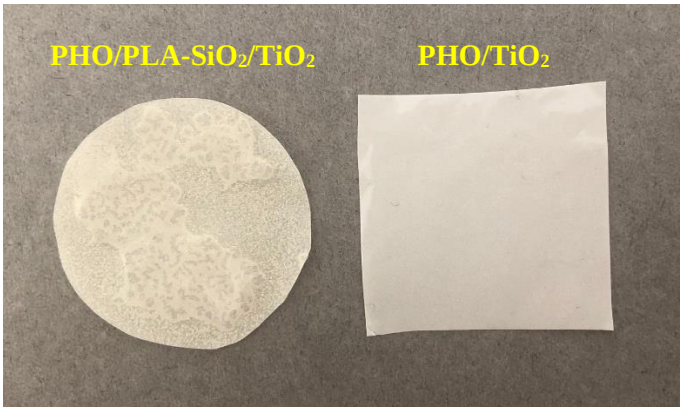

Figure 1. PHO-based solvent cast films.

2. Characterization of PHO-based biomaterials

The obtained nanocomposite films were characterized using scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), powder X-ray diffraction (XRD), thermogravimetric analysis and differential scanning calorimetry (TGA-DSC).

Internal structure and surface of the PHO/TiO_2 nanocomposites with increasing TiO_2 content were investigated by SEM (**Fig. 2)**. The incorporation of TiO_2 NFs has clearly affected both morphology and thickness of the nanocomposite films. The higher the TiO_2 content, the thicker and more uniform in terms of nanophase distribution the films were.

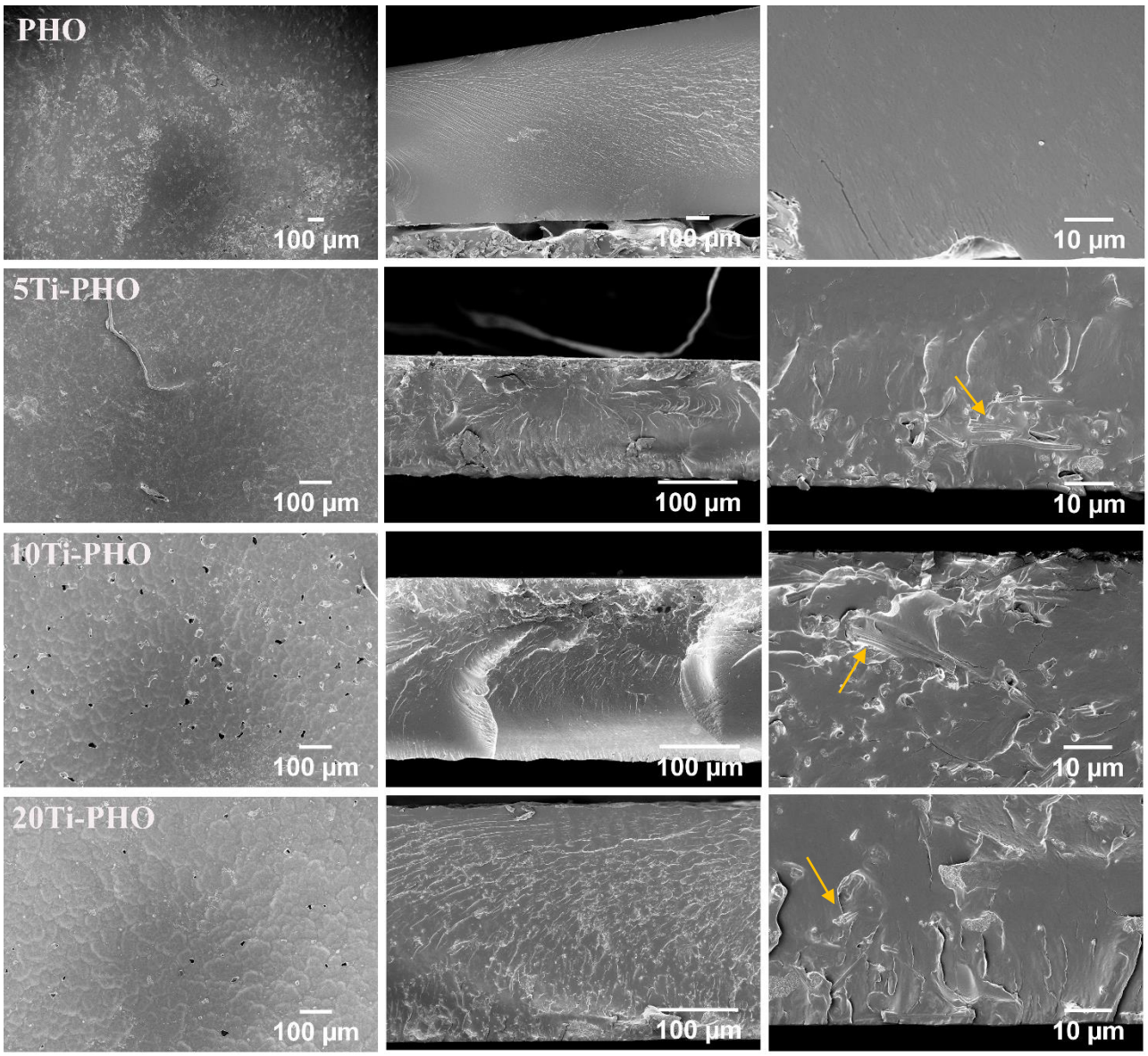

Figure 2. SEM micrographs of the surface and corresponding cross-sections of neat PHO and PHO/TiO_2 films. The TiO_2 NF bundles within biopolymer matrix are indicated by arrows.

Biocompatibility and degradation rate of a biomaterial are determined by its crystallinity, so in order to evaluate changes in the PHO degree of crystallinity in the nanocomposite samples, powder XRD was performed (**Table 1**).

Table 1. Sample abbreviations and corresponding degree of crystallinity (χ_c), glass transition (T_g), melting (T_m) and onset of thermal degradation temperature (T_d).

Sample	χ_c [%]	T_g [°C]	T_m [°C]	T_d [°C]
PHO	37.38	- 39	52.29	252.61
5Ti-PHO	24.55	- 39	50.19	254.67
10Ti-PHO	14.21	- 39	49.53	260.92
20Ti-PHO	11.21	- 39	50.02	274.34

As it can be seen the PHO degree of crystallinity decreases with increasing TiO_2 loading, showing that the presence of TiO_2 has significantly influenced polymer chains mobility and packaging. Thermal analysis indicated that both neat biopolymer and nanocomposite samples had similar glass and melting temperatures, however the onset of thermal degradation was shifted towards higher temperatures for the nanocomposite samples (**Table 1**).

Potential interactions between components of the nanocomposite samples were analyzed by FTIR (**Fig. 3**).

