**P-058** **Adipose derived stem cells on silk fibroin film as feeder layer for bioengineered skin**

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## INTRODUCTION AND OBJECTIVES

Keratinocytes are used to obtain bioengineered skin for wound dressing. For this purpose cells must be seeded into a scaffold after their isolation. The limits of this procedure are the need of feeder layer, which is often of animal origin, and the slow scaffold *in vivo* biodegradation (Sugiyama 2008). Silk fibroin, extracted from *Bombyx mori* cocoons, is an excellent material for tissue engineering because it is biocompatible, biodegradable, elastic and resistant (Vepari 2007). The aim of this work is to prepare an adequate film based on silk fibroin, pectin and glycerol and to evaluate adipose-derived stem cells (ADSCs) adhesion and proliferation on it, in order to use them as a feeder layer for regenerative medicine purpose.

## MATERIALS AND METHODS

*Bombyx mori* cocoons were degummed in autoclave, washed and dried. Fibroin fibers were solubilized in calcium nitrate/methanol solution. Eighteen different films were obtained using different fibroin-pectin-glycerol ratios by casting method. Films were sterilized by autoclave, and characterized before/after sterilization by differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR). Morphological investigations were performed by scanning electron microscopy (SEM). Human adipose stromal vascular fraction was plated on plastic surface and adherent ADSC were expanded till the 3rd passage. Cells were then cultured on a selected film (#18, table 1) for 15 days and characterized by electronic microscopy (SEM and TEM).

## RESULTS AND DISCUSSION

The compositions of the eighteen prepared films are reported in table 1. In the first films group pectin was added to fibroin because it allows fibroin conformational transition. Results suggests that 6% of pectin is required for fibroin stable conformation, but all fibroin-pectin films were stiff and fragile, not adequate for tissue engineering employ. To improve film characteristics, glycerol was added to the composition. The best results were obtained using pectin:glycerol in rate 1:2 and the best formulation was 82% fibroin, 6% pectin and 12% glycerol. Results of DSC (fig.1-2), FTIR (fig.3) and SEM analysis (fig.4) demonstrate that sterilization does not induce fibroin degradation and films maintained their structural integrity with homogeneous and smooth surfaces.

**Table 1 : percentage weight composition of films**

| Film | Fibroin | Pectin | Glycerol |
|---|---|---|---|
| 1 | 99 | 1 | - |
| 2 | 98 | 2 | - |
| 3 | 96 | 4 | - |
| 4 | 94 | 6 | - |
| 5 | 92 | 8 | - |
| 6 | 90 | 10 | - |
| 7 | 98 | 1 | 1 |
| 8 | 96 | 2 | 2 |
| 9 | 92 | 4 | 4 |
| 10 | 88 | 6 | 6 |
| 11 | 84 | 8 | 8 |
| 12 | 80 | 10 | 10 |
| 13 | 97 | 1 | 2 |
| 14 | 94 | 2 | 4 |
| 15 | 88 | 4 | 8 |
| 16 | 82 | 6 | 12 |
| 17 | 76 | 8 | 16 |
| 18 | 70 | 10 | 20 |

