Electrospun silk fibroin meshes combined with graphene oxide as novel biomaterials for tissue engineering applications

Salvador Aznar-Cervantes [a*], Ana Pagán [a*], M. Ángeles García-Pérez [a], Luis Meseguer-Olmo[b], Abel Lozano-Pérez[a], Juan I. Paredes[c], Jose L. Cenis[a]

[a] *Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA), Department of Biotechnology La Alberca (Murcia), E-30150, Spain.*

[b] *Biomaterials & Tissue Engineering Unit & Orthopaedic Surgery Service. V. Arrixaca University Hospital, El Palmar E-30150 & Universidad Católica of Murcia, Guadalupe, E-30107 , Spain.*

[c] *Instituto Nacional del Carbón, INCAR-CSIC, Apartado 73, 33080 Oviedo, Spain.*

** Both authors have contributed equally to the work*

Introduction

Graphene based materials are being studied as emerging scaffolds for tissue engineering applications due to their excellent properties in terms of biocompatibility. Numerous reports have noted the optimal adhesion and proliferation of diverse type of cells growing on graphene oxide (GO) materials, moreover the chemical nature of graphene seems to act also on the differentiation of stem cells.

In this work we explore the possibility to produce electrospun silk fibroin (SF) scaffolds with GO incorporated by means of two different methodologies in order to combine the excellent properties of both biomaterials.

Materials & Methods

Cocoons of *Bombyx mori* were degummed (boiling in 0.02N Na_2CO_3) and SF was dissolved in 9.3M LiBr and dialyzed against distilled water water (3.500 Da MWCO) for 3 days. Then resultant 7 % (w/v) SF solution was concentrated by dialysis against 30 % (w/v) PEG for 24h and the final 18 % (w/v) dissolution was employed for the electrospinning. The electrospinning conditions were adjusted so that the Taylor cone was stable and the electrospun meshes were annealed by immersion in methanol.

The GO was added either by absorption of GO on the surface of the fibres or incorporation of GO in the electrospinning solution. The first one was performed by means of one dipping cycle of the SF electrospun mats in GO aqueous suspension (1 $mg.mL^{-1}$) and the second through the electrospinning of a 18% (w/v) SF aqueous solution containing GO (SF:GO 1000:1 ratio).

Murine fibroblasts (L929 cells) were seeded on the materials at a density of 10.000 $cells \cdot cm^{-2}$ in DMEM expansion medium (10% FBS, 100 $U \cdot mL^{-1}$ penicillin and 100 $\mu g \cdot mL^{-1}$ streptomycin) at 37 ºC and 5% CO_2. Adhesion and proliferation of L929 fibroblasts growing onto these materials were studied by SEM and MTT assay, respectively.

Results

The microscopic images of the materials, obtained by SEM, showed a framework constituted by randomly-oriented fibres. In all cases the surface of the fibres was homogenous and smooth. The only difference observed was related to the increase in the diameter of the fibres (table 1) as a consequence of the addition of GO.

The adhesion of the L929 fibroblasts was excellent; the cells appeared well spread with thin filopodia - either attached to the fibre surface or connecting to neighbouring cells (figure 1)

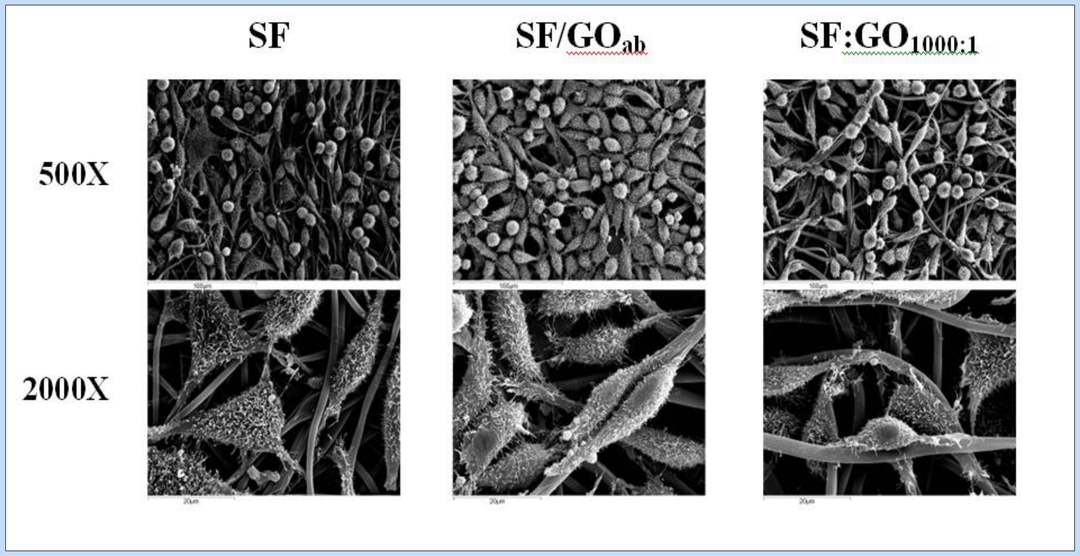

Figure 1. SEM micrographs of L929 cells growing on different materials 4 days after the seeding.

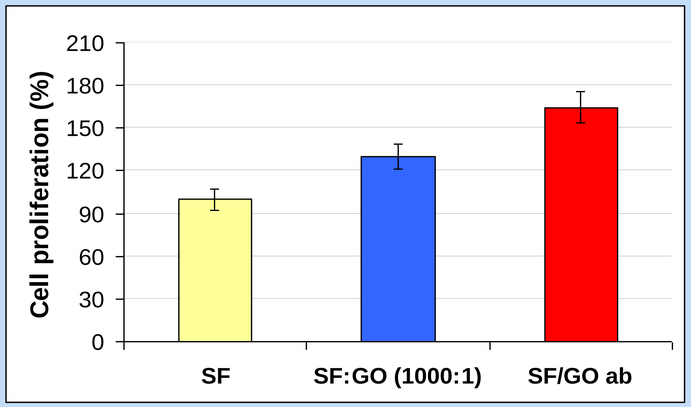

Figure 2. Cell proliferation studied by MTT assay.

As can be observed in figure 2 both materials showed a significant stimulatory effect in the proliferation of L929 cell cultures 4 days after the seeding (Tukey, $p<0.05$). Considering pure SF electrospun mats (SF) as negative controls the proliferation increased by 64.7 % in meshes with GO adsorbed (SF/GOab) and by 30.3 % in meshes with GO incorporated in the electrospinning solution (SF:GO 1000:1).

Table 1.

Material	Average diameter of fibres (nm)
SF	1293 ± 207
SF/GO_{ab}	1903 ± 374
$SF:GO_{1000:1}$	2534 ± 618

Conclusion

This work suggests the potential use of electrospun SF-GO scaffolds for applications in biomedicine due to their excellent biocompatibility and the stimulatory effect on cell proliferation.

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