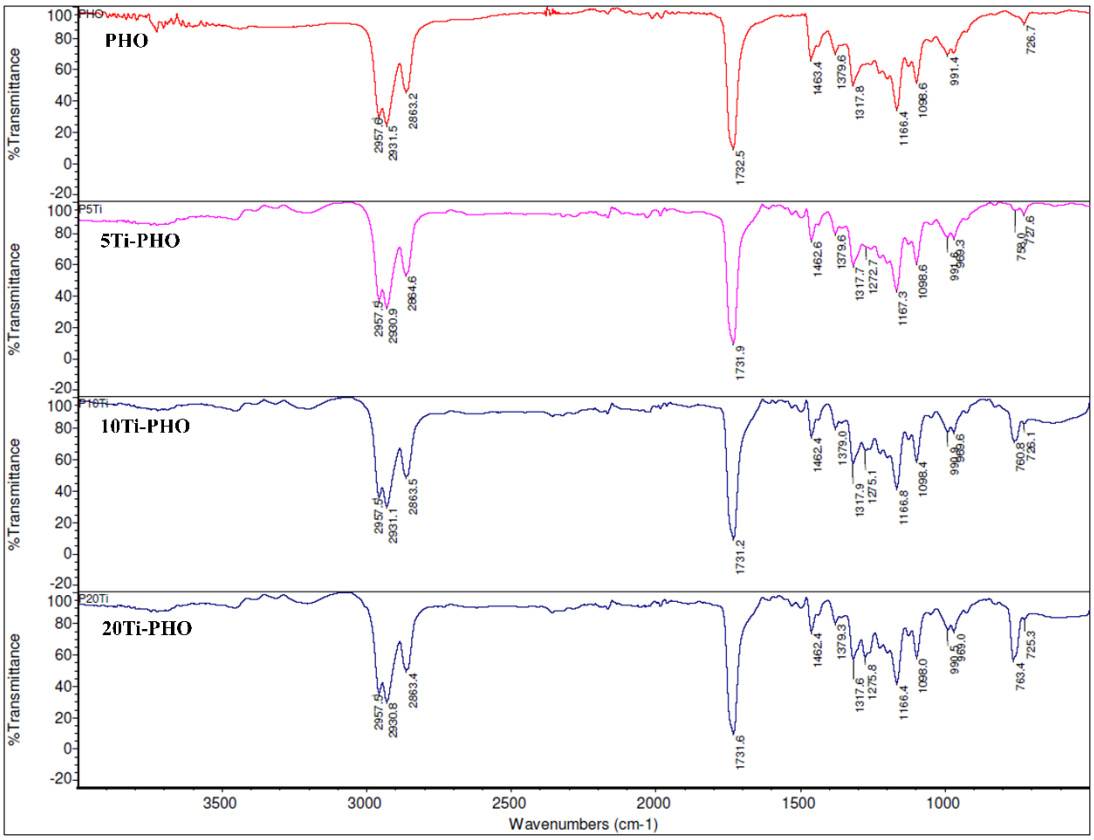

Figure 3. FTIR spectra of the neat PHO and nanocomposite samples with increasing TiO_2 content.

In addition, *in vitro* cytotoxicity evaluation of both TiO_2 NF and nanocomposite films was performed by the standard MTT assay using normal human fibroblasts cell line (MRC5). The results of the MTT assay have shown that all PHO/TiO_2 films did not have cytotoxic effect and that addition of TiO_2 NF to the biopolymer has even stimulated cell proliferation.

The obtained results have indicated that the incorporation of TiO_2 NF into PHO matrix has had a significant effect on the nanocomposite sample properties. Regarding the potential application, it is expected that a decrease in biopolymer crystallinity may positively affect degradation rates of the samples, making them attractive as transient, biodegradable implants.

The samples will be further analyzed regarding mechanical properties and degradation profiles (accompanied with pH changes) in a physiological-like environment. The work will be continued at the Faculty of Technology and Metallurgy in Belgrade (Serbia).

It is envisioned that the results of this STSM, when completed, will lead to the development of a novel, improved biomaterial that can be used in biodegradable ureteral stent design and will be submitted for a publication in a peer reviewed journal. The established collaboration between researchers and two institutions will continue.

Literature :

[1] T. Zou et al., J. Mech. Behav. Biomed. 38 (2014) 17-25.

[2] K. Yin, P. Divakar, U.G.K. Wegst, Acta Biomater. 84 (2019) 231-241.

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[6] J. Nikodinovic-Runic et al., Adv. Appl. Microbiol. 84 (2013) 139-200.

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Ivana Malagurski

Ivana Malagurski

SHORT TERM SCIENTIFIC MISSION (STSM) – SCIENTIFIC REPORT

Action number: CA16217
STSM title: Urinary stents made of biodegradable and drug-eluting organic/inorganic composite.

STSM start and end date: 01/08/2019 to 31/08/2019
Grantee name: Elena Dragoni

PURPOSE OF THE STSM

The main purpose of this STSM was to develop the method for the synthesis of innovative antibacterial and drug-eluting stent coatings using porous zinc oxide (ZnO) nanomaterials developed by Politecnico di Torino (in a flower-like morphology) 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. The aim of this month of the STSM was to load individually two different drugs inside the samples previously prepared (Ibuprofen and Diclofenac) and then to study the release in artificial urine.

In order to measure the amount of drug absorbed and released, both balance and UV-spectroscopy have been used. The aim of this study was to compare the release trend of the same material with different percentages of ZnO powders.

The completed STSM is strongly aligned with the key aims of the ENIUS Action.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM

The two types of materials considered for the uptake were polyHEMA and the copolymer poly(HEMA-co-AAc), each with 3 different percentages of ZnO inside (none, 0.1% and 1%).

I started my STSM learning the best concentration of the solution with drug for the uptake, from which to begin.
To do that, I took into account the maximum solubility written in the datasheet of both drugs as the reference for the starting concentration.

Drug	Maximum solubility
Ibuprofen	100 mg/mL
Diclofenac	50 mg/mL

Then, I used one of the polyHEMA pure samples to make the first trial of drug absorption and to find the best time of uptake, from 1 h up to 4 h.
Duting this time, the concentration of the solution was measured each hours, in order to controll the development of the uptake. Moreover, also the sample was monitored for all the time, to see if it remained intact.
I noticed that the drug absorpion continued to increase all along, but at the end of the trial, the sample

appeared too soft. That was due to the swelling property of the polymer, which is at the basis of the mechanism by which the drug is imbided.
Hence, if the polymer is kept in the solution for too long, there is a risk that its meshes would widens excessivelly or that the hydrolysis process would start. These two events should lead to the leakage of drug from the sample and consequently to the failure of the uptake process.

For that reason, I choose 4 hours of immersion as standard time of uptake, both for ibuprofen and diclofenac.

For what concern the release in artificial urine at physiological pH (around 7.3) has been occurred including the first 2 samples of each material, previously loaded with both drugs; thus in total it has been analyzed 24 samples (12 samples with Diclofenac and 12 samples with Ibuprofen).
After the uptake, I used simply a balance to measure the amount of drug absorbed, weighing each sample before and after the uptake.

First of all, I prepared 12 falcons with 50 mL of artificial urine and I put them inside a MultiTherm™ Vortex incubator, in order to maintain the temperature at 37 °C and to keep the solutions stirred. Then I took from each falcon 100 µL of solution and I inserted this volumes in the 96-well quartz plate, to be used as background.

Then, I put each sample inside 1 falcon and the release analyses started, controlling the release trend by collecting the solution from each falcon every hour, during the first 6 hours. Then, the follow-up has continued measuring the concentrations after 24 h, 48h, 168 h (1 week) and 336 h (2 weeks).
Even in this case, to prepare the qartz plate for the following UV-Vis analysis it has been taken 200 µL from each falcon, in order to fill 2 wells and to have duplicate data.

