Double porous membrane as bio-artificial vascular patch: development, characterization and understanding cell-matrix interaction by varying pore size and morphology

Pritam Das[1,2], Jean-Christophe Remigy[1], Jean-François Lahitte[1], Simona Salerno[2], Loredana De Bartolo[2], Patrice Bacchin[1]

[1]Laboratoire de Génie Chimique, Université de Toulouse, CNRS, INPT, UPS, Toulouse, France
[2]Institute on Membrane Technology, National Research Council of Italy (ITM-CNR), Rende (CS), Italy
Correspondence to: pritamdas005@gmail.com

INTRODUCTION

An artificial vascular patch of poly (ε-caprolactone) (PCL) and Chitosan (CHT) blend membrane have been developed to mimic a three dimensional bio-architecture with double porous morphology[1]: (i) surface macrovoids that could be easily accessible for cell proliferation and differentiation; (ii) macroporous spongy network to transfer essential nutrients, gas, growth factor between the macrovoids and the supernatant and also to provide sufficient adhesion between the cell-matrix interface. The developed membranes were characterized in order to evaluate their structural, biodegradation and permeability properties. Then they were used for the culture of human Mesenchymal stem cells (hMSCs) in order to understand the role of pore size and morphology of the scaffolds for high cell viability[2].

EXPERIMENTAL METHODS

The membranes were developed by modified version of conventional liquid induced phase inversion method by diffusion between solvent as formic acid/ acetic acid mixture and non-solvent NaOH solution. PCL and CHT with a ratio 100/0, 90/10, 80/20 and 70/30 were prepared. The PCL/CHT casted solution was covered by the isoporous PET macrovoid-generator membrane of pore diameter 10 µm in order to locate the non-solvent inlet and in turn to achieve the double porous structure **(Fig. 1)**. After preparation, membranes were characterised in order to evaluate their structural, physico-chemical and bio-degradation properties. SEM images of the cross section of the scaffolds and the mercury porosimetry confirmed that the macrovoids are connected with each other through the spongy macroporous network. ATR-FTIR, XRD and DSC were done to check the miscibility of the blend. The mechanical properties were observed in dry and in wet condition.

We investigated cell proliferation by MTT assay, cell morphology by SEM analysis and the cell metabolism by oxygen uptake rate. We investigated the cell-matrix interaction from the surface towards the bulk of the scaffolds by immunostaining and observed at CLSM. We marked the cytoskeleton protein vimentin and CD90, which are specific markers for MSCs **(Fig. 2)**.

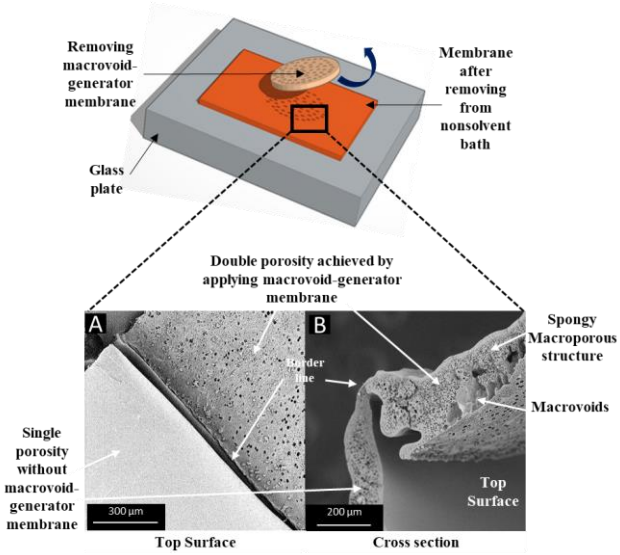


Fig. 1. *Double porosity development using macrovoid-generator membrane by modified liquid induced phase inversion process. SEM images of PCL/CHT 100/0 at the either side of the border line represent double porosity and single porosity on the (A) top surface and the (B) cross section.*

