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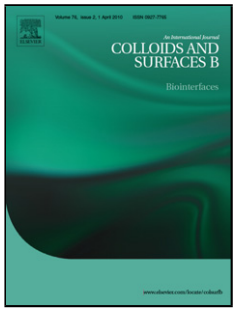

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Antimicrobial PHAs coatings for solid and porous Tantalum implants

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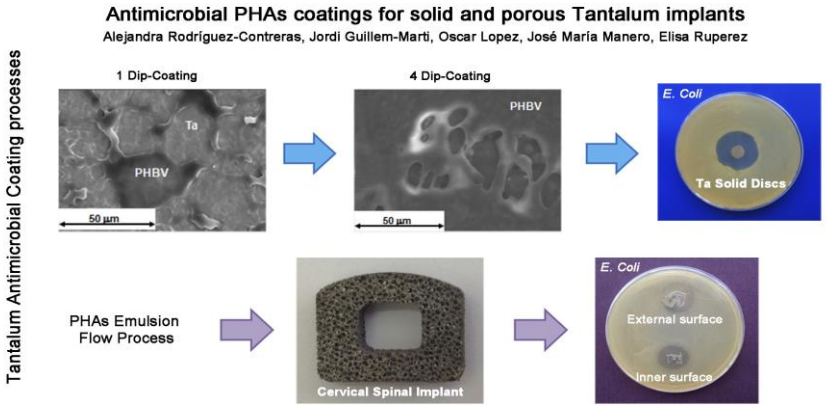
Graphical abstract



Highlights

- Drug delivery systems for Ta implants coating were developed with diverse PHAs
- The dip-coating technique was successfully used for covering solid Ta discs
- A new PHA emulsion flow process was developed for the coating of porous Ta
- PHAs coatings proved to provide Ta surfaces with antimicrobial effect
- Antibacterial, non-toxic, homogeneous PHAs coatings were attained

Abstract

Biomaterial-associated infections (BAI) are the major cause of failure of indwelling medical devices. The risk of BAI can end dramatically in the surgical removal of the affected device. Therefore, a major effort must be undertaken to guarantee the permanence of the implant. In this regard, we have developed antimicrobial coatings for tantalum (Ta) implants, using polyhydroxyalkanoates (PHAs) as matrices for carrying an active principle. The dip-coating technique was successfully used for covering solid Ta discs. An original PHA emulsion flow process was developed for the coating of porous Ta structures, specially for the inner surfaces. The complete characterization of the biopolymer coatings, their antibacterial properties, toxicity and biointegration were analyzed. Thus, non-toxic, well-biointegrated homogeneous biopolymer coatings were attained, which showed antibacterial properties. By using biodegradable PHAs, the resulting drug delivery system assured the protection of Ta against bacterial infections for a period of time.

Keywords: Porous Tantalum, Biodegradable biopolymers, Gentamicin, Dip-coating technique, Polyhydroxyalkanoates, Trabecular Metal

Introduction

Since the number of joint replacements, spinal surgeries, and dental implantations has increased in the past few decades, an urgent need has arisen to prevent implant-associated infections (Hickok, Shapiro, and Chen 2018; Kwakman et al. 2006). These infections are caused not only by post-operative bacterial proliferation in the wound but also by microorganisms introduced on the surfaces of metallic implants and surgical instruments, a result of inadequate sterilization (Ibrahim et al. 2017). An effective way to prevent biomaterial-associated infections (BAI) is by the creation of new implants with antimicrobial properties.

Tantalum (Ta) is one of the most chemically stable and biologically inert biometal used in orthopedic implants, and is characterized by its excellent anticorrosion resistance and biocompatibility. However, its significantly high elastic modulus (186 GPa), and density (16.6 g/cm^3), among other mechanical properties, make it incompatible with bone tissue, and unsuitable for load-bearing implants (Prasad et al. 2017). By designing porous structures with Ta, more appropriate and appealing mechanical properties are obtained. The elastic modulus varies depending on the porosity of these structures (Paganias et al. 2012), and can be found between 2 and 20 GPa. These closely match with that of human cortical bone, which possess a modulus in the range of 3 to 20 GPa (Dhawan et al. 2017; Paganias et al. 2012). Modulus matching has the potential to reduce stress-shielding and related problems in load bearing metallic implants.

In a previous work (Rodríguez-Contreras et al. 2017), we developed original antibiotic-loaded biopolymer coatings on titanium (Ti) implants that were designed to serve as devices to prevent implant-associated infections by a gentamicin-controlled release phase. The biopolymers used were poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) from the family of the polyhydroxyalkanoates (PHAs). These bio-polyester are naturally produced by microorganisms, and are characterized by their biodegradability, biocompatibility and non-toxicity (Rodríguez-Contreras, Koller, et al. 2013). The use of these biopolymers provides a prolonged drug release through their degradation with time and under physiological conditions (Chen and Wu 2005; Wu, Wang, and Chen 2009). Thus, the drug delivery system (DDS) designed not only assured the avoidance of the first stage of BAI, bacterial adhesion, but also their proliferation and biofilm formation. Now, by using PHB and two of its copolymers, PHBV and Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (PHB4HB), we were able to create protective layers on Ta surfaces, which provided the implant with an antibacterial character. We also attained in this work the covering of

porous Ta (Zimmer Biomet's Trabecular Metal) by a novel biopolymer emulsion flow process. The challenge was the coating of the inner surfaces, with the objective of the generating antimicrobial coverage on porous Ta surfaces, while allowing the proliferation of eukaryotic cells, such as osteoblasts.

Materials and methods

Materials

Solid 10 mm diameter and 1 mm thick Ta discs were supplied by Goodfellow company (Goodfellow Bros., Inc., Washington, USA), while the porous Ta samples for cervical spinal implant (marketed under the name of Trabecular Metal™ Material) were supplied by the Zimmer Biomet company (Indiana, USA). The PHA's used for the study, poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and (PHB4HB), were kindly provided by Dr. Martin Koller from the Institute of Biotechnology and Biomedical Engineering of Graz (Austria). The antibiotic used was gentamicin sulfate (Genta, with a molecular mass of 477.596 g/mol, freely soluble in water) purchased from Sigma-Aldrich (Missouri, USA). Chemical products such as hydrogen peroxide, Trichloromethane, poly(vinyl alcohol) (PVA) were also purchased from Sigma-Aldrich, and used without further purification.