As already said, in order to measure the concentration of the solution both during the uptake and the release, I used the UV-Vis Spectroscopy.

This technique provides the absorbance of the solution, from which shall be obtain the concentration according to the Lambert-Beer Law, after having known the equation of the calibration line for each drug in each solvent used.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

Concerning the uptake, following are shown the amount of drug absorbed from each sample.

DICLO uptake weight	Uptake (mg)		
	Sample 1 (mg)	Sample 2 (mg)	Sample 3 (mg)
PolyHEMA pure	7,4	11,4	12,9
PolyHEMA + 0.1% ZnO	9,6	7,3	9,1
PolyHEMA + 1% ZnO	3,4	9,7	8,9
Poly(HEMA-co-Aac) pure	13,4	15,1	10,2
Poly(HEMA-co-Aac) + 0.1% ZnO	10,8	11,8	10,6
Poly(HEMA-co-Aac) + 1% ZnO	13,5	10,7	11,7

IBU uptake weight	Uptake (mg)		
	Sample 1 (mg)	Sample 2 (mg)	Sample 3 (mg)
PolyHEMA pure	13,6	14,8	57,1
PolyHEMA + 0.1% ZnO	18,1	14	11,4
PolyHEMA + 1% ZnO	13,1	26,2	9,7
Poly(HEMA-co-Aac) pure	27,6	19,6	14,8
Poly(HEMA-co-Aac) + 0.1% ZnO	7,2	2,8	56,9
Poly(HEMA-co-Aac) + 1% ZnO	10,3	8,6	29,8

I used only the first 2 samples for each material to study the release in artificial urine at physiological pH (7.3).

Concerning the calibration line of drugs, needed to calculate the concentrations from the absorptions, I firstly found the peak of absorbance of each drug:

- Diclofenac: 272 nm in artificial urine.
- Ibuprofen: 223 nm in artificial urine.

After that, it was possible to obtain the equation of the calibration lines of the drugs in artificial urine, in order to study the release.

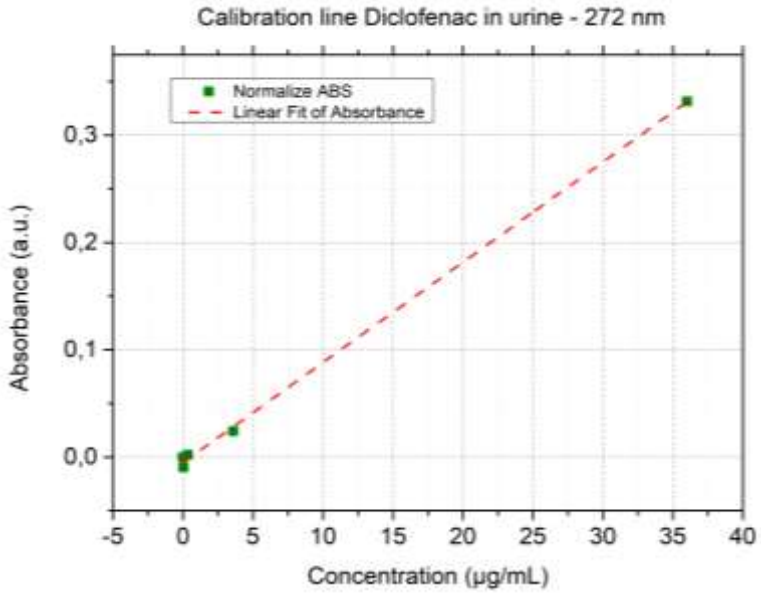

Figure 1 Diclofenac calibration line in Urine.

Equation: y = -0,00511 + 0,00934x			
Adj. R-Square 0,99872			
DICLO in Urine		Value	Standard Error
Abs	Intercept	-0,00511	0,0027
Abs	Slope	9,34E-03	1,67E-04

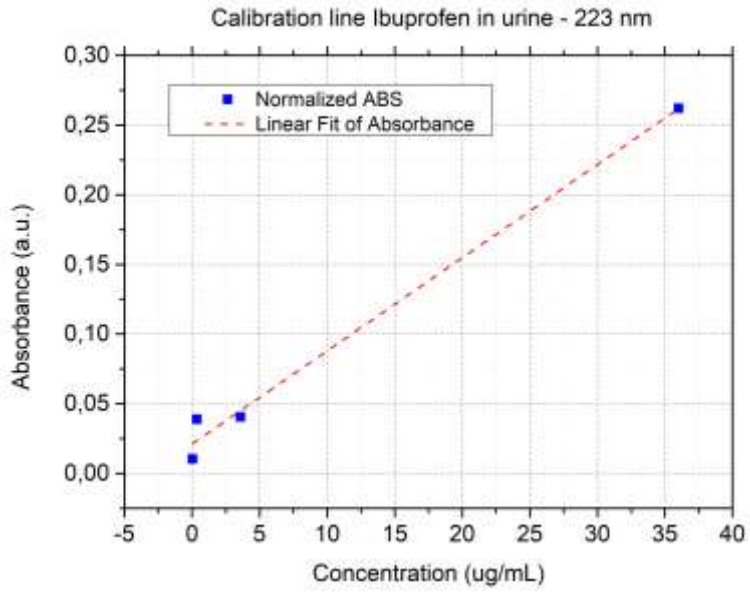

Figure 2 *Ibuprofen calibration line in urine, at 223 nm*

Concerning the release, firstly I collected the the UV-Vis absorbance spectra in the range 200–800 nm, in order to evaluate the trend of the absorbance peak of drugs. Following is reported the Diclofenac graph that collects all the spectra as an example.

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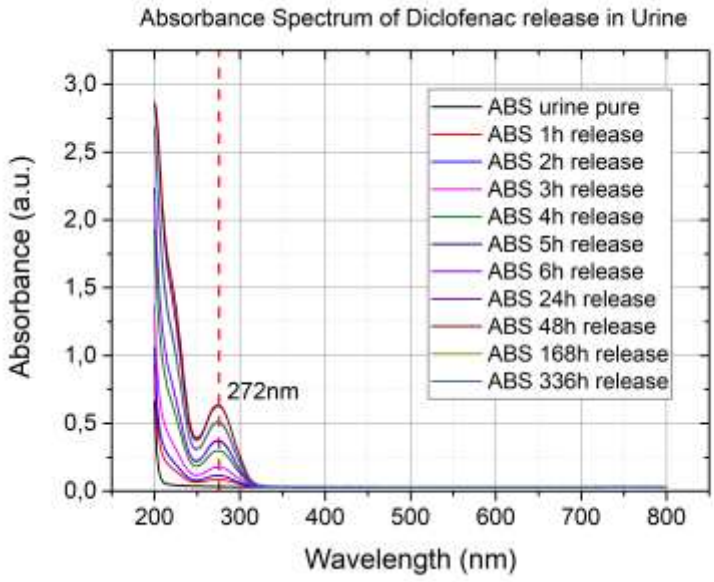

Figure 3 *Spectrum of sample 1, poly(HEMA) + 1% ZnO.*

Then, I collected the second series of scans at characteristic wavelength of each drug, to obtain the absorbances and then the amount of drug released.
Knowing the amount of drug uptaken by each sample and comparing it with the concentrations obtained, it was possible to calculate the % Release. Then, in order to obtain more precision percentage values, it has been made the normalization of the % Release as compared with each maximum value within each

sample.

