Restorable polymers activated by cold plasma for chitosan coating aimed for cell adhesion enhancement

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Abstract: The aim of this work is to develop an innovative 3-D matrix for tissue engineering allowing the regeneration by replacement or reconstruction of living tissues. For this, a knitted scaffold made out of polysaccharide (chitosan) coated polyester (PLA) is developed. In order to overcome the surface adhesion incompatibility between the chitosan and the poly(lactic acid) (PLA), an atmospheric pressure plasma treatment is used. We show that plasma treatment is able to graft new functionalities on the polyester surface.

Keywords: Atmospheric Pressure Plasma jet, Chitosan, Poly(lactic acid), adhesion.

1. General

The aim of the project is to develop a resorbable scaffold, with adequate mechanical properties for tissue engineering based on PLGA (Poly-Lactide-co-Glycolide). Due to its biocompatibility; tailored biodegradation rate; and potential to modify surface properties to provide better interaction with biological materials, this synthetic polymer has found great success as a base material for biomedical applications. In parallel with the synthesis of the PLGA and the restorability study, we began the work with the PLA, also for cost reasons. However, the surface of synthetic polymers is significantly limited because they are inherently hydrophobic and have no cell-adhesive domains. On the other hand, chitosan coating is expected to enhance bio-functionalities of the final scaffold. Chitosan is widely used in biomedical applications, food processing, and other, because it is reactive, biodegradable, nontoxic, antimicrobial and biocompatible [1], [2]. Recently, chitosan has also got great interest in textile applications for antibacterial treatment of textiles [3].

In tissue engineering, a scaffold is required to provide cell anchorage sites, mechanical stability, and to serve as a structural guidance for tissue to grow in three dimensions [4]. While, chitosan provide cell anchorage sites, its poor adhesion to synthetic polymers is mainly due to the hydrophilicity of this surface of their surfaces. Therefore, this interface problem is somehow moved from cell / polyester to chitosan / polyester adhesion weakness. To overcome this problem, there is some ways to improve the interface of PLA and PLGA copolymers in general [5]. Plasma treatments have been also recently used on these synthetic polymers for the cell adhesion improvement [6]. Only few works reported the use of plasma treatment for the chitosan- PLA couple [7].

In this work, atmospheric pressure plasma jet is used to activate the PLA surface, and further ensure chitosan adhesion. Grafting of new amine and hydroxyl functionalities is the main ground of improving chitosan adhesion. The presence of chitosan after plasma treatment was investigated by Fourier transform infra-red spectroscopy (FTIR) and X-ray photon spectroscopy (XPS). Finally, the antibacterial action was evaluated with *gram-positive* strains.

2. Experimental

Materials: the substrate material is an amorphous polylactide from INGEO (PLA 4060D). Its glass temperature transition (T_g) is around 55°C and has no melting temperature. The chitosan is commercial from shrimp (Sigma Aldrich) with a deacetylation degree of 75% and a molar mass of 300 kg/mol. Acetic acid (ACS reagent, 99.7% pure) was taken from Sigma Aldrich and used as received. The pellets of PLA were dried under vacuum for one night before thermo-compression at 180°C to obtain samples of 200 µm thick. The chitosan was used as received and solubilise in aqueous medium with the help of acetic acid (1wt% ratio with chitosan). The final concentration of chitosan was 1mg/ml.

Method: Plasma treatment setup is shown in (Fig. 1). The Plasma-Gun from Thermo fisher developed by GREMI [8] has been adapted to generate the plasma within an open T-tube (quartz, 10 mm diameter) at is both extremities. In order to reproduce the filament plasma treatment conditions, which will be used for the knitting of the matrix, 8×120 mm^2 samples were cut and inserted into the tube. Plasma treatment time was 30, 60 and 120 s with 5 and 8 l/min helium flux. The samples were then dip coated in the chitosan solution and air dried for 24h.

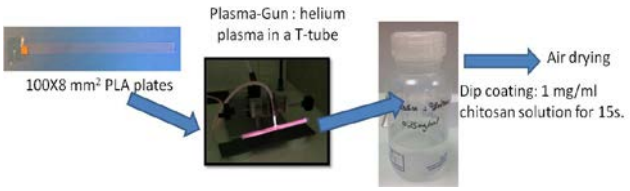

Fig. 1. Experimental set-up

Anti-microbial activity against the clinical strain *Staphylococcus aureus* SH1000 was evaluated as described previously [9]. 500 µL of minimal medium containing about 10^6 CFU/mL, controlled by enumeration, were deposited on functionalized PLA for 24 h at 37 °C. Serial dilutions of detached bacteria were plated, and colony counts were performed to evaluate adhesive activity.