PHA's characterization

Biopolymers molecular masses (Mw) and their corresponding polydispersity indices (PDI) were determined with gel performance chromatography (GPC), using a Waters Styragel HT column (Waters Corporation, Massachusetts, USA). Chloroform was used as an eluent at a flow rate of 0.80 ml/min with a stabilization pressure of 35 bars, and a sample concentration of 1.5 mg ml^{-1}. Polystyrene with different Mw's were used as standard. The chemical structure of the three natural PHAs, and monomer compositions of PHBV and PHB4HB were determined by proton nuclear magnetic resonance (^{1}H NMR) (Jagur-Grodzinski 2006). Spectra were recorded at 25 °C on a Bruker AM300 spectrometer (Bruker, Massachusetts, USA). The biopolymer samples were dissolved in deutered chloroform, and a drop of tetra methyl silane was added as internal standard for the calibration of the ^{1}H chemical shift. Proton spectra were recorded at 300.1 MHz with a spectrum of 32 K data points. A total of 64 scans were utilized with a relaxation delay of 1 s.

Surface treatment procedures

Smooth, mirror-like surfaces were achieved by grinding with SiC papers of decreasing grit size (from P800 to P4000, European grade standard), followed by polishing with diamond crystal suspension (1 μm particle size) on cotton cloths. Samples were then ultrasonically rinsed with distilled water, ethanol and acetone. Finally, they were stored under a vacuum.

Sodium hydroxide treatment (NaOH) was carried out after polishing. Ta disks were immersed in 2 and 5 M NaOH freshly prepared solution in closed poly(propylene) flasks. They were then placed into a furnace at 60 °C for 24 h, after which the samples were cleaned and immersed in Milli-Q water for 30 min, and rinsed with water of the same quality and acetone. They were dried with nitrogen, and stored in a vacuum.

Preparation of the water in oil (W/O) emulsions and their viscosity

Based on our previously reported studies (Rodríguez-Contreras, Canal, et al. 2013; Rodríguez-Contreras et al. 2016; Rodríguez-Contreras et al. 2017), we used the following methodology for the preparation of biopolymers emulsions containing gentamicin sulfate. A Bandelin Sonopuls ultrasonic homogenizer with a micro-tip MS 73 (Bandelin, Germany) was used at 5 W for 3 min to sonicate a concentrated biopolymer solution (40 mg/mL) in chloroform (organic phase), with an aqueous solution of Genta with 2% PVA as an emulsifier. Different Genta concentrations 10% and 30% (w/ w) of the polymer mass were used to form the simple W/O emulsion. In regards to the porous Ta samples, the emulsion used for their coating was prepared with a 10% diluted PHBV solution.

The viscosity of the emulsions was determined for their characterization. A rotational viscometer Haake Mars (Thermo Fisher Scientific, Massachusetts, USA) was used at 25 ºC and with a fixed rotation speed of 0,6 mm/min to determine the viscosity of the biopolymer emulsions.

Coating processes for Ta samples

Dip-coating process for solid Ta discs: The daily fresh prepared W/O emulsion was used to coat Ta surfaces *via* dip-coating following a methodology previously reported from studies with Ti (Rodríguez-Contreras et al. 2017). A dip coater KSV (Nima instruments, Stockholm, Sweden) was used with a single vessel and KSV NIMA Dip Coaters software. Shortly, the substrate was immersed in the W/O emulsion at room temperature (22 °C) with a constant rate of 10 mm/min and extracted with a controlled output rate of 70 mm/min. Different numbers of immersions were tested to obtain a totally coated surface.

PHAs emulsion flow process for porous Ta: Preliminary studies for the coating of porous Ta samples were carried out with PHBV emulsified in chloroform. The usual PHBV concentrated solution and a 10% diluted one were used to prepare the emulsion. The samples were initially place into chloroform, and then briefly subjected to ultrasound, so as to eliminate the air entrapped in the sample pores and to improve their wettability with the biopolymer emulsion. Samples were placed inside a syringe. With the syringe capped, a volume of biopolymer emulsion was poured into the syringe body, in an amount sufficient to cover the sample. The plunger was inserted back into the syringe body, and the trapped air was eliminated by tilting the syringe upwards and applying pressure. With the syringe capped, a force of approximately 2.20 kN (7kPa of pressure) was executed for 5 min. Then, the stopper of the cannula was removed to let the emulsion flow though the sample until the plunger reached the sample. Finally, the sample was collected and left to dry in the air.

Wettability and Contact Angle (CA)

Contact angles with water were measured for the characterization of the Ta surface hydrophilicity, and with biopolymer concentrate solutions (40 mg/mL in chloroform) to study the wettability (affinity) of the biopolymer to the substrate. Static contact angles were estimated using the sessile drop method (Contact Angle System OCA15 Plus; Dataphysics, Germany). Measurements were acquired in triplicate for three samples at room temperature, with a volume of 3 μL, and a dose of 1 μL/s.

Field Emission Scanning Electron Microscope (FESEM)

A field emission scanning electron microscope JEOL JSM-7001F (Toyo, Japan) operating at 20 kV was used to study the morphology and topography of the PHAs coatings. Back scattered electron (BSE) images were used to analyze and confirm that the Ta surfaces were completely covered by the dip-coating method. Energy Dispersive X-ray Spectroscopy (EDS) was also used for the elemental analysis of the surfaces. High-resolution images allowed pore diameters to be measured using Photoshop (Adobe Systems, San Jose, CA, USA).

Interferometry and profilometer

White light interferometry (Wyko NT1100 Optical Profiler, Veeco Instruments, USA) was used to evaluate the roughness of the samples. The average height (Ra) of each sample was measured in three randomly

chosen points on the disk. The thickness of the coatings was measured with a Dektak 150 profilometer (Veeco Instruments, USA) with a scan length of 6 mm and a measuring range of 524 μm.

Cytotoxicity

The methodology used to analyze the toxicity of coated Ta surfaces was an indirect *in vitro* test carried out with human osteoblast-like SaOS-2 cells (ATCC, Manassas, VA). Although SaOS-2 cells are an osteosarcoma cell line, their suitability as osteoblast cell model has been widely demonstrated (Pautke et al. 2004; Czekanska et al. 2012). Each specimen was immersed in Dulbecco's modified Eagle's medium (DMEM) for 72 h at 37 °C with an extraction medium area/volume ratio of 0.5 cm^2/mL (according to ISO 10993-5). Afterwards, the extraction medium was removed and diluted with DMEM (dilution 1/0; 1/ 1; 1/10; 1/100; 1/1000) and added to previously-seeded SaOS-2 cells. After 24h of incubation, cells were lysed with M-PER (Pierce, USA) and the activity of the lactate dehydrogenase (LDH) enzyme was analyzed using the Cytotoxicity Detection Kit LDH (Cytotoxicity Detection Kit - LDH, Roche Diagnostics, Mannheim, Germany). Absorbance's were recorded at 492 nm using a Synergy HTX Multi-Mode Reader (Bio-Tek, USA). As a reference for 100% maximum survival, cells were placed in TCPS (Tissue culture polystyrene).

