2D and 3D electrospun silk fibroin gelatin coatings to improve scaffold performances in cardiovascular applications

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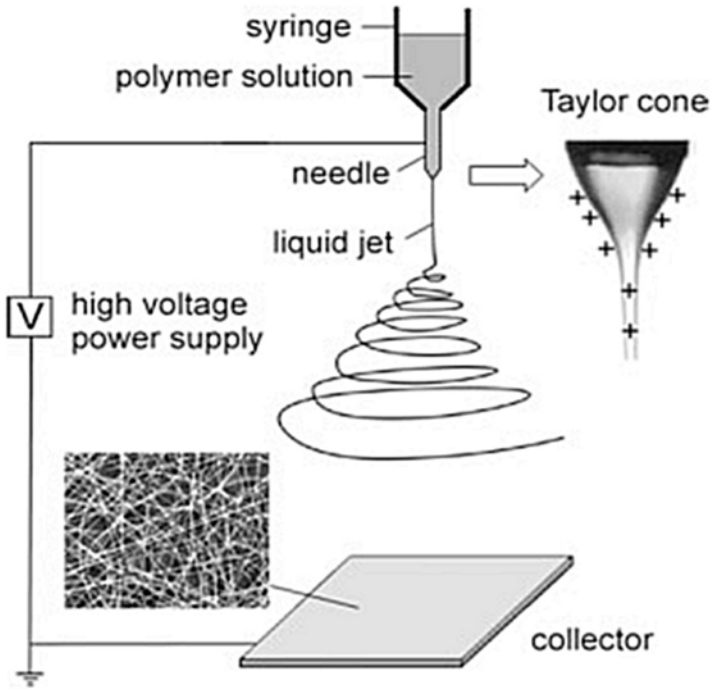
The concept



Schematic for
ELECTROSPINNING

3D scaffolds and 2D matrices fabricated by use of the **electrospinning** technique show morphology similar to that of native ECM**!**

However, their mechanical and biological properties are often inadequate for the specific tissue to be restored

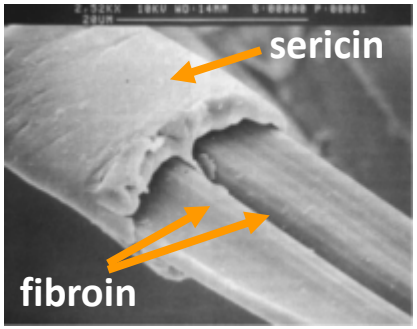
A coating with biomacromolecules can improve their performance, , particularly in applications in contact with blood

Aim of this work:

Apply and characterize a coating of
crosslinked gelatin on **electrospun silk fibroin** (ESF)
mats and tubes
intended for the regeneration of cardiovascular tissues

Electrospun silk fibroin (ESF)

SILK:



Fibroin:

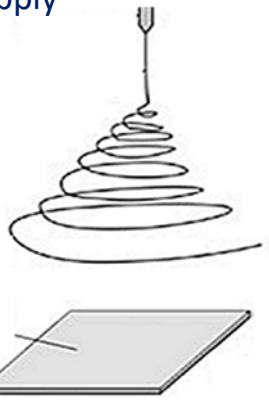
- biocompatible
- electrospinnable
- good mechanical properties
- no thrombogenic effects

ELECTROSPINNING: SF solution in formic acid on a plane or rotating mandrel stainless steel collector

high voltage supply

high voltage supply

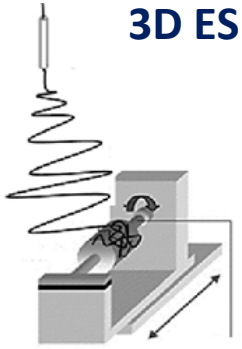
2D ESF



(10 x 10cm)

Ø = 6 mm, l = 10 cm

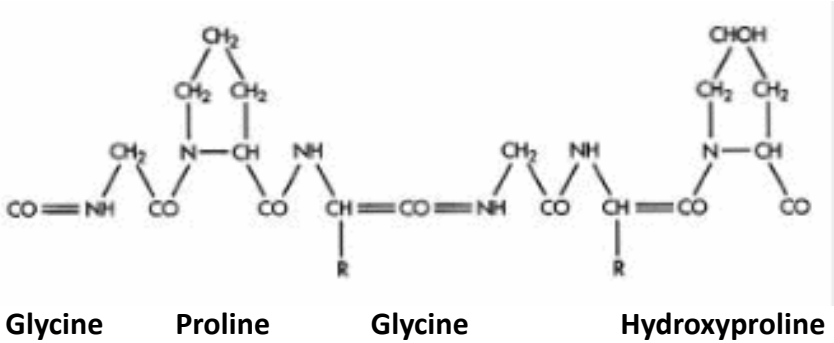
3D ESF



[Alessandrino, A. et Al, Engineering in Life Sciences, 2008, 8 (3), 219-225; Marelli B., et Al., Acta Biomater. 2010, 6:4019–26]

Why gelatin?