Following I put the graphs that show the release trend of each drug from the different materials.

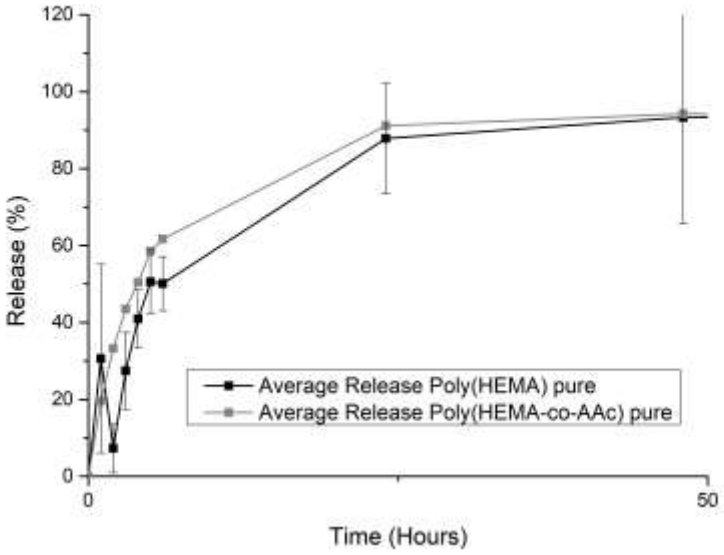

Figure 4 *Diclo release. Confrontation of pure pHEMA and copolymer release.*

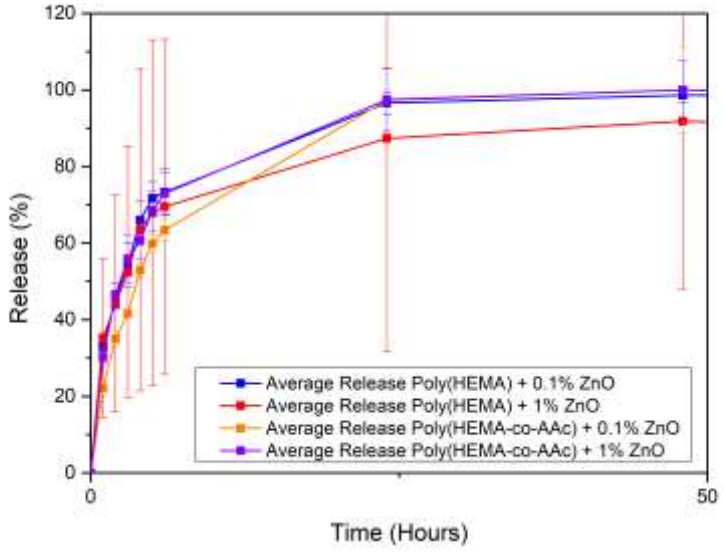

Figure 5 *Diclo release of materials with 0.1% and 1% ZnO.*

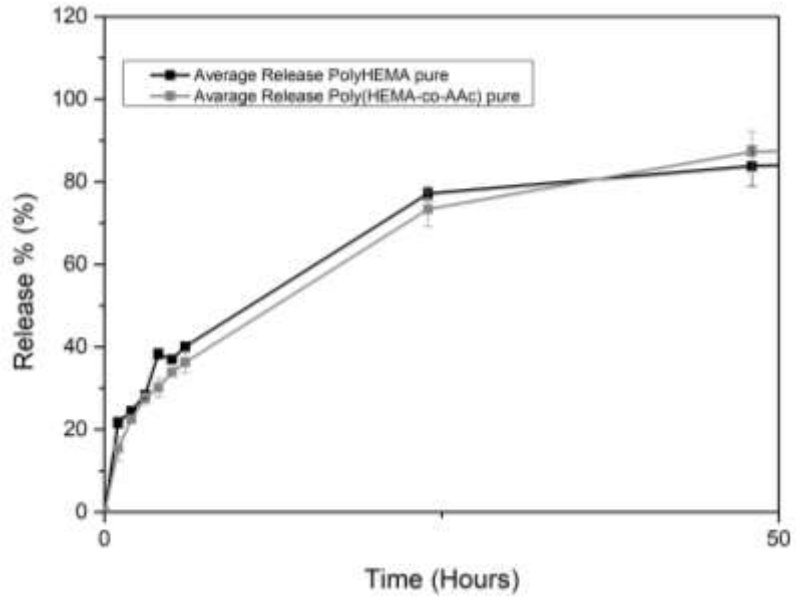

Figure 6 *Ibu release. Confrontation of pure pHEMA and copolymer release.*

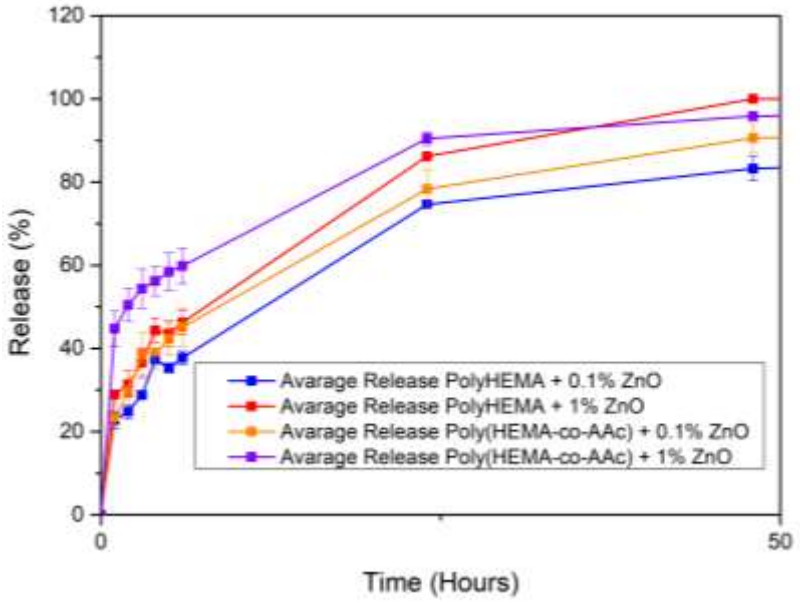

Figure 7 *Ibu release of materials with 0.1% and 1% ZnO.*

FUTURE COLLABORATIONS

In the next work in Turin it is planned to use Sample 3 of each material to study the release in pathological conditions of acid (around 5) and alkaline (around 9) pH

I will take the samples charged with drug in Turin to analyse the release and then the degradation of the materials.

SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA 16217
STSM title: Time-dependent quantitative assessment of encrustation/biofilm growth on ureteral stents
STSM start and end date: 01/03/2020 to 18/03/2020
Grantee name: Emile Talon

PURPOSE OF THE STSM:

(max.200 words)

This STSM is aimed to the the study and the analysis of the ureteral stents. Ureteral stents are used to restore urine drainage from the kidneys to the bladder but, despite their wide use today in urology, involve many side effects. The focus is set on the analysis of the encrustation formation, considered one of the main cause of stent failure. The critic role of fluid dynamics in a stetned ureter has only recently been asserted in term of encrustation formation. In this STSM the main objective is to develop a simple *in-vitro* system including a silicone ureter model and a pumping element and calculate the volumes of the crystal deposition inside the stent by means of µCT scans and assert the distribution of the encrustation. The development of a novel protocol for the experimental *set-up* and for the scanning methods will be presented.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

(max.500 words)

The ureder model was built before the STSM start at the University of Bern, CH. It consist in a silicone cylinder with a hollow straight lumen of 4 mm in diameter, mimicking the ureter dimensions. The cylinder is contained in a plexiglass holder and at the extremities, two luer connector are placed to facilitate the inlet and outlet tubing connections.
At the beginning of the STSM the µCT needed to chosen among all the machines at disposal of the µ-VIS X-Ray Imaging Center at the University of Southampton. Due to the large dimensions of the ureter model, it was decided to proceed with the HUTCH System, a customized walk-in bay designed for versatility. Indeed this mahine can scan specimens whose dimensions range from a few millimetres in cross-section up to 1 x 1 x 1.5 m. A reference scan of the ureter model filled with water is performed, in order to set the different scanning parameters. Voxel size for the scans is set at 20 µm.
Once the access to the Bioengineering Lab was obtained, the *in-vitro* set-up was built. In order to properly seal the ureter model and create the proper connectors, two caps were manufactured in the workshop, together with two silicone gaskets realized in the lab.
A ureteral stent is placed inside the ureter model, with the renal and bladder pigtail placed in the designed enlargements (Black Loop-E, Pure Medical Device, Switzerland).

The set-up is shown in Figure 1. The pump used is a roller pump, MINIPULS 3 Peristaltic Pump (Gilson®, Middleton, WI, USA). The artificial urine is not recirculated in the loop, in order to not change the crystal concentrations. The ureter model is placed vertically (differently from what shown in Figure 1).

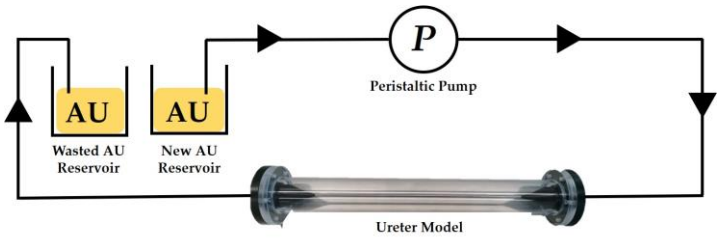

Figure 1: Schematic representation of the in-vitro set-up realized. The artificial urine is stored and kept on a hotplate stirrer, then pumped into the ureter model by mens of a roller pump with a flow rate set a 1 ml/min. The AU is then collected.

The artificial urine (AU) recipe, which does not include bacteria presence, for 1,08 L of water is here reported:

Chemical Component	Molecular Weight [g/mol]	Concentration [mmol/L]	Weight [g]
Lactic Acid	90,08	1,10	0,10
Citric Acid	192,12	2,00	0,41
Sodium Bicarbonate	84,01	25,00	2,27
Urea	60,06	170,00	11,02
Calcium Chloride Dihydrate	147,00	2,50	0,40
Sodium Chloride	58,44	90,00	5,68
Magnesium Sulfate Heptahydrate	246,47	2,00	0,53
Sodium Sulfate Decahydrate	142,05	10,00	1,53
Potassium Dihydrogen Phosphate	136,09	7,00	1,03
Ammonium Chloride	53,49	25,00	1,44

AU was kept in hot stirring plate at 37°C for all the duration of the experiment.
The AU was let circulated in the *in-vitro* platform for 16 hours, in order to let the crystals deposit on the ureteral stent.
After the end the experiment, the ureter model was detached from the set-up and kept filled with AU by means of three way valves. The model is then brought to the µCT for scan. The goal is to identify and then possibly segment the encrustation deposits on the ureteral stents. Through segmentation, the measurements of crust volumes could be possible.
The scan of the entire ureter model was divided in 13 different scans then reconstructed together.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

The scan of the reference clean stent showed how some bubbles re present inside the lumen of the stent, despite all the silicone model was filled with water, as it can be seen in Figure 2.

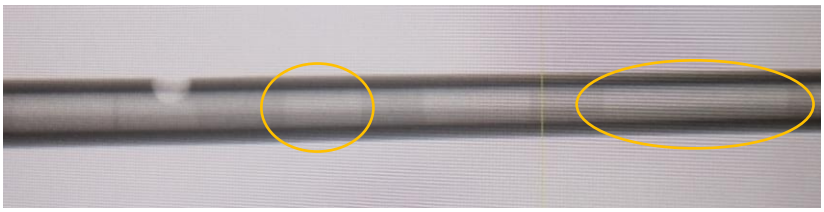

Figure 2: Reference stent scan. Highlighted bubbles of air can be seen inside the stent lumen. The difference between air and water can be appreciated.

These bubbles, if formed during the implantation of the stent, can cause an increase in the resistnce for the flow draining from the kidneys to the bladder and can enhance the extraluminal flow with respect to the intraluminal one.

The experiment ran without any leakage for 16 hours. During this time crystals could easily be seen bare eye flowing smoothly from the top (renal end) to the bottom (bladder end). It was let running overnight, and the morning after the flow diminished down to 0.8 ml/min, sinche the under pump tubing got partially obstructed by crystal depositions. Referring to the volume collected during these 16 hours, it was calculated that the mean flow rate was 0.85 ml/min.
The ureter model filled with AU was scanned with the same parameters of the reference one. The scan took 6 hours more or less. A first look at the raw images showed that air bubbles were still present even in this case. However no big encrustation agglomeration could be identified on the lower regions of the stent. Also in this case the stent was divided in 13 regions to scan and, unfortunately, the 13th scan, the one referring to the renal pigtail region failed due to unknow circumstances.
The absence of big deposit of crystals could suggest that bacteria could be necessary to allow the adhesion of these crystals to the stent wall. Other solution to properly obtain deposits could be to let the experiment run for more time or to saturate the AU solution even more.

FUTURE COLLABORATIONS (if applicable)

The STSM was unfortunately interrupted due to the Covid19 outbreak and all the consequent safety measures. Therefore no segmentation of the images has been performed yet. The STSM succeded however in demonstrating how the in-vitro set-up is properly designed and a first test on the ureteral model succeed, suggesting that this model can be applied for future works.
Next steps will involve the production of artificial urine including bacteria, which will allow the formation of biofilm and so enhance the encrustation process. The scanning technique also resulted positive and will be used in the next experiments.
A collaboration will be set in place between the Universitiy of Southampton and the ARTORG Center for Biomedical Engineering Research at the University of Bern, CH. This collaboration will generally help to carry these experiments and bring significant apport in the ureteral stents research and development.

SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA16217
STSM title: Bioreactor design for dynamic flow through small calibre tubes with/without cell cultured on their lumen
STSM start and end date: 07/02/2020 – 14/02/2020
Grantee name: Yannis Missirlis

PURPOSE OF THE STSM:

The purpose of this very short term mission to Ege University was to continue with current collaboration I have with the Dept. of Bioengineering in designing dynamic bioreactors for small caliber tubes, and extend thedesign for ureter models, physiological/pathological and for stented cases (stent through the lumen).

