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Fibroin non-woven mats as support of adipose stem cells and pancreatic islets for diabetes treatment

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INTRODUCTION AND OBJECTIVES

Allogeneic pancreatic islet (PI) transplantation is used to treat type I diabetes mellitus. This technique has some limits, as the number of donor pancreas required and the immediate inflammation reaction after transplantation. This pitfalls could be solved by the use of a co-transplantation of PI and mesenchymal stem cells, which are able to promote neo-vascularization, preventing graft rejection (Sordi 2010). The aim of this work is to obtain a silk fibroin medical device useful for the co-transplantation of mesenchymal stem cells and PI. Silk fibroin was chosen because it is a natural material, widely used in biomedical field and tissue engineering (Vepari 2007). Three fibroin non-woven mats with different thickness were designed and used for separate culture of adipose derived stem cells (ADSCs) and PI, in order to permit their assemblage at the time of transplantation.

MATERIALS AND METHODS

Thin, medium and thick fibroin non-woven mats were produced with the water entanglement method after degumming process. Fibroin non-woven scaffolds were sterilized by heat in autoclave or with gamma rays. After sterilization, scaffolds (thin and thick mats) were characterized by scanning electron microscopy (SEM), energy dispersive X-ray (EDX) analysis, tensile strength and stretching, Fourier transform infrared microscopy (FTIR) and differential scanning calorimetry (DSC).

Stem cells from adipose tissue of informed donors, were cultured on 1 cm^2-fibroin scaffolds for 15 days, while pancreatic islets, isolated from cadaver donors, were cultured for one day. After the culture, cells were morphologically investigated by SEM. An immunostaining analysis was also performed for PI to assess the glucagon-insulin positivity.

RESULTS AND DISCUSSION

The macroscopical aspect of the three silk fibroin non-woven mats is appreciable in fig.1.

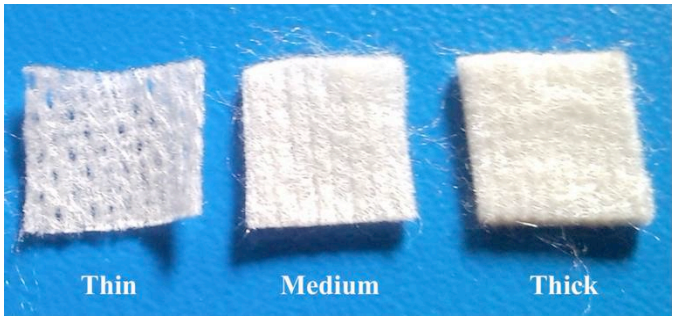

Figure 1 : Photographs of fibroin non-woven mats: each sample was cut at 1x1 cm.

Fibers orientation and distribution were not influenced by scaffold thickness. The SEM investigation was performed on thin and thick scaffolds and revealed a similar microstructure characterized by evident porous, homogeneous aspect and paralleled fibres with smooth surface (Fig.2). Their mechanical properties are suitable for the employ in tissue engineering (data not shown).

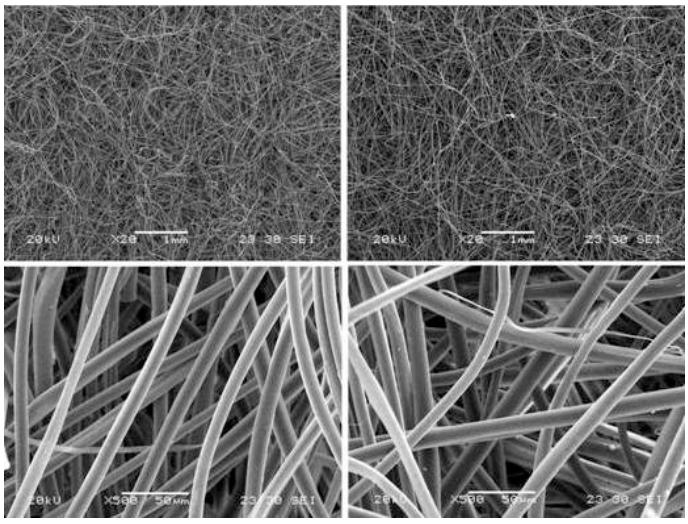

Figure 2 : Scanning electron microphotographs of thin (left column) and thick (right column) non-woven mats at different magnifications

The EDX analysis underlines the high fibroin purity, no contaminant heavy metals from water entanglement process were detected (data not shown). FTIR and DSC analyses (fig.3 and 4) demonstrate that sterilization does not induced fibroin degradation.

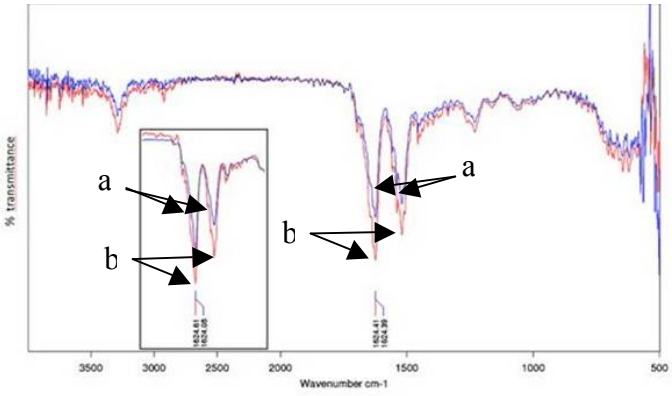

Figure 3 : FTIR spectra of thin non-woven mats before (b) and after gamma-ray sterilization at 60 kGy (a). Inset: FTIR spectra of thin non-woven mats before (red) and after autoclave sterilization (blue).