Protein derived from **collagen** (most abundant structural protein in animals)



Advantages

- improves cell adhesion
- non-immunogenic
- cheap

Disadvantages

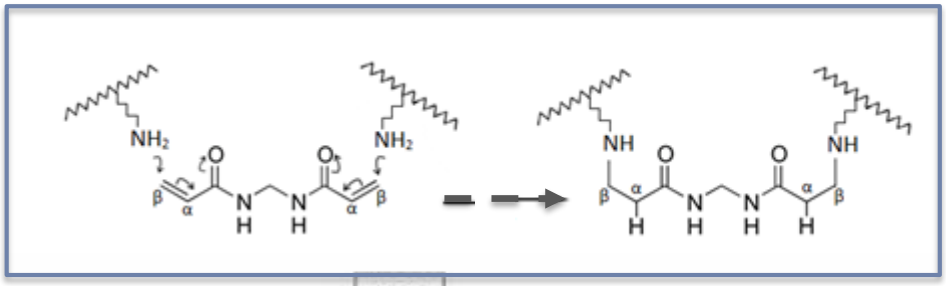
- Inadequate mechanical properties
- dissolution at 37°C (sol-gel transition)

CROSSLINKING is needed

limitations of common crosslinking methods:

- toxicity, possible release of toxic compounds
- concerns about thermal and mechanical stability

Our method for crosslinking gelatin

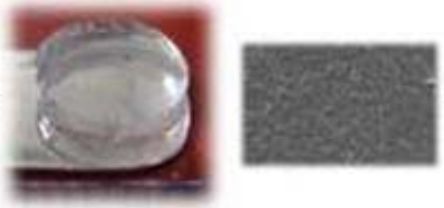


Michael-type addition: formation of covalent bonds between gelatin amino groups and β-carbons of MBA (*N-N'-methylene bis-acrylamide*)

Gelatin water solution @ 50 °C

Add crosslinker @ 50 °C, pH > 10

GELATIDE hydrogel



GELATIDE microspheres

GELATIDE 3D printing

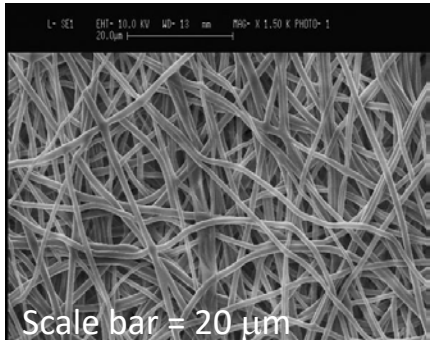
GELATIDE as coating

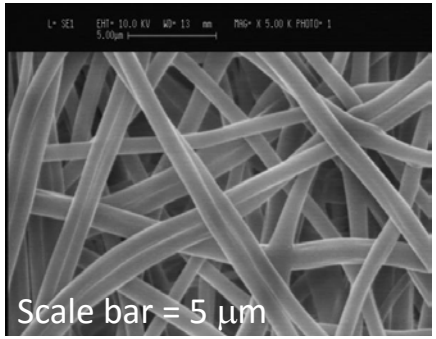
Possible applications of the crosslinked gel

ESF mat morphology and coating

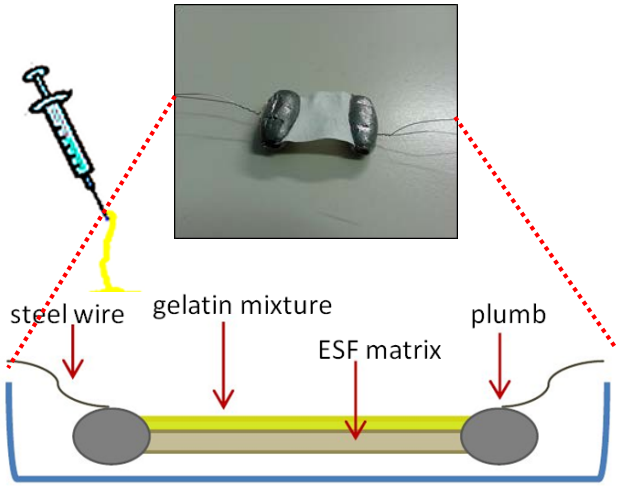
ESF samples treated with methanol (> 99.9%) to induce silk fibroin crystallization (silk I → silk II)