Cell adhesion

Human osteoblast-like SaOS-2 cells were seeded at a density of 20,000 cells/disk onto sterilized and coated Ta discs loaded with antibiotic, and incubated in an atmosphere at 37 °C containing 5% (v/v) CO_2. After 4 h, non-adherent cells were washed off by gently rinsing with PBS, and remaining cells were lysed with 200 μl/well of M-PER® (Pierce, Rockford, IL, USA). Enzymatic activity of lactate dehydrogenase (LDH) was quantified by colorimetric assay (Cytotoxicity Detec-tion Kit - LDH, Roche Diagnostics, Mannheim, Germany), using a multimode microplate reader (Infinite M200 PRO, Tecan GroupLtd., Männedorf, Switzerland). Cell number was obtained using a standard curve. Tests were carried out with total coatings of the surfaces and with 30% of Genta incorporated. Cell adhesion on PHAs (antibiotic free) and on activated Ta were also evaluated as controls.

Antibacterial assay: diameter of growth inhibition

Porous and solid Ta samples were sterilized by washing them three times for 5 min in ethanol (70%, v/v), and they were then kept under UV overnight. A commonly used procedure for studying the antibacterial action of the coated surfaces was followed (Madigan, Martinko, and Parcker 2000; Nonsee, Supitchaya, and Thawien

2011). The assay was performed with both Gram-positive and Gram-negative bacteria (*Staphylococcus aureus* and *Escherichia coli*, respectively). The overnight-incubated bacteria solutions were diluted with sterile PBS solution to reache an absorbance value of 0.2 at 600 nm (bacterial concentration about 10^8 CFU/ml). The solutions were used to inoculate (100 µL) the Petri plates with Chromocult® (Merck) media for *E. coli* and Triptone Soy Agar (TSA) media for *S. aureus*. The samples were placed in the middle of the inoculated surface. The plates were incubated at 37 °C for 24 h, and the diameter of the growth inhibition halo was then measured. Antibiotic-free coated Ta samples were used as controls. Tests were carried out in triplicate.

Statistical studies

Surface characterization and biological results were expressed as a mean value of standard deviation (SD) for each sample. T-test was used with a 95% confidence interval to evaluate statistical differences.

Results and discussion

In previously reported studies (Rodríguez-Contreras et al. 2016; Rodríguez-Contreras et al. 2017), we demonstrated that biodegradable PHAs are excellent biopolymers for their use in biomedicine, especially as drug carriers. By designing the appropriate DDS, it is possible to adopt different strategies to achieve an antibacterial effect on medical device surfaces. Thus, PHB and its copolymers were chosen as matrixes for drug bearers, and to develop antibacterial coatings on solid and porous Ta surfaces.

Characterization of the PHAs

The Mw and the PDI are fundamental parameters for the understanding of the biopolymer properties and their behavior. While PHB and PHBV show very similar Mw, the Mw of PHB4HB is about 5 times higher (**Figure 1a**). The three PHAs show large PDIs of their molecular masses and, therefore, open distribution hoods. This is common for natural biopolymers produced *via* fermentation (Rodríguez-Contreras, Koller, et al. 2013). The chemical structures and the quantification of the monomeric composition of the natural copolymers were determined by H^1NMR (**figure 1b, c and d**). All copolymer spectra show the main peaks corresponding to the protons of the 3HB monomer: a doublet at 1.274 ppm attributed to the methyl group ($-CH_3$) coupled with one proton, a doublet of quadruplet at 2.520 ppm, which is attributed to a methylene group ($-CH_2$) adjacent to an asymmetric carbon atom bearing a single proton, and a multiplet at 5.260 ppm characteristic of the methane

group (-CH) (Rodríguez-Contreras et al. 2017). The characteristic peaks at 0.99 ppm attributed to the methyl group for valerate monomers, and at 4.05 ppm attributed to the methylene group of 4-hydroxybutyrate were used to accurately determine their composition (Valentin and Dennis 1997). The integration data of these peaks revealed that PHBV is composed of 19 mol.% of the 3HV monomer, and PHB4HB is formed by a 10% of the 4HB monomer.

Ta activation treatment

When Ta was treated with 5 M alkali concentration, a more hydrophilic surface and greater roughness (compare **figure 2a, b and c**) was attained compared to the treatment with 2 M NaOH (**figure 2d**). The chemical treatment with NaOH removes organic contaminants and promotes surface oxidation and hydroxylation (OH groups) by increasing surface wettability (Paredes et al. 2014). Moreover, it imparts submicron or nano-roughness (Kim et al. 1996). Thus, the alkali treatment provided the surface with more hydroxyl groups and a greater superficial area, representing more echoing points for linking the biopolymers to the surface. As demonstrated by **Kato et al. (2000) (Miyazaki et al. 2000b),** a thermochemical treatment of Ta with NaOH favors the direct union between metal and bone. By this process, the formation of a layer of sodium tantalate is achieved, which provides Ta-OH groups on the surface of the implant material. These groups induce the nucleation of apatite in the same way as occurs with Ti or Si **(Miyazaki et al. 2000b)**. Thus, this treatment will help to prepare a suitable surface for the deposition of cells once the biopolymer coating is degraded.

Contact angles with PHAs solutions

Concentrated PHA's solutions were prepared to study their wettability and affinity to the activated Ta surfaces (**Figure 2e**). The biopolymer affinity to the surface is referred to the linking of the biopolymers chain with OH from the activated Ta surfaces via Van der Waals bounds (Rodríguez-Contreras et al. 2017). PHB shows greater affinity to the Ta surfaces compared to the other PHA's, especially when the Ta surfaces were treated with 5 M NaOH. Contact angle of PHBV solution does not show a significant difference in wettability on the three Ta surfaces tested. PHB4HB solutions show low affinity to Ta surfaces, however, when they are treated with NaOH, and this affinity improves by decreasing the contact angle. The highest wettability and affinity observed for PHB solutions and the lowest ones for PHB4HB are related to their molecular structure and their Mw. While PHB shows more polar groups, specially more OH at the end of the chains due to its

lower molecular mass, 4HB monomer introduces more nonpolar groups such as methylene ($-CH_2$) to the copolymer.