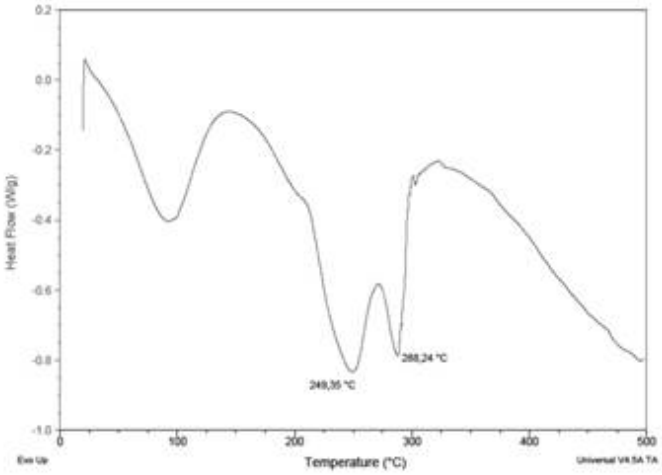


**Figure 1 : DSC patterns of film before sterilization.**

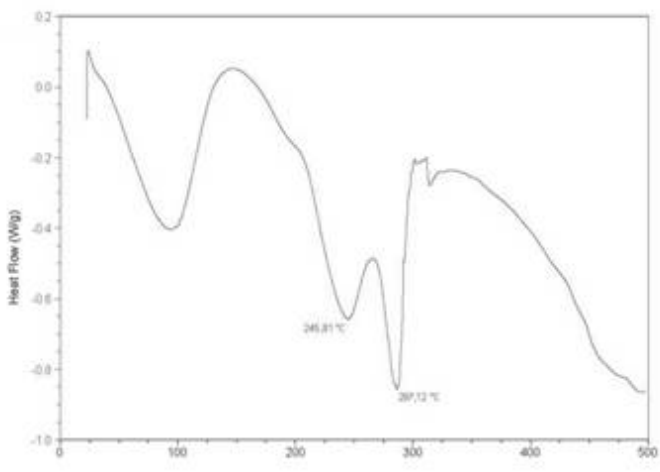


**Figure 2 : DSC patterns of film after sterilization.**

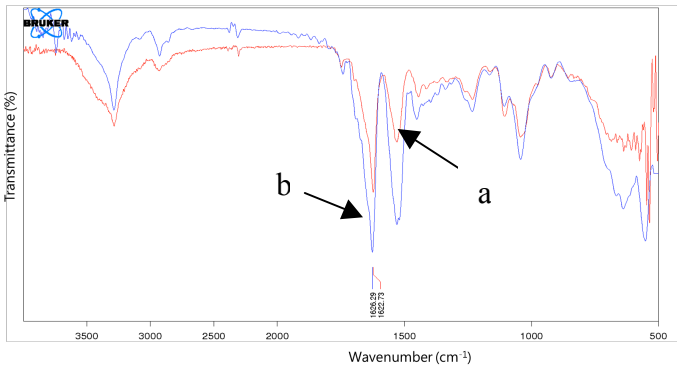


**Figure 3 : FTIR spectra of film before (b) and after (a) sterilization**

After sterilization film n.18 was used as scaffold for ADSCs culture. Cells adhere to the support, with a simil-fibroblastic shape and reach confluence (fig. 5). The ultrastructural analysis performed with TEM shows the presence of adhesion proteins that promote cell anchorage to the film, forming a multilayered cell structure; moreover, typical active-cell features as nuclei, mitochondria, rough endoplasmic reticulum, lysosomes and vacuoles were observed (fig.6).

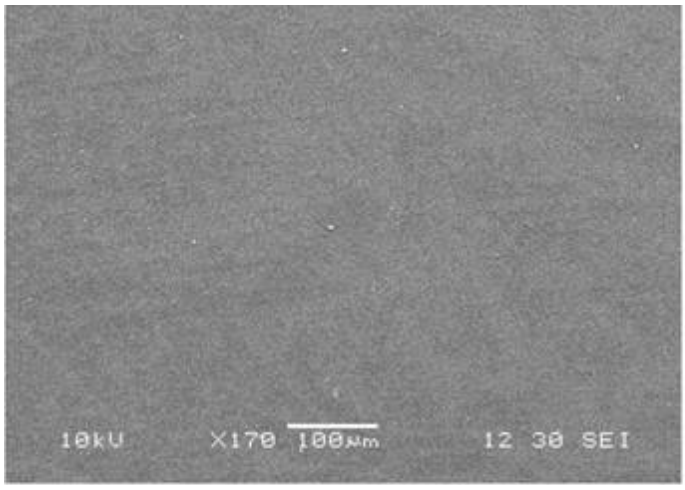


**Figure 4 : Scanning electron microphotographs of fibroin film after sterilization**

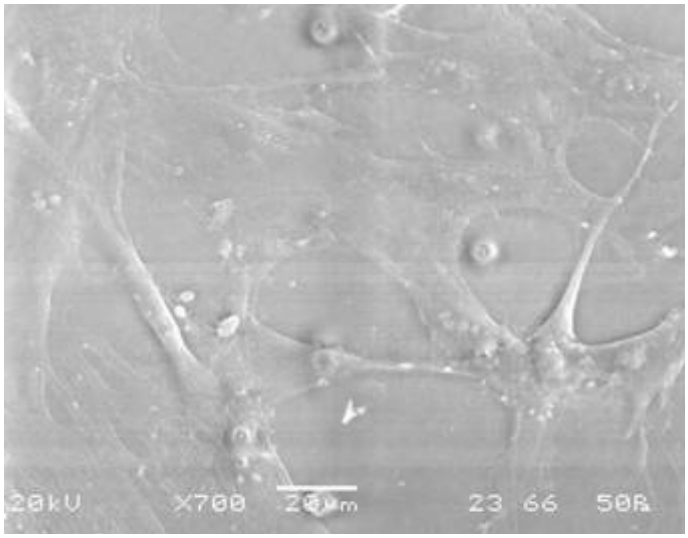


**Figure 5 : Scanning electron microphotographs of fibroin film after cell culture**

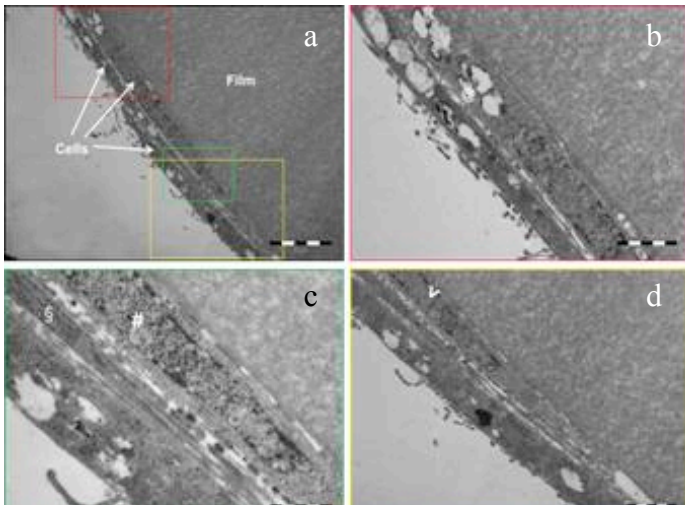


**Fig. 6 : TEM analysis of cells on films. (b), (c) and (d) are magnification of (a) sections. (a) lysosomes (@); (b) rough endoplasmic reticulum (§), nuclei (#), (c) adhesion proteins (>).**

## CONCLUSION

In conclusion, we obtain silk fibroin films with the right characteristics for the employment as scaffold for wound dressing and regenerative medicine. As a matter of fact, films have a smooth surface and good mechanical properties, and are stabile after sterilization process. Furthermore ADSCs are able to adhere on them and after culture they express typical vital cell patterns.

## REFERENCES


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- Sugiyama H. et al. (2008). *Human adipose tissue-derived mesenchymal stem cells as a novel feeder layer for epithelial cells*. Journal of Tissue Engineering and regenerative medicine 2 445-449.