SEM images:
- random fiber distribution,
- fiber Ø = 856 ± 147 nm
- thickness (WET) = 122.5 ±20.6 μm

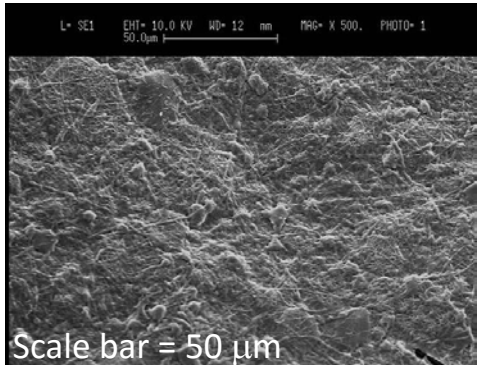




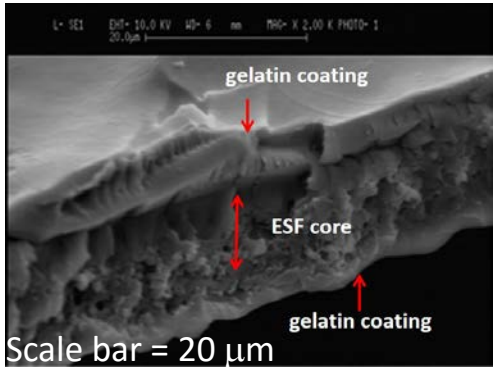
COATING: ESF mat suspended on a glass container by fixing the ends with plumbs. Crosslinking solution deposited by use of a syringe. Crosslinking = 24h at 50°C. Consecutive washing steps for purification



COATED SURFACE



COATED CROSS SECTION

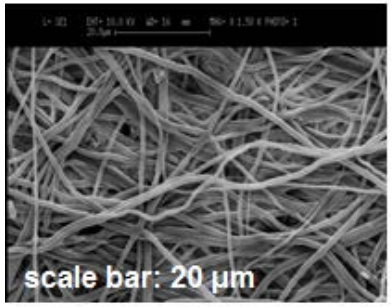


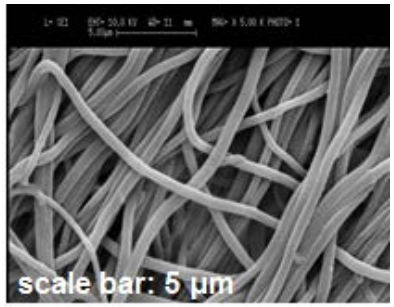
- thickness (WET) = 176.8 ± 29.7 μm

ESF tube: morphology and coating

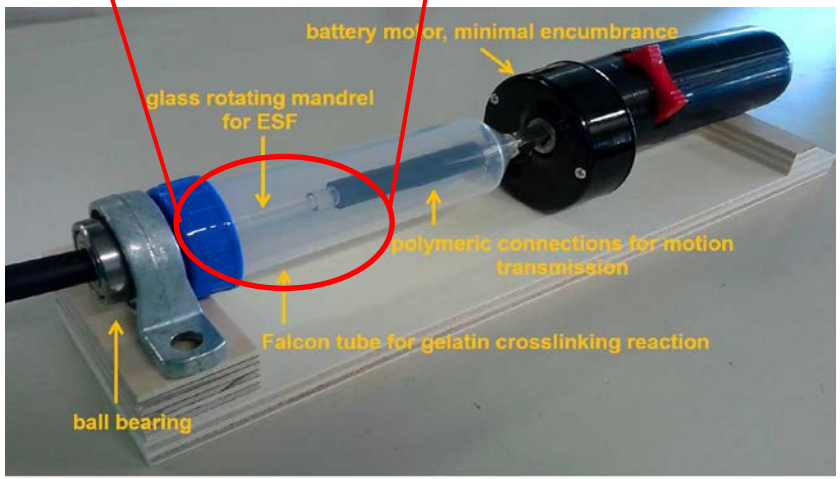
SEM images:

- random fiber distribution
- fiber Ø = 855 ± 236 nm
- thickness (WET) = 212.5 ± 43.1 µm