Characterization of the biopolymer emulsions, viscosity

We demonstrated in previously reported studies **(Rodríguez-Contreras et al. 2017)** that the incorporation of the antibiotic, gentamicin sulfate, as a dispersion inside the biopolymer solution in chloroform (PHB and PHBV) did not result in a homogeneous coating. Since gentamicin is not soluble in the same solvents as the PHAs, and aiming for a homogeneously distribution of the antibiotic on the whole Ta surface, W/O emulsions of the biopolymers diluted in chloroform and the active principle diluted in water together provided an appealing alternative for the coating of Ta surfaces.

Preliminary tests (data not shown) confirmed that PHAs emulsions have a non-Newtonian behavior, regardless of the emulsifier concentration. In non-Newtonian fluids, the shear stress is not directly proportional to the deformation rate, and their constitutive equation is expressed by the equation: $\tau = K\,\gamma^n$, where k (consistency index) and n (index behavior) are constants. The parameter n is known as apparent viscosity by its similarity to Newton's law. When $n < 1$ this model corresponds to thinning fluids. If $n > 1$ this model refers to dilatant fluids, and when $n = 1$ the behavior matches with a Newtonian fluid and the apparent viscosity coincide with the viscosity ($n = \mu$) **(Derkach 2009)**. The three co-polymer emulsions are shear thinning liquids ($n < 1$), whose viscosity decreases with the rate of shear strain (**figure 2f**). For the evaluation of the degree of viscosity of the emulsion, the consistency index is the parameter of analysis. Therefore, PHB4HB emulsion is the most viscous of the three, supported by Mw results. Consequently, PHB4HB was discarded for the coating process of the porous Ta structures, since its dense emulsion would have difficulties to enter through the porous network of the material, therefore requiring more pressure for the coating of its inner walls.

Studies on solid Ta samples

Dip-coating technique and coating characterization

Coatings produced by different immersions in the biopolymer emulsions were analyzed via FESEM (**Figure 3a and b**), aiming to obtain a total coating of the surface. Although two antibiotic concentrations (10 and 30% Genta) were evaluated, there were no visual changes on the coatings topography. For PHB and PHBV, four

immersions were necessary to completely coat the Ta surface, while only three immersions in PHB4HB emulsion were sufficient to totally cover it (**Figure 3b**). BSE analyses and micrographs at higher magnifications (**Figure 3c**) confirm the complete covering of the substrate. Most likely, this difference is caused by the higher viscosity and, therefore, higher density of PHB4HB compared to that of PHB and PHBV. Compared to previous work (Rodríguez-Contreras et al. 2017), Ti needs more immersions in PHB and PHBV emulsions for an entire coating of its surface. This can be related to the molecular interaction between the PHAs and the two different biometals, Ti and Ta.

Regarding the topography, PHBV coatings on Ti showed a highly porous surface, while the PHB coatings were solid and smooth (Rodríguez-Contreras et al. 2017). However, PHB coatings on Ta show some porosity, and this feature of PHBV coatings is more moderated than on Ti. This difference on the polymeric surface topography is probably due to the number of immersions and the evaporation process of the solvents containing the biopolymer and the active principle. In the case of PHB4HB coatings, they showed the greater porosity with the highest roughness of the three PHAs tested (**Figure 3d**). PHB4HB also forms the thicker coating, this being related to the volume that the porosity provides. The fact that PHB4HB emulsion is the more viscous and denser explains the difference on coating thickness and its porosity.

For the characterization of the biopolymer coatings, the water contact angles were analyzed. However, they were not possible to measure since the drops of water were immediately absorbed as soon as they touched the biopolymer surfaces, as it previously happened with polymeric coatings on Ti (Rodríguez-Contreras et al. 2017),

Cytotoxicity and cell adhesion

The conditions studied were the ones exhibiting the greatest risk of toxicity: coatings with 4DC for PHB and PHBV, and 3DC for PHB4HB, with the highest content of antibiotic (30% w/w Genta). The toxicity of the PHAs, and activated Ta (alkali reaction with 5M NaOH) was also evaluated. According to UNE-EN ISO 10993-5, the percentage of cell survival should exceed 80% in the first dilutions to ensure the non-toxicity of the coatings. The average cell number exposed to extracts from the different conditions tested exceed this limit in all cases and for all dilutions (**Figure 4a**). This indicates that the solution proposed in this study to combat BAI does not cause any problem in regards to cellular toxicity. This test also confirms that chloroform

from biopolymer solutions evaporates entirely, as confirmed by the elemental analysis via EDS (data not shown).

The objective of the cell adhesion test was to verify that the proposed DDS had an appropriate behavior concerning osteoblastic cell adhesion, as well as in the subsequent growth of bone tissue on the device surface. Although the cell number average shows that more osteoblasts adhered to activated Ta than on PHAs surfaces, there are no significant differences between the metal surfaces and most of the biopolymer coatings (**Figure 4b**). Only PHB and PHB4HB coatings without Genta show significant differences on cell number compared to Ta surfaces. PHAs surfaces do not show significant differences between them, regardless of the presence of Genta. On one hand, the slight increase in the number of cells in coatings with Genta compared to the corresponding controls (biopolymers alone) confirm the results obtained in the cytotoxicity test (the combination of PHAs with the antibiotic is non-toxic). On the other hand, a greater number of cells adhered to Ta was expected since its biocompatibility was improved by the surface activation treatment (with NaOH). Miyazaki et al. (Miyazaki et al. 2000a) already determined the relation between live bone and Ta without the formation of a fibrous capsule that would prevent direct cell adhesion to the metal tissue. They already attributed this kind of osteoinductive and osteoconductive properties to NaOH treatment.

