Fibroin therapeutic platform for co-transplantation of adipose stem cells and pancreatic islets

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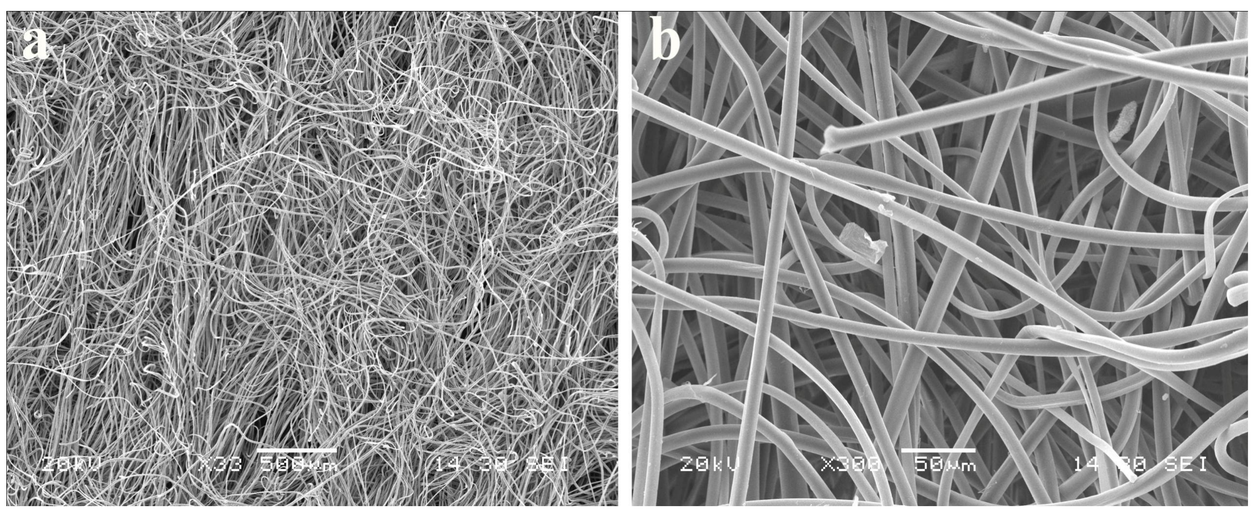
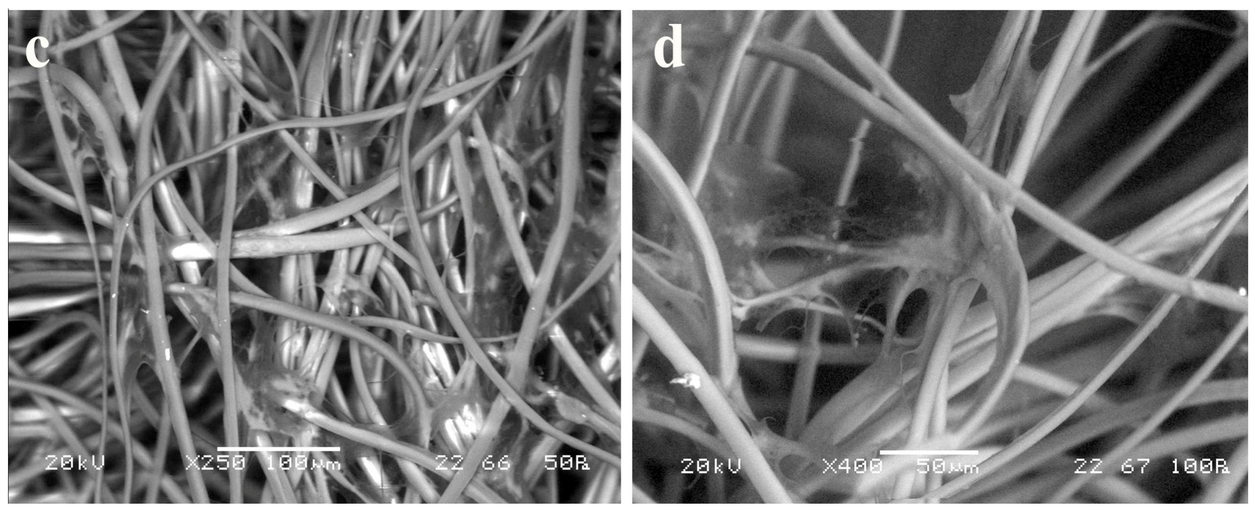
Allogeneic islet transplantation aims to a constant physiological glycemic control in type I diabetes, although it presents some limits: the scarcity of donor pancreas, the need of two or three donors for a single transplant and the loss of islet function due to an immediate inflammatory reaction. Mesenchymal stem cells have been proposed to promote graft vascularization and modulation of immune response, preventing the rejection of the graft by reducing the inflammatory cytokine production. The aim of this work was to evaluate adipose derived-stem cells (ADSCs) and pancreatic islets attachment on non-woven silk fibroin scaffolds as a novel therapeutic platform for co-transplantation. The idea is based on the separately culture of these two cell lines and the assemblage of the two modules at the time of transplantation; stem cells could be isolated from patient and cultured before pancreatic islet availability.