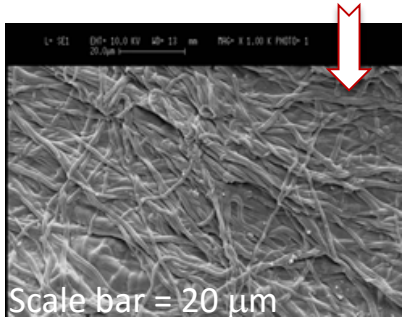


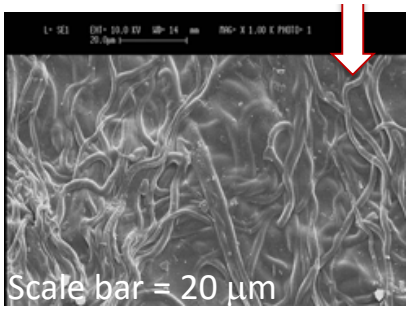
COATING: impregnation with the gelatin solution by two methods: static and dynamic, using the home-made apparatus of left image.
Crosslinking = 24h at 50°C. Consecutive washing steps for purification



static dipping

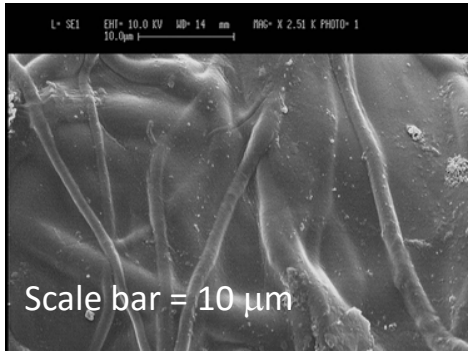
dynamic dipping





Characterization analyses

- As a more efficient method, the "dynamic dipping" was chosen for coating the ESF tubes with cross-linked gelatin



COATING FEATURES (on both 2D and 3D samples):

- homogeneous coating
- fibres still visible under the gel layer
- > 40% weight increase in coated samples (wet conditions)

Characterization (tubular samples):

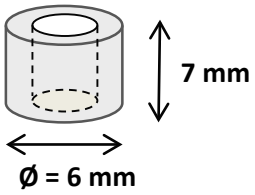
- swelling and stability in water at 37°C
- Circumferential tensile tests

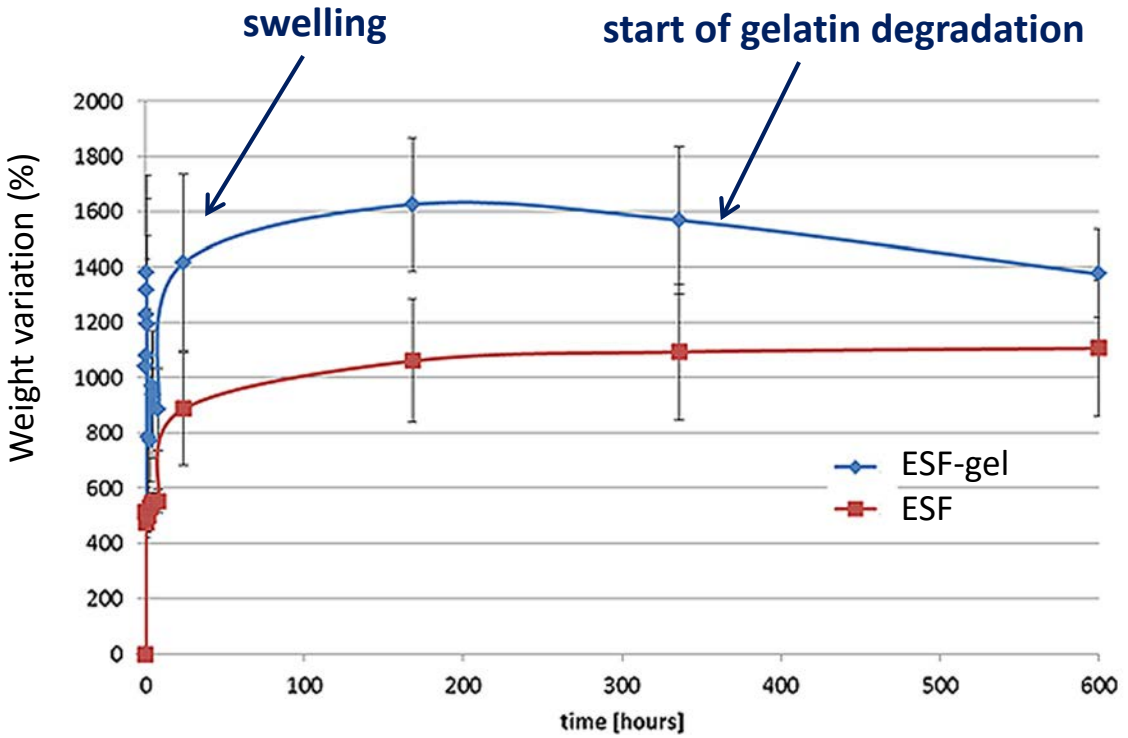
Characterization (2D and 3D samples):