Antibacterial properties of the PHAs coatings

Determination of bacterial growth inhibition was performed with both Gram-positive (*S. aureus*), and Gram-negative (*E. coli*) bacteria. While polymeric controls and Ta surfaces did not show any zone of growth inhibition, all cases tested (PHB, PHBV PHB4HB with 10 and 30 % Genta and with only 1DC and with the surface completely covered) showed different diameters of growth inhibition halos for both microorganisms. These halos are higher for the Gram-positive strain, especially when Genta was used in a higher concentration (30% w/w) (**Figure 5**). These results are indicative that Genta is released above 0.0005 mg/mL, which is the Minimum Inhibitory Concentration for Genta (Salmon and Watts 2000). *S. aureus* has become a major pathogen involved in hospital-acquired infections (Lemaître et al. 1998), and it has proved to be susceptible to Genta (Brehm-Stecher and Johnson 2003). The zone of inhibition growth was slightly higher when the number of dip-coatings increased, i.e. when the Genta concentration was higher. The halo diameter is therefore proportional to the percentage of drug concentration and the number of immersions (Rodríguez-Contreras et al. 2017).

Studies on porous Ta samples

The cervical spinal implant, Trabecular Metal™ Material (TM), is up to 80% porous and has a fully open interconnected pore structure, which offers an osteoconductive scaffold to allow for bone in-growth into the implant. TM's high coefficient of friction provides initial fixation and modulus of elasticity similar to cancellous bone **(Company)**.

Biopolymer coating characterization

The pore size was estimated between 400 and 600 μm (**Figure 6b**), enough for the cells to proliferate thorough the sample. The coating process of these samples was first carried out with the usual concentrated solution, however, the pores were obstructed once the emulsion dried (**Figure 6a inset**). Because the PHAs are characterized by being biodegradable under physiological conditions (Chen and Wu 2005; Wu, Wang, and Chen 2009), the porous space would eventually be freed, allowing the proliferation of the eukaryotic cells. However, by preparing a 10% less-concentrated PHBV solution, the emulsion viscosity decreased (**Table in figure 6c**), allowing the fluid to flow better through the pores during the coating process. Consequently, the coating was more homogeneous, and the pores were not blocked once the emulsion was dry (**Figure 6a**). The morphology of the biopolymer coating of the TM samples differs from the solid Ta discs. The PHBV coatings appear to be formed by rounded shapes (**Figure 6d**), while on the discs, the coatings morphology was flatter, simply showing some porosities. This difference on biopolymer coating topographies could be attributed to the roughness that TM initially shows on its surface (**Figure bI**).

Antibacterial properties

The porous Ta samples coated were exposed to *E. coli* and *S. aureus* to confirm the antibacterial properties provided to the implants. Not only the outer surface was tested but also the inner one (**Figure 6e**). Both surfaces, the external and the cross section showed growth inhibition halo with both microorganisms. Although the implant samples were not exactly the same, due to the process for obtaining the cross section, the halo of growth inhibition could be estimated (**Figure 6e**). The halo diameter was of (2.07±0.70) cm for the outer surface and (2.16± 0.35) cm for the cross section with *E. coli*, and (2.53±0.76) cm and (2.83±0.42) cm, respectively for *E. aureus*. As it occurs with Ta discs, the diameters of the growth inhibition halo of the *S. aureus* are slightly greater than the ones from *E. coli*. These results reveal a new protective character of the cervical spinal implant, provided from the drug-bearer biopolymer coating.

Conclusion

Porous Ta with its good biocompatibility and elastic modulus is a biomaterial that was introduced in orthopedics to overcome problems related to implant loosening. Finding a simple way to protect implants from BAI is challenging. By means of the dip-coating technique, we have found a way to optimally coat solid Ta discs with a biopolymer matrix carrying an antibiotic. Porous Ta surfaces were also successfully covered by a biopolymer emulsion flow process. PHAs coatings containing Genta proved to provide Ta surfaces with antimicrobial effect, protecting the implant from Gram-positive and -negative bacteria. The results on cytotoxicity and cell adhesion confirmed the biocompatibility of the designed DDS, and suggest their suitable osteointegration. The fact that the PHAs used for the developed coatings degrade in human bodily fluids assures the release of the antibiotic in the time frame within which the biopolymer will be degraded. Thus, they will provide a protection from microorganisms and BAI for a longer period of time. Once again, we demonstrate that using these natural biodegradable biopolymers, it is possible to adopt different strategies to achieve an antibacterial effect on medical device surfaces. Furthermore, the alkali treatment with NaOH to which Ta surfaces were exposed was shown to increase the contact surface, allowing for more binding points between the substrate and the coating. Thus, a more bioactive material is obtained that favors cell adhesion and proliferation once the coating is degraded.

Author contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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Figure captions

a)