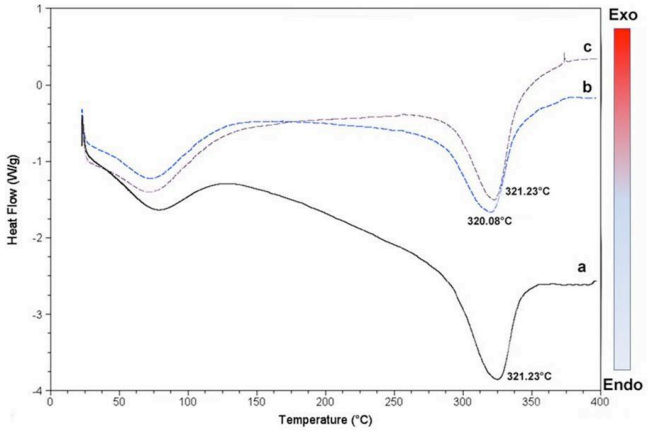

Figure 4 : DSC patterns of thin non-woven mats: (a) before sterilization; (b) after autoclave sterilization; (c) after gamma-ray sterilization at 60 kGy.

Adipose-derived stem cells and pancreatic islets are isolated and separately cultured on thin scaffolds. When stem cells are cultured for 15 days, both abundant extracellular matrix and adhered cells can be appreciated; the cells migrate inside the scaffold and colonize it (fig.5).

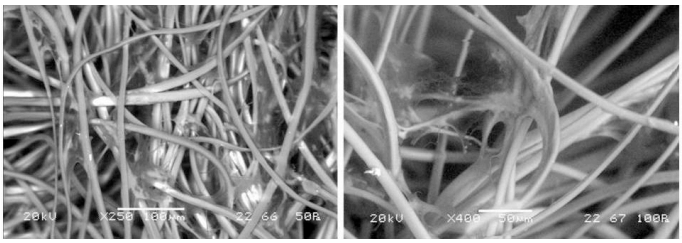

Figure 5 : Scanning electron microphotographs of the non-woven scaffold after 15 days of adipose-derived stem cell culture.

The peripheral cells of the islets after a 1-day culture on non-woven fibroin scaffold closely adhere to the fibers, although the culture timespan was limited (figure 6); as well as for the ADSCs the distance between the fibers allows the distribution of islets inside the scaffold. The immunostaining of cultured islets shows a slight positivity to glucagon (figure 7a) and a more marked one to insulin (figure 7b).

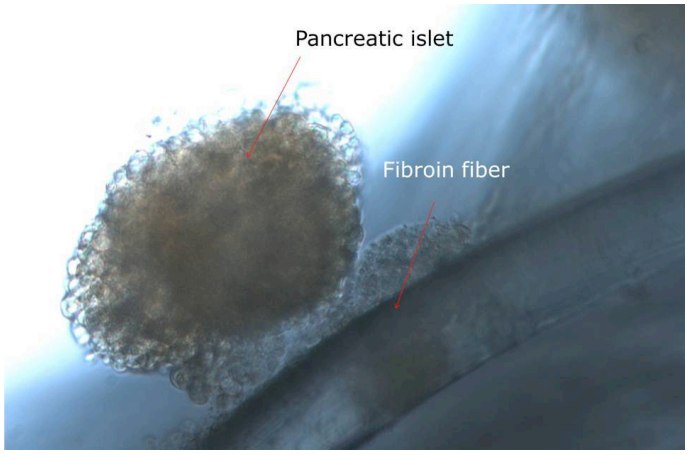

Fig. 6 : Stereomicrograph of a pancreatic islet after one day of culture on non-woven fibroin scaffold. 20x magnification

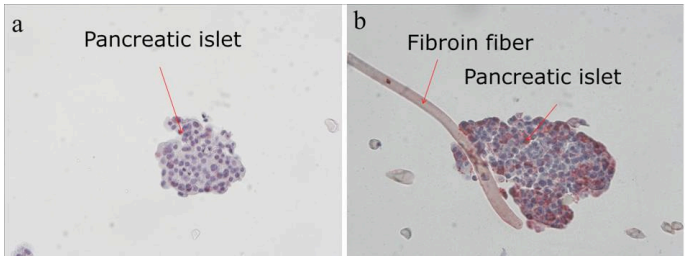

Fig. 7 : Immunostaining for glucagon (a) and insulin (b) of islets after one day of culture on nonwoven fibroin scaffold. Counterstaining hematoxylin/eosin, magnification 20x.

CONCLUSIONS

Non-woven mats support both ADSC and PI cultures. Using this approach, ADSCs could be isolated from the patient before PI availability and the system developed may improve the follow-up of diabetes treatment.

REFERENCES

- Sordi V. et al. (2010) *Mesenchymal stem cells as feeder cells for pancreatic islet transplants.* Rev Diabet Stud 7(2) 173-184.
- Vepari C et al. (2007). *Silk as biomaterial*. Progress in polymer science 32(8-9) 991-1007.