- Indirect contact cytocompatibility, L929 cell line
- Direct contact cytocompatibility: L929 line and primary HUVEC

Swelling and weight loss

Tubular samples
distilled water at 37°C

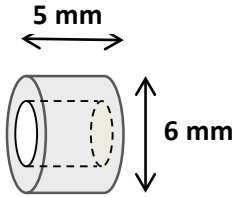


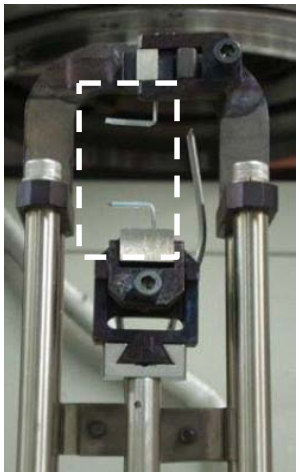


ESF samples: rapid swelling within the first 10 min, reaching their maximum water uptake (≈500%) and maintaining this value of plateau until the 25th day

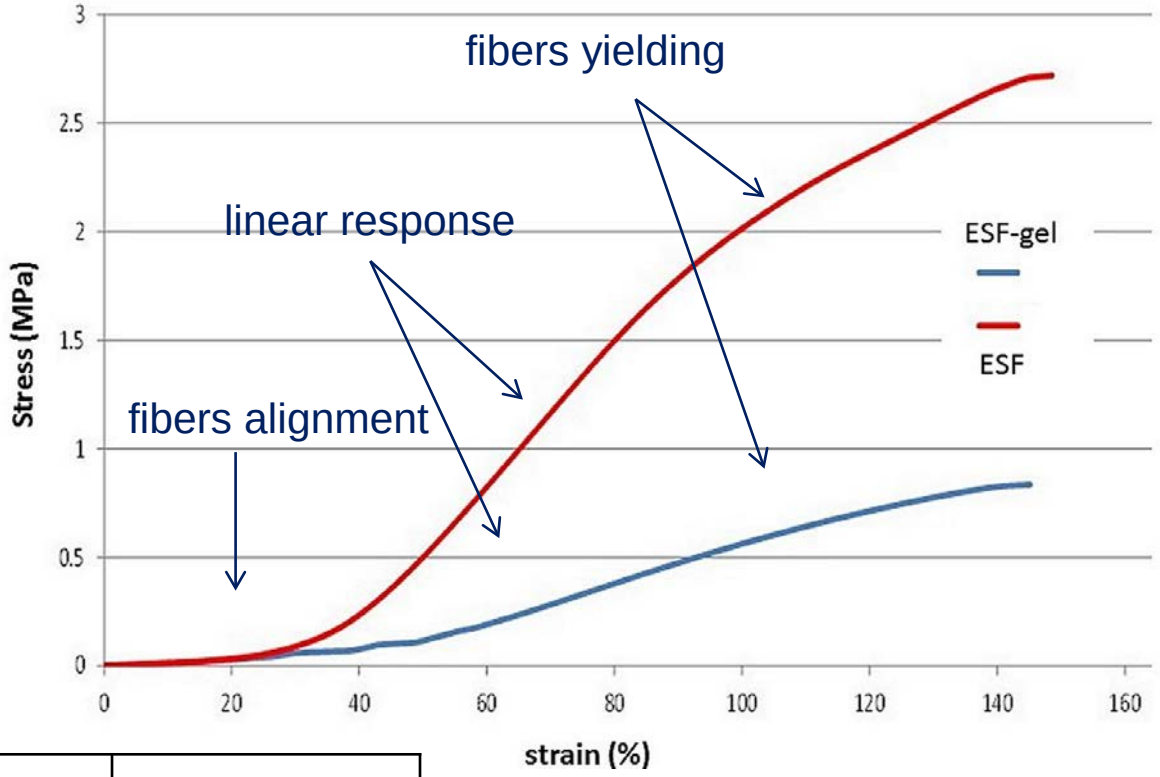
ESF-gel samples: higher swelling values ($p < 0.05$), increasing at 1000% after 60 min; weight variation still increases to 1600% up to 14 days and then starts decreasing probably due to the beginning of the gelatin gel degradation.

Mechanical proprties: circumferential tensile test





n = 3