PHA	M_w (KDa)	PDI
PHB	240	8.00
PHBV	270	3.37
PHB4HB	1315	7.90

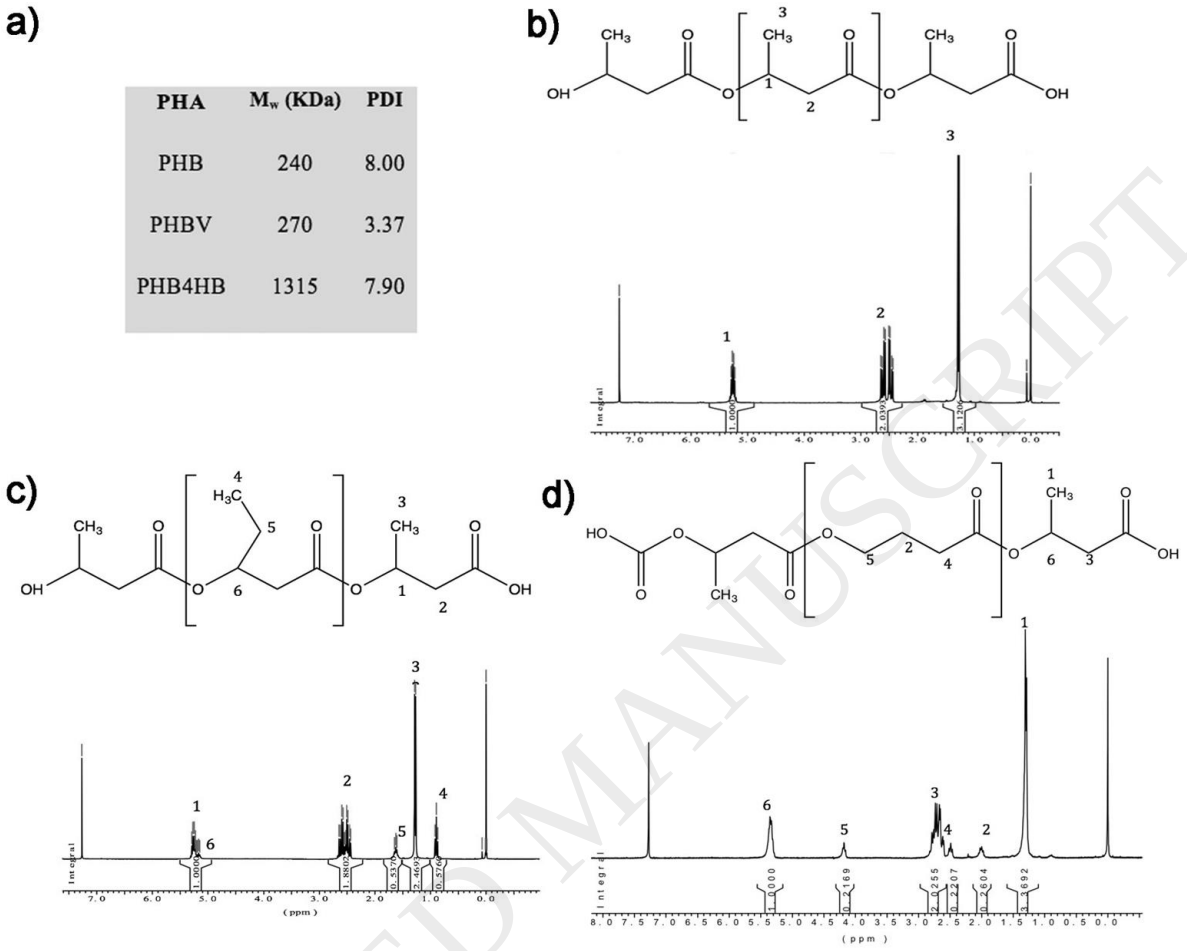
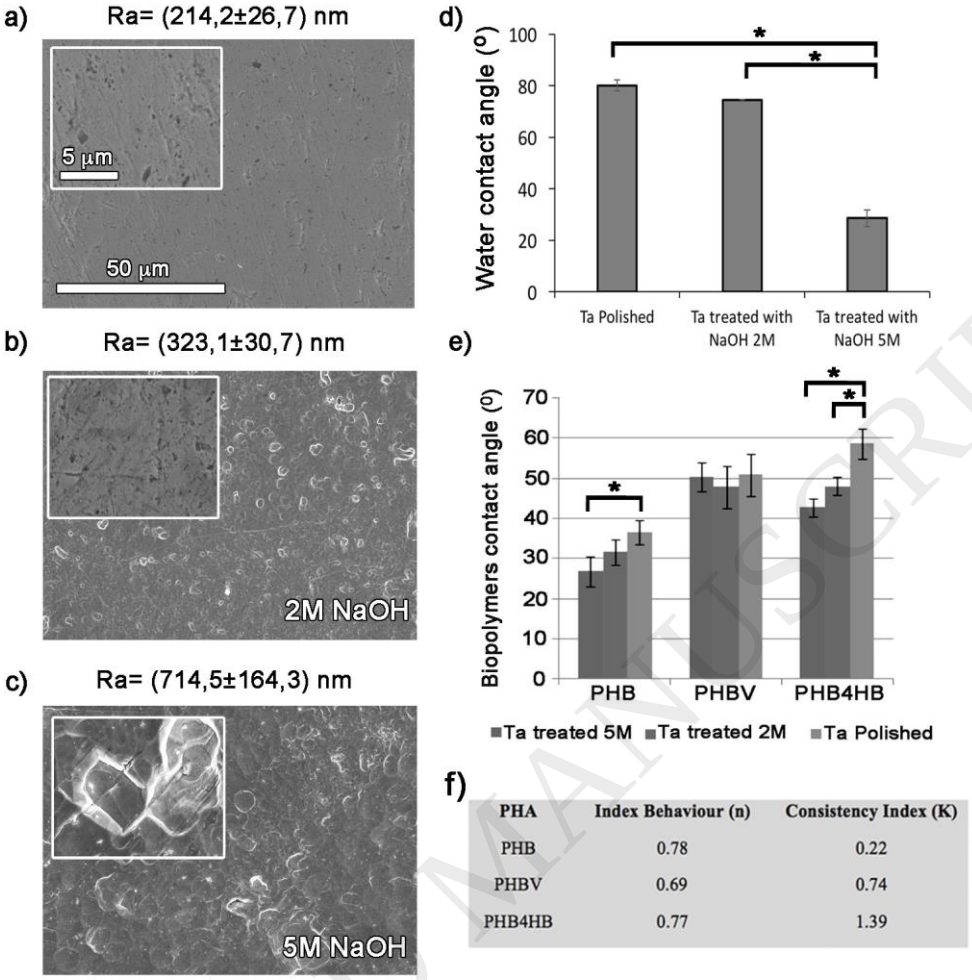
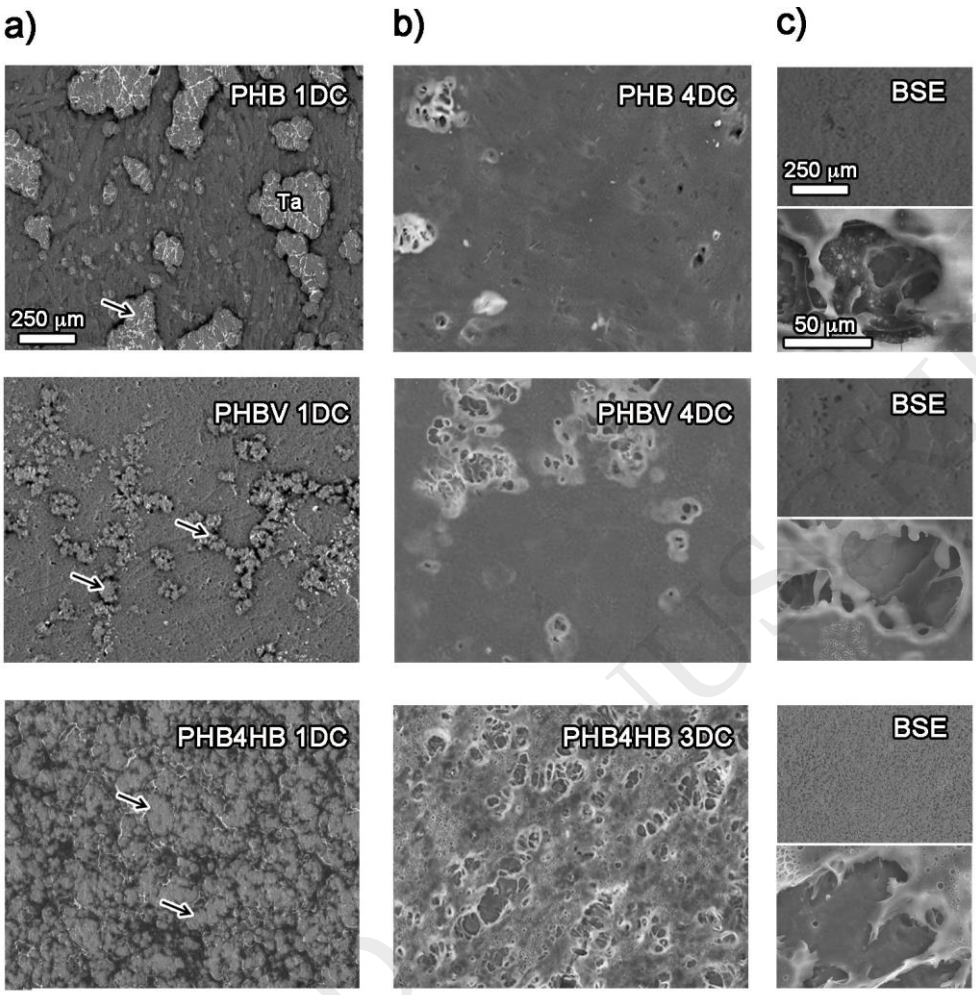