3. Results and discussion

In order to check of the plasma treatment on the functionalization of the PLA surface, the chemical signature was first investigated by FTIR analysis. Absorbance FTIR spectra in the 4000–400 cm^{-1} range collected in transmission mode are shown in (Fig. 2) for PLA, PLA chitosan coated without plasma treatment and with treatment. The FTIR spectra reveal the importance of the plasma activation in the fixation of chitosan on the PLA surface. Indeed, the spectrum corresponding to a non-activated PLA surface before coating don't show any trace of the amine or amide bands of the chitosan [10]. These bands only appear with the plasma activation.
The bands attributed to chitosan are those at 1420−1477 cm^{-1} resulting from the coupling of C–N axial stretching and N–H angular deformation, the one around 1580 cm^{-1} attributable to the angular deformation of N–H bonds of the amino groups. In the left side of the spectrum, the absorption centered at 3440 cm^{-1}, is attributed to the O–H and N–H axial stretching, generally related to water residues of chitosan.

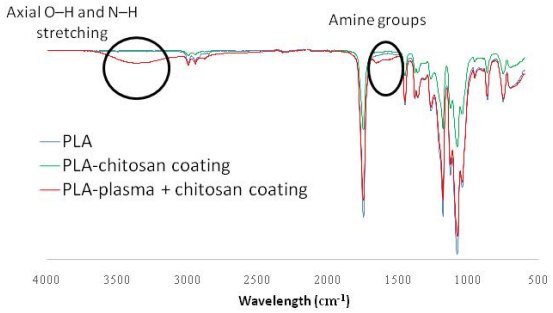

Fig. 2. FTIR spectra of the plates: reference, no plasma and with plasma treatment, before chitosan coating

The presence of chitosan was then confirmed by X-ray photoelectron spectroscopy (XPS) analyzes. The C1s spectrum after chitosan coating shows the presence of the chitosan on the surface by the appearance of the energy band of the O-C-O. Here, we show only the high resolution spectrum of nitrogen around 400 eV: whereas the nitrogen spectra of the PLA sample without plasma treatment results on a weak and noisy signals, the high resolution spectrum of nitrogen for the sample with plasma pre-treatment, clearly shows nitrogen bonds (Fig. 3). The deconvolution of the N1s spectrum exhibited two characteristic peaks at 398and 402 eV that correspond to the $–NH_2$ and $N+(CH_3)_3$ bonds [11], respectively. Chitosan structure is characterized by both reactive amino and hydroxyl (-OH) groups: it is supposed that amino groups of chitosan can be covalently bonded to the polyester surface through hydroxyl groups introduced to PLA surface after plasma activation. Nonetheless, no information about the type of interaction or link (Glucosamine for example) happening in this interface were identified for the moment, more work to understand the nature of these interaction must be done in the future to fully understand the process.

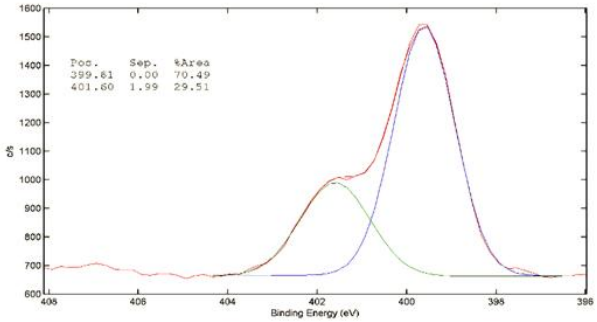

Fig. 3. XPS spectrum of the nitrogen N1s after plasma activation and chitosan coating.

Otherwise, before engaging in cell cultures on this PLA-Chitosan system, we started with the validation of biocompatibility. The anti-bacterial properties of chitosan seem to be preserved after coating (Fig. 4), with a reduction in bacterial adhesion by a factor of 2.7 compared to uncoated PLA.

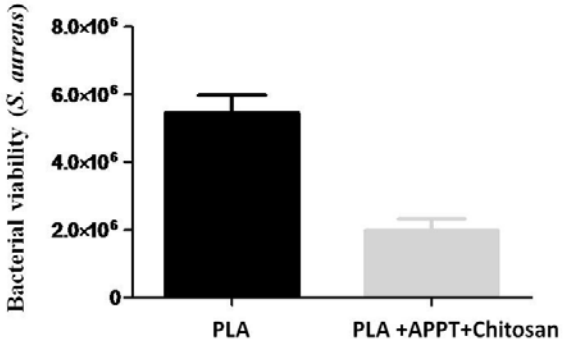

Fig. 4. Bacterial adhesion

4. Conclusion

The final goal of the overall project is to develop a bio-resorbable polyester knitted matrix for tissue engineering. In this paper, we have demonstrated the need for a surface activation treatment to ensure immobilization of chitosan as a bio-functional film on PLA. In our case, the treatment is provided by atmospheric pressure plasma generated in a tube, which is supposed to reproduce the filament processing conditions for producing the knitted matrix. Therefore, the mechanical strength would be provided by the knitted structure while the chitosan achieves additional biofunctionality.

The effect of plasma treatment on chitosan adhesion has been demonstrated by FTIR and XPS characterizations where a surface modification has been made. Thereafter, the inhibitory activity of chitosan was shown on gram-positive bacteria. On the other hand, target cell (osteoblast) adhesion thanks to chitosan is also expected. This strategy seems promising for the implementation of the 3D matrix by textile process. Cell adhesion assays are under progress on PLA and PLA-Hydroxyapatite composite substrates with chitosan coatings as a bioactive layer.

5. Acknowledgement

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6. References

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