RESULTS AND DISCUSSION

The area where the polymer solution was covered by the macrovoid-generator membrane, two different solvent exchange rate was observed leading to the formation of double porous structure **(Fig. 1)**. The macrovoids were formed where nonsolvent has a direct access with the polymer solution through the pores of the macrovoid-generator membrane. In addition, the interconnected macropores were formed where the nonsolvent could not make a direct connection with the polymer solution. ATR-FTIR chemical spectra confirms the presence of secondary interaction between PCL and Chitosan. From the FTIR chemical mapping, we found slight segregation of polymer along the thickness in case of high CHT wt.%, which is quite natural due to the high affinity of Chitosan with the solvents and chain length difference between the two polymers. From the XRD and DSC study, we have found an increase of crystallinity and melting point respectively by increasing CHT wt.%. By increasing the CHT wt.%, we found that the mechanical property was decreasing and also the mechanical property in the wet condition was reduced than the dry condition. The enzymatic degradation of the scaffolds was increasing by increasing CHT wt.% in both the cases of lipase and lysozyme where in case of lysozyme the degradation was much slower than the lipase (wt. loss% of PCL/CHT 70/30 was 9% after 94 days). In the MTT assay, OUR experiments and SEM images, all the membranes shown high cell proliferation especially on pure PCL and PCL/CHT 90/10 membranes. In CLSM by immunostaining **(Fig. 2)**, cells cultured on the membranes expressed cytoskeleton proteins such as vimentin and CD90, which are specific markers of MSCs. On PCL/CHT 70/30 membranes, cells adhered on the surface area and appeared entrapped into the macrovoids (diameter of 35 µM). Cells on the surface exhibited high degree of spreading while in the macrovoids they acquired a very globular morphology due to the limited area available for cell adhesion and organization. On the contrary, on PCL and PCL/CHT 90/10 cells adhered only on the surface area and spread as highlighted by the distribution of vimentin in microfilaments.

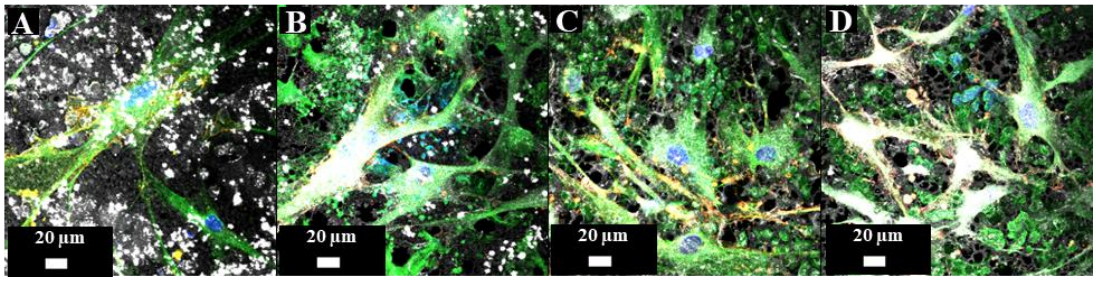


Fig. 2. *CLSM images of hMSCs on different blends of membranes after 7 days where A) PCL/CHT 100/0, B) PCL/CHT 90/10, c) PCL/CHT 80/20, D) PCL/CHT 70/30 respectively. (Nucleus marked by DAPI in blue, Cytoskeleton marked by vimentin in green, cell surface marked by CD90 in red and membrane in black-grey).*

CONCLUSION

In this study a well-developed double porous morphology blended with PCL/CHT polymeric scaffolds were developed, miscibility was characterised and role of pore size and morphology of the scaffolds was investigated by static cell culture. We found a linear change in macrovoid size and surface morphology by increasing CHT wt.% which has a direct impact not only on the scaffold physico-chemical and biodegradation properties but also on the cellular viability.

REFERENCES

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