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

Several meetings were organized during my stay at the Department of Bioengineering, by Professor Aylin Sendemir and her team both general and more specific, related to different bioreactors that we have collaborately designed. Work is going on there for a model of the blood-brain-barrier, and the effort is to apply to the system the relevant mechanical environment.
The meetings/discussions were not only in meeting rooms but also in the laboratory, observing and attempting to improve the systems.
More specifically I had meetings with the M.Sc. student Mr. Mert Sahinler, alone or with Prof. Sendemir joining us and introduced to him the various problems and knowledge I have about the urinary stents and the ENIUS Action.
I had already gathered as much literature as I could on the subject, we discussed about their content, and initially we limited ourselves to two papers:
A paper by Kim et al on flow through different models of ureters (https://doi.org/10.1155/2017/5172641)

and a paper by Francesco Cavila, Dario Carugo and colleagues (https://doi.org/10.1371/journal.pone.0087433)
closely related to experimental designs for our bioreactor considerations.
Already Mert asked several questions, understood a lot and started using free available software to produce the first drafts.
I might ask later on for an STSM for him to visit either Francisco or Dario for collaboration.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

There are not really any real results obtained from this short 5-day visit, but I am happy that the basis for collaboration on this project: bioreactor for ureter and/or stented one experimental simulation under real pulsatile conditions has been understood and work started.

FUTURE COLLABORATIONS (if applicable)

I plan to discuss and check on the progress of this endeavor frequently, discuss with Dr. Kallidonis in Patras about the real problems, and learn more about these, and, as mentioned earlier, if things progress well I might ask for an STSM for Mert to collaborate with Francesco or Dario.

SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA16217 - European network of multidisciplinary research to improve the urinary stents
STSM title: Scientific stay at Universitätsmedizin Rostock. Collaboration in the project "surface-modified ureteral catheter in domestic pig model".
STSM start and end date: 04/05/2019 to 04/08/2019
Grantee name: Julia Estibaliz de la Cruz Conty

PURPOSE OF THE STSM:

(max.200 words)
The purpose of this STSM is the collaboration of the Grantee in the project "Investigations on the interactive effect of surface-modified polyurethane catheters on the ureteral tissue to inhibit cell adhesion and encrustation of urological implants in the domestic pig animal model". On the one hand, considering that the Host Institution has plenty of experience in the study of encrustation related to urologic implants, the aim is to acquire new skills and knowledge in the study of this encrustation and its risk factors, including tools to quantify and characterize it. On the other hand, the Grantee contributes with the experimental assessment of a new stent in the porcine model, as her main research and surgical training is related to endourology, specially in the porcine species. The involvement of JE de la Cruz in this project results in the development, along with Dr Kram and Prof. Hakenberg, of the last stage of this project, where a novel Double-J stent with an anti-encrustation and antibacterial coating is tested both *in vitro* and *in vivo*. Additional to this task, the Grantee assists in Dr Kram's complementary research and clinical activity during the STSM period. This complementary activity includes animal model surgeries, support in training activities and the qualitative study of stones and encrustations.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

(max.500 words)
The work carried during the STSM can be outlined:

1- The *in vivo* assessment of a novel surface-modified Double-J stent in the porcine model. This study consisted in the endourologic placement of the novel stent vs. a standard double-J stent and the follow-up of the animals, in order to study the variables involved in the biocompatibility of the device and it's anti-encrustation effect. There were two study groups. A short term follow-up group of one week, where the main target was the release of the anti-encrustation agent into the urine and it's safety. The second group, the long-term follow-up of six weeks, is focused in the study of biocompatibility and anti-encrustation effect of the stent. During this study, the involvement of the Grantee included the performance, along with the research team (Dr. Wolfgang Kram), of the surgeries for both endourologic placement of stents and end-of-study sampling for histologic assessment, as well as the performance of the follow-ups and the analysis of the variables of study. However, some of the results are still pending.
2- The assessment of materials and coatings for stent development in the murine model. Simultaneously to the performance of the aforementioned experiments, a study of the interaction between the urothelium and different materials and coatings was performed by introducing via

cystotomy proportional samples of stents in male murine models and their respective follow-up and end-of-study. These animals underwent also the induction of encrustation for the study of the antifouling capability of these materials and coatings. The involvement of the Grantee consisted in training for the surgical procedures as well as assisting Dr Kram in the study of the encrustation on the probes and learning the induction of encrustation in animal models.

3- Contact with the multidisciplinary group, composed by clinical figures, translational researchers and engineers, involved in the development of this ureteral stent and receiving and overview of the complete development and assessment of a medical device.

4- Theoretical and practical training and assistance in the quantitative and qualitative study of urinary stones and encrustation. The expertise of Dr Wolfgang Kram has been of high value to acquire knowledge and skills in this area. The Grantee has received practical training in the use of FTIR (Fourier Transform Infrared Spectroscopy) and polarized microscopy for the evaluation of crystals and stones in urine. Theoretical knowledge regarding stone development and treatment has also been acquired.

5- Additional to this research activity, the Grantee had the opportunity to collaborate and attend as observer in the 21st Practical Course of Visceral Surgery, held at the Rudolf-Zenker für Experimentelle Chirurgie from the 20th to the 23rd May 2019.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

The main results obtained for the moment are regarding safety of the urologic device, materials and coatings assessed in the animal models. However, the complete results concering the effectiveness of the antifouling effect of the novel stent are still pending, as encrustation assessment and histologic study of the urinary tract samples will be performed in the following months. After obtaining all the complete data, statistical analysis will be carried out for proper dissemination of the results under the framework of this ENIUS.

FUTURE COLLABORATIONS (if applicable)

Future collaborations will take place for joint publication of the results of these studies. Besides, both Institutions are keen on future partnership for development and assessment of urinary stents and devices.

SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA16217
STSM title: Mathematical Modelling of Urine Flow Degradation of Biodegradable Urinary Stents
STSM start and end date: 17/06/2019 to 27/09/2019
Grantee name: Nikita Greenidge

PURPOSE OF THE STSM:

(max.200 words)

This STSM purpose was to bridge the gap between the computational simulation work of WG2 and the study of biomaterials exposed to the urinary environment of WG4. The biodegradable ureteral stents developed by Hydrustent have the unique ability to degrade homogeneously during deployment, eliminating the need for a second surgical procedure.
The aim was to develop a mathematical model that can predict the change in stent dimensions due to swelling and the time taken for the stent to degrade in vitro.
This model, once validated, will allow for a reduction in preclinical trials involved in the design of the biodegradable urinary stents and accelerate stent development. It will also give more insight into the mechanism of degradation which is not yet completely understood.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

(max.500 words)
Firstly, a literature review was conducted on the current swelling and degradation models as well as to determine the swelling and degradation mechanism of the stent's particular material.
A theoretical model was developed at the University of Oxford. This model was developed to capture the main dominant mechanisms involved in the swelling process.

In the second portion of the STSM, swelling experiments were conducted at the 3B's Research Group, Portugal to allow for model fitting. Constant communication was kept with the University of Oxford to enable iterative improvement of the model.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

A swelling model was developed which once calibrated will be able to predict the swelling of the hydrogel. Swelling data was obtained and will allow for an informed model to be developed.

FUTURE COLLABORATIONS (if applicable)
The two groups will continue to do work on this project. The next steps are to calibrate the model, make

predictions and validate the model.

SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA16217
STSM title: **Synthesis and characterization of carbon quantum dots hydrogels for ureteral stents**
STSM start and end date: 19/01/2020 to 13/02/2020
Grantee name: Biljana Todorović Marković

PURPOSE OF THE STSM:

Ideal ureteral stent should have the following features: optimal urine flow characteristics, biocompatibility, radiopacity, visibility on ultrasound, ease of insertion and removal, resistance to infection, corrosion, encrustation and should be well tolerated by patient. Apart from metal mesh stents, tail and dual durometer stents, composite materials i.e. polymers can be used as stents. Stent surface can be coated by hydrogel mostly in ureteral stents that allows the anchoring of water molecules on the stent's surface. Combination of hydrophillic matrix and hydrophobic drugs could have a promising future. Advantages of hydrogel-coated stents are improved material biocompatibility, hydrophilization and lubrication.
In the last few years we investigated dominantly structural, antibacterial and cytotoxic properties of graphene oxide (GO), graphene quantum dots (GQDs), carbon quantum dots (CQDs) and N-doped CQDs and polymers encapsulated by CQDs. The aim of this study is to synthesize material which can be coated on the surface of polymer based nanocomposites and enhanced their antibacterial properties. We prepared hydrogels based on biocellulose and doped by GQDs, chitosan and nanochitosan dots. During this STSM, detailed characterization of these samples has been conducted. We have investigated surface morphology (surface roughness), chemical composition, mechanical properties as well as photoluminescence of biocellulose hydrogels.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

During STSM four types of samples have been characterized by different techniques: nanochitosan dots, biocellulose hydrogels doped by chitosan, nanochitosan dots and GQDs, respectively.
1. Nanochitosan dots (NChiD) were prepared by the following procedure: chitosan powder was dissolved in 1wt.% acetic solution. The concentration of chitosan powder was 2 wt. %. Chitosan/acetic solution was exposed to gamma irradiation at 20 kGy, 40 kGy and 60 kGy, respectively.
Biocellulose or bacterial cellulose is a biocompatible nanomaterial with a three-dimensional network structure composed of microfibrils having nanometric diameters obtained by the *Gluconacetobacter xylinus* bacteria. Biocellulose pellicles were donated by Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine.
2. Biocellulose hydrogels doped by chitosan (ChiBC) have been prepared by the following: small pieces of biocellulose (diameter of 6 mm) were dipped into chitosan/1 wt. % acetic solution. The concentrations of chitosan in 1 wt. % acetic solution were 0.2 and 2 wt. %, respectively.
3. Biocellulose hydrogels doped by nanochitosan dots (NChiDBC) have been prepared by the following: small pieces of biocellulose (diameter of 6 mm) were dipped into the solution of nanochitosan dots. The concentrations of NChiD were 0.2 and 2 wt. %, respectively.
4. Biocellulose hydrogels doped by GQDs (GQDBC) have been prepared by the following: small pieces of

biocellulose (diameter of 6 mm) were dipped into GQDs solution in acetone. The concentration of GQDs was 1 mg/ml. GQDs have been produced by exfoliation of graphite foil in NaOH/ethanol solution.
All samples have been prepared in Vinča Institute of Nuclear Sciences, Belgrade, Serbia and characterized during STSM project in Polymer Institute of Slovak Academy of Sciences, Bratislava, Slovakia by the following techniques: atomic force microscopy (AFM), FTIR, dynamic mechanical analysis (DMA), XPS and photoluminescence (PL) measurements. For all characterizations, the biocellulose samples put on round glasses and dried in vacuum furnace at 60℃ for 12 h whereas the NChiD samples have been drop-casted on mica (AFM measurements) and Al foils for all other characterizations.

1. AFM measurements have been conducted on atomic force microscopy (Bruker-Germany) operated in air at room temperature. Surface roughness, diameters and height of biocellulose hydrogels and NChiD have been calculated by Gwiddion software.
2. FTIR measurements have been conducted on a Nicolet 8700 spectrometer operated in ATR mode. Measurement range was from 400–4000 cm^{-1} whereas spectral resolution was 4 cm^{-1}.
3. XPS measurements were performed on a Thermo Scientific K-Alpha XPS system (Thermo Fisher Scientific, UK).
4. DMA measurements were conducted using a TA Instrument dynamic mechanical analyser DMA Q800. Temperature range was from 5°C to 105°C. The samples of uniform shapes were measured in the modul tensile multifrequency strain at 1 Hz and heating rate 1 °C•min^{-1}.
5. PL measurements were performed on a RF-5301PC spectrofluorometer (Shimadzu, Japan). The excitation wavelengths were 380, 430, 480 and 530 nm, respectively.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

1. AFM measurements have been used to observe the surface morphology of all samples. Diameters of NChiD exposed to 20, 40 and 60 kGy were 66, 46 and 65 nm. The heights of were 2,1 and 1 nm, respectively-Fig. 1 (a–c).

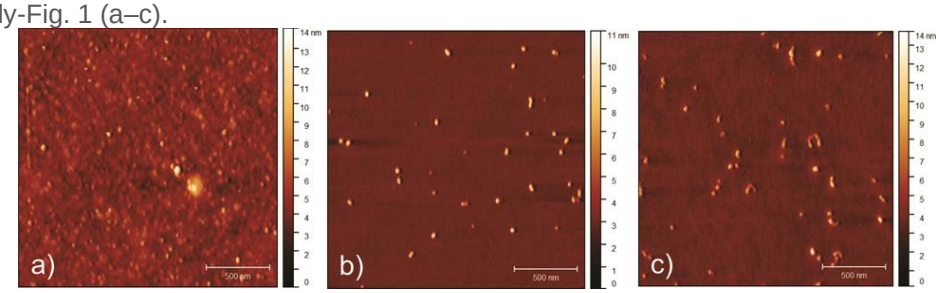

Fig. 1. AFM images of NChiD produced by gamma rays at a) 20 KGy, b) 40 kGy, c) 60 kGy, respectively.

Surface morphology of pure and doped BC samples have been presented in Fig. 2 (a–f). All samples have fibrous structure except of ChiBC-Fig. 2b. This sample has granular structure. GQDs and NChiD coated the BC microfibers. Average surface roughnesses are 10.56 nm for pure BC, 0.8 nm for ChiBC sample, 18.94 nm for GQDBC sample, 24.56, 32.05, 38.35 nm for NChiDBC samples, respectively.