Figure 1. Biopolymer Characterization: (a) Molecular masses (M_w) of the biopolymers with their respective polydispersity indices (PDI). H^1NMR spectra of (b) PHB, (c) PHBV and (d) PHB4HB.



PHA	Index Behaviour (n)	Consistency Index (K)
PHB	0.78	0.22
PHBV	0.69	0.74
PHB4HB	0.77	1.39

Figure 2. Ta Surface and Emulsion Characterizations: (a) FESEM micrograph and arithmetic average of the roughness profile (Ra) of Ta surface polished, after NaOH treatment with (b) 2 M NaOH and (c) 5 M NaOH. (d) Water contact angle of Ta surfaces with the different NaOH concentrations. There are significant differences between the contact angle formed on Ta surfaces treated with 5 M NaOH and with 2 M NaOH (*$p < 0.05$: statistically significant). (e) PHAs emulsions contact angles on Ta surfaces polished and treated with 5 M or 2 M NaOH solutions (*$p < 0.05$: statistically significant). (f) Table of viscosity with index behavior and consistency index from the viscosity constitutive equation for non-Newtonian fluids.



d)

	PHB		PHBV		PHB4HB	
Genta Concentration	10%	30%	10%	30%	10%	30%
Thickness (µm)	21,75 ± 3,25	22,54 ± 4,25	28,32 ± 4,84	34,01 ± 3,60	53,54 ± 4,53	78,73 ± 6,69
Ra (µm)	2,03 ± 0,44		7,37 ± 0,80		10,05 ± 1,35	

Figure 3. Dip-Coating Characterization: FESEM micrographies of Ta surfaces coated with PHB, PHBV and PHB4HB showing (a) the surface uncoated by 1 immersion (1DC) and (b) surfaces totally coated by 4 immersions (4DC) for PHB and PHBV, and 3 immersions (3DC) for PHB4HB. Black arrows show some of the non-coated sections of the sample. (c) BSE surfaces analyses (images above) and micrographies at higher magnifications (images below) demonstrate that the Ta surfaces in c are totally covered by the biopolymer.

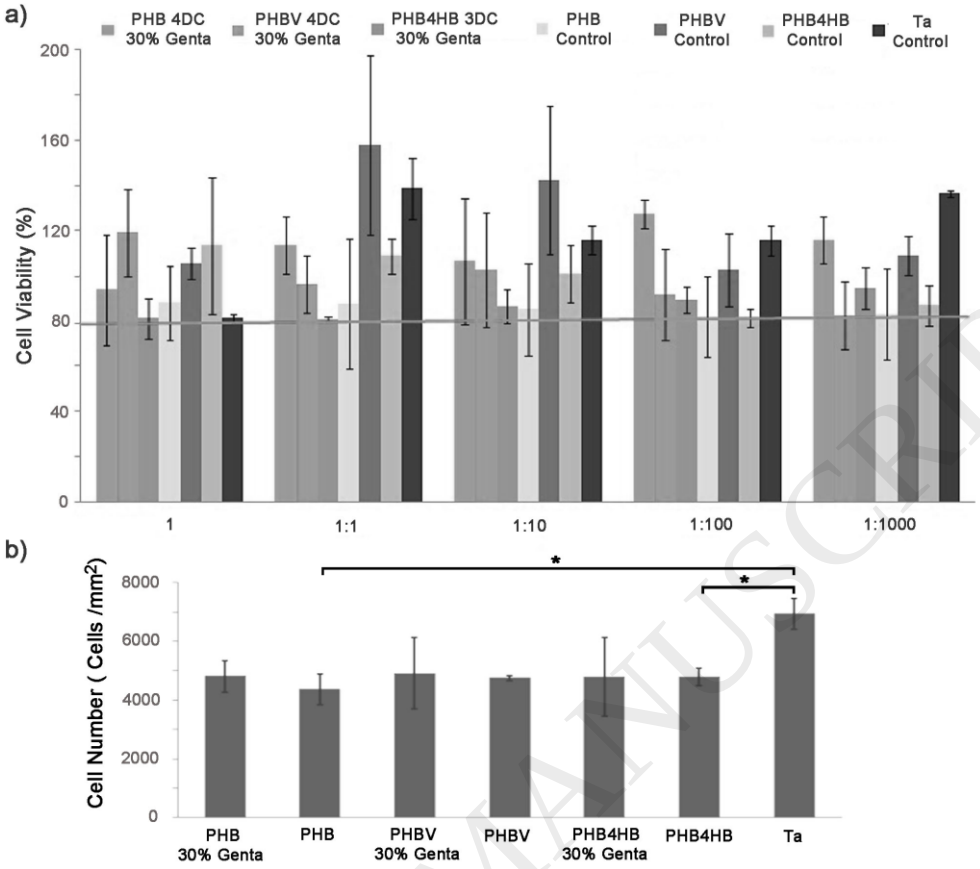


Figure 4. Cytotoxicity and Cell Adhesion: (a) Percentage of cell survival in contact with the biopolymeric coatings of PHB, PHBV and PHB4HB. The horizontal line indicates the limit of toxicity per UNE-EN ISO 10993-5. (b) Number of adhered cells to the biopolymer coatings and controls (*$p < 0.05$: statistically significant).