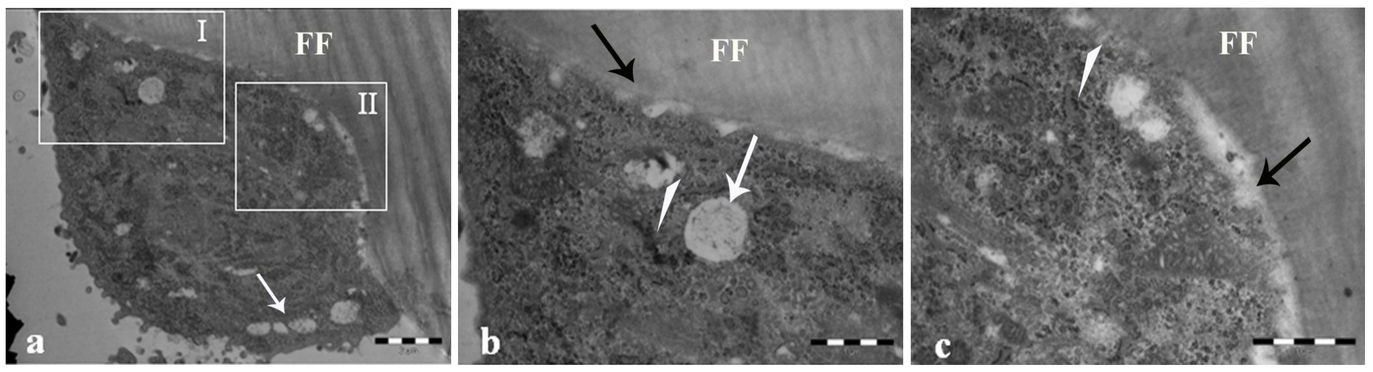
Silk fibroin scaffolds were produced with the water entanglement method (Faragò & Lorenzotti, Italian Application ITMI20080500).
SEM images - Non-woven fibroin scaffold appears as a compact, tangled network (figure a); at higher magnifications, mat-forming fibers are smooth and isodiametrical (figure b). These indicates that degumming process efficiency was high.



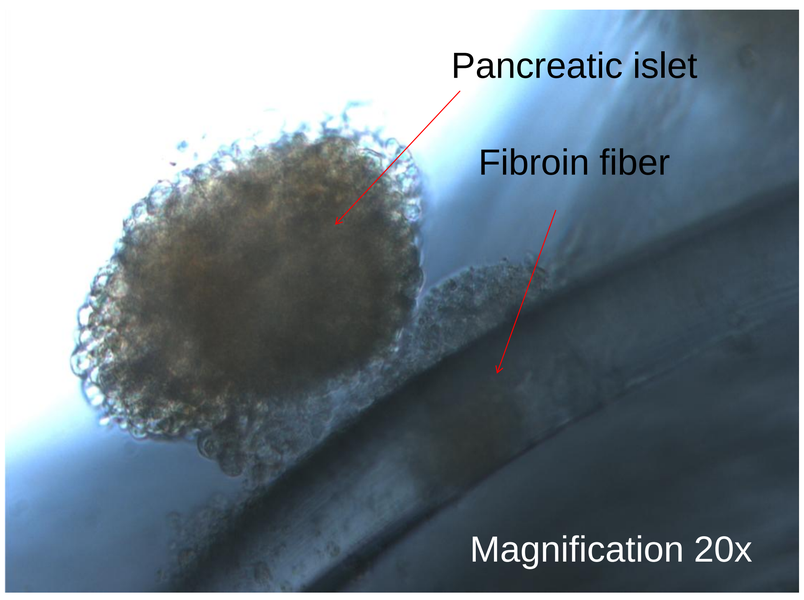


When ADSCs are cultured (40.000 cells/cm^2 of scaffold) for 15 days, both abundant extracellular matrix and adhered cells can be appreciated (figure c and d). The cells migrate inside the scaffold and colonize it by using their cytoplasmic extensions. Notwithstanding its compactness, the mat porosity seems to be optimal to host expanding cell clusters.

TEM images - The typical fibroblast-like shape of ADSCs can be observed; the overall cytoplasmic pattern is typical for fully active cells, cell membrane appears continuous, regular and well delimited. Several vesicles with an inhomogeneous, granular content are present in the cytoplasm: four of them are visible in close proximity of the plasmalemma (figure a), where an active exocytosis process could be speculated, since the vesicles are located near the indented margins. A network of rough endoplasmic reticule can be observed, mainly at higher magnifications (figure a, b and c). The cell-scaffold space is minimal, and interrupted by adherence zonulae, where a real interface can be identified (figure b and c).

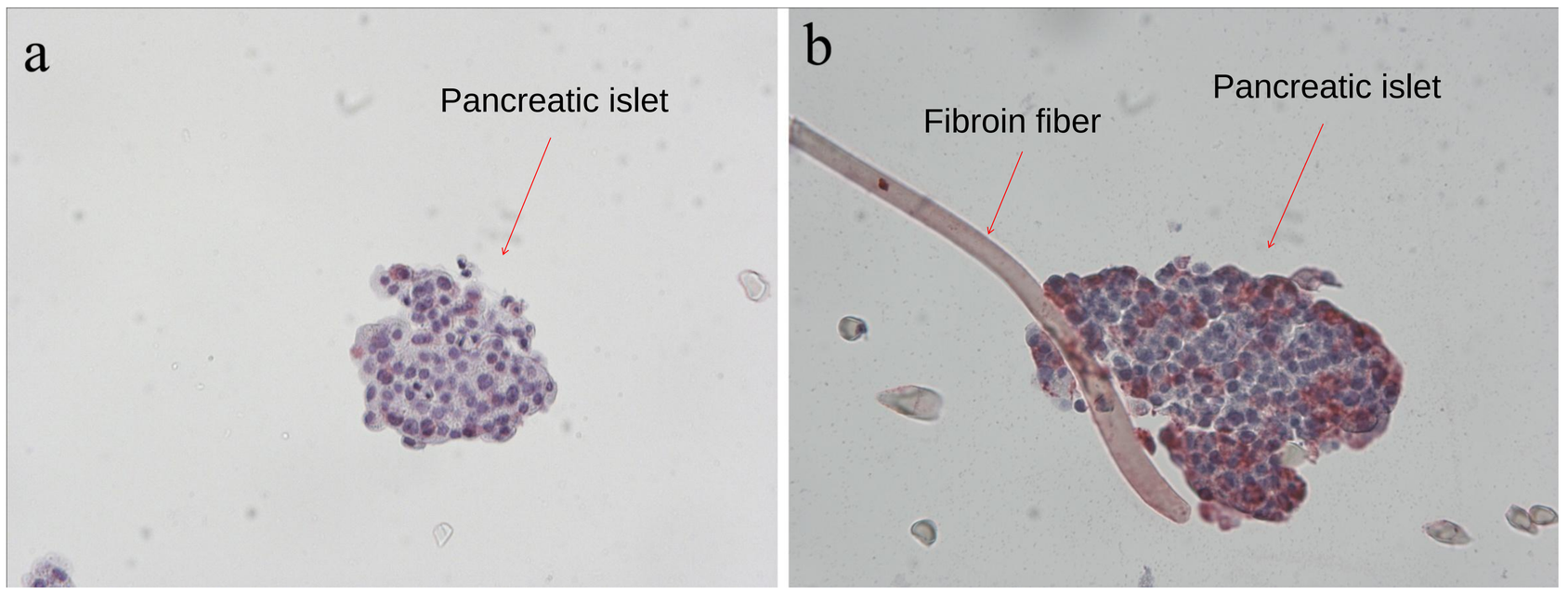


The insets I and II in figure a are magnified in figures b and c, respectively. FF: fibroin fibers; White arrows: vesicles; black arrow: cell-fibroin fibers interface; white arrowheads: rough endoplasmic reticule. Bars in figure a: 2 µm; bars in figures b and c: 1 µm.



The islets after a 1-day culture on non-woven fibroin scaffold (30.000 Ieq/cm^2 of scaffold) closely adhere to the fibers, although the culture timespan was limited.

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 a) and a more marked one to insulin (figure b) (magnification 20x).



Results suggest that fibroin is a promising support for the culture both of ADSCs and islets; the feasibility of culturing separately cells and assembling them before transplantation may improve the follow-up of diabetes treatment, reducing the inflammatory response and the number of requested islets. The prototypical formulation represents a novel platform which may be modified to meet various clinical requirements.

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