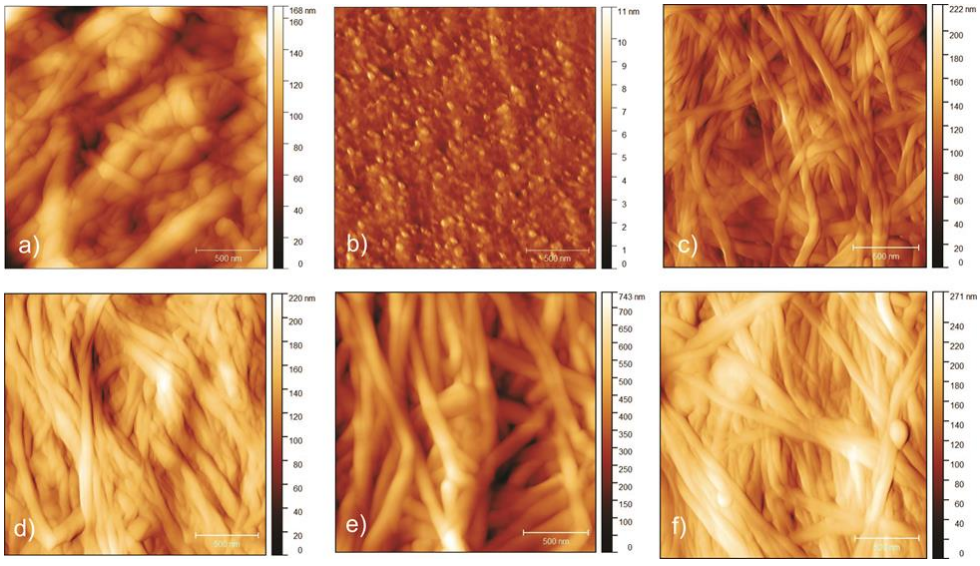

Fig. 2. AFM images of a) BC, b) 2% ChiBC, c) GQDBC, d) 20kGy NChiDBC, e) 40 kGy NChiDBC, f) 60 kGy NChiDBC samples.

2.Fig. 3 presents FTIR spectra of pure BC and NChiDBC samples. In the FTIR spectra of BC we detected all peaks characteristic for BC: 3347 cm^{-1} (O-H vibrations), 2897 cm^{-1} (C-H vibrations), 1652 cm^{-1} stem from water molecules in the amorphous region, 1427 cm^{-1} (C-H vibrations),1168 cm^{-1} (vibration from the β-anomeric link). As for peaks refeers to NChiDBC samples we detected the following peaks: 3353 cm^{-1} (O-H vibrations), 3096 cm^{-1} (N-H vibration), 2891 cm^{-1} (C-H vibrations), 1639 cm^{-1} (C=O vibrations), 1594 cm^{-1} (N-H vibrations), 1380 and 1322 cm^{-1} (C-H vibrations) 895 cm^{-1} (C-H bending vibrations). The peaks at 1038, 1058 and 1156 cm^{-1} originates from C-O-C vibrations.

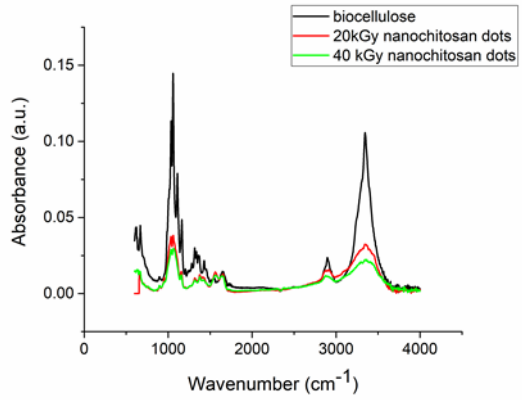

Fig. 3. FTIR spectra of BC (black curve) and NChiDBC (red and green curves).

3.XPS measurements

XPS measurements have been conducted for all prepared samples. Processing of results is underway.

4.DMA analysis

DMA testing was performed to characterize the viscoelastic characteristics of the NChiDBC samples-Fig. 4. Fig. 4a shows that the storage modulus of both samples increases with temperature. But for sample of NChiD (20kGy) storage modulus starts to decrease. The same trend can be observed for loss modulus vs. temperature-Fig. 4b. The tang δ is the ratio of loss modulus to storage modulus-Fig. 4c. The fact that the transition occurs over a broad temperature interval suggests that the fibrous network is highly inhomogeneous.

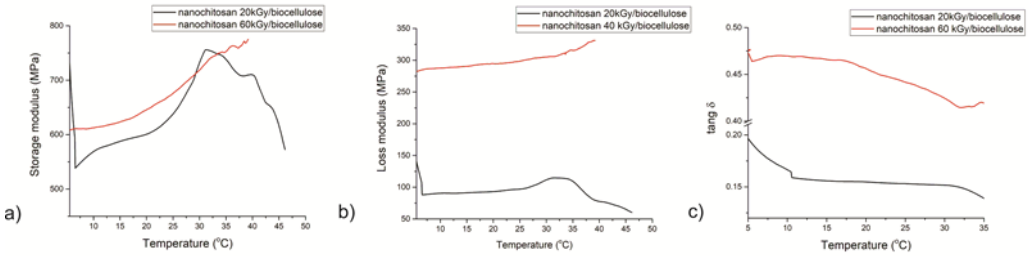

Fig. 4. a) Storage modulus b) loss modulus and c) tang δ of NChiDBC samples.

DMA analysis has been conducted for ChiBC and GQDBC samples, too.

5.PL measurements

PL measurements have shown that NChiD and NChiBC had photoluminescence at exctitation wavelengths (380, 430, 480 and 530 nm)-Fig. 5a,b. The highest emission intensity peaks (525 nm) have detected at wavelength of 480 nm for both samples. The existence of PL for NChiDBC samples indicates the incorporation of NChiD into BC. The similar spectra has been recorded for other NChiDBC samples.

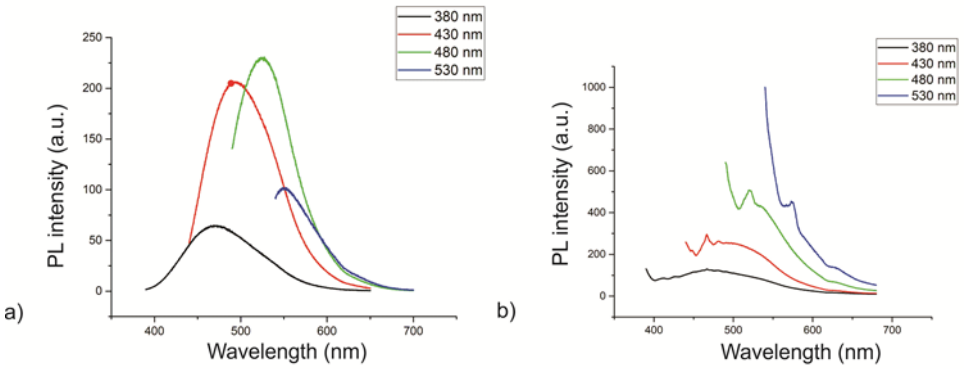

Fig. 5. PL spectra of a) NChiD and b) NChiDBC hydrogel.

FUTURE COLLABORATIONS (if applicable)

I am very grateful to my host Dr Zdeno Spitalsky and his colleagues for their warm hospitality and to COST Action 16217 for giving me the opportunity to visit Polymer Institute of Slovak academy of Sciences in Bratislava, Slovakia. This STSM was highly beneficial as it enabled efficient knowledge transfer and it is my sincere hope that this visit will further strengthen bilateral collaboration.
What remains is to continue with a joint work on the optimization parameters of produced biocellulose hydrogels doped by chitosan, nanochitosan dots and GQDs, respectively with possibility to use them as a material for urinary stents. It is planned to present the obtained results in a joint paper prepared for a peer-reviewed journal and through conference paper(s). This STSM experience as well as the obtained results and planned future work could also serve as a a starting point for future research project proposals involving Polymer Institute, Slovak Academy of Sciences in Bratislava, Slovakia and Vinča Institute of Nuclear Science, University in Belgrade, Serbia.