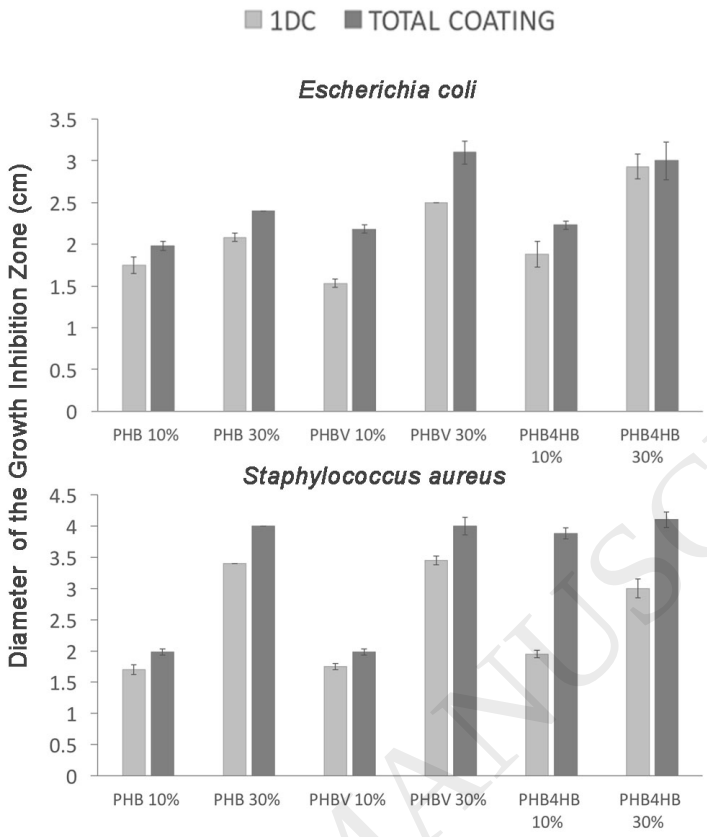
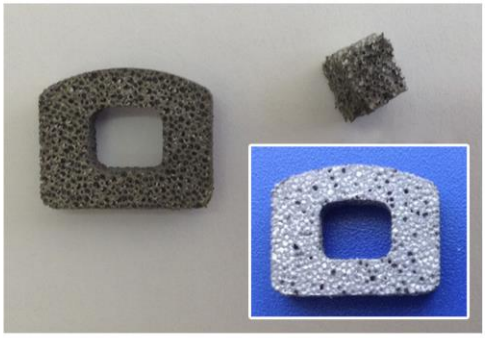
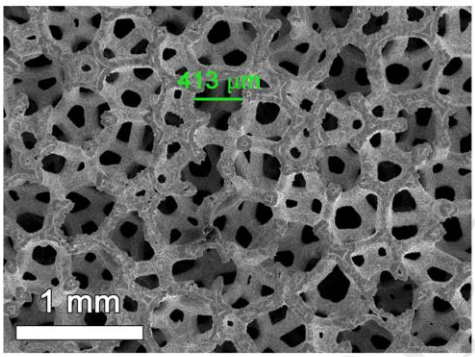


Figure 5. In vitro antibacterial assay: Diameter of the bacterial growth inhibition zone produce by PHAs coatings with different antibiotic concentration, 10 and 30 % Genta. Controls of the biopolymers and active Ta produce no growth inhibition.

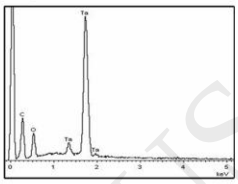
a)



b)



bI)



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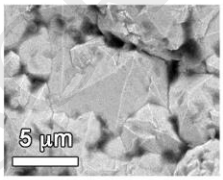
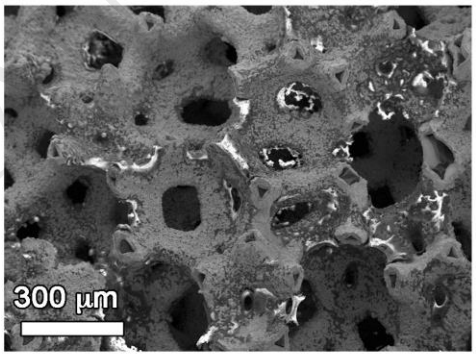


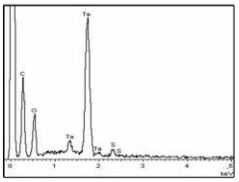
c)

Emulsion	Index Behavior (n)	Consistency Index (K)
PHBV concentrated	0.694	0.744
PHBV 10% diluted	0.697	0.308

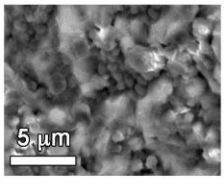
d)



dI)



dII)



e)

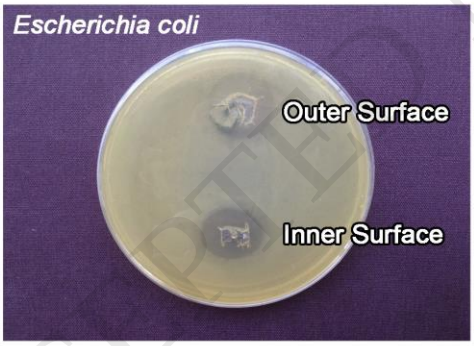


Figure 6. Porous Ta TM. (a) Entire TM sample and its cross section coated with a PHBV emulsion produced with 10% diluted biopolymer solution in chloroform. The inset image shows the TM sample coated with the PHBV emulsion produced with the usual biopolymer emulsion. (b) SEM micrograph of the TM inner surface. (bI) Elemental analyse spectra by EDS of the Ta surface and (bII) SEM micrograph of its surface. (c) Table of the viscosity parameters with index behavior, and consistency index of the PHBV solution used to prepare the emulsion. (d) SEM micrograph of the TM inner surface coated with PHBV prepared with a biopolymer solution 10% diluted from the concentrated one. (dI) Elemental analyse spectra by EDS of the coated Ta

surface, showing the presence of sulfur, corresponding to gentamicin sulfate. (dII) SEM micrograph of the coated Ta surface. (e) Photograph of a petri plate covered by biofilm of *E. coli* and showing the halo of growth inhibition from the porous Ta sample: half of the sample (outer surface) and the cross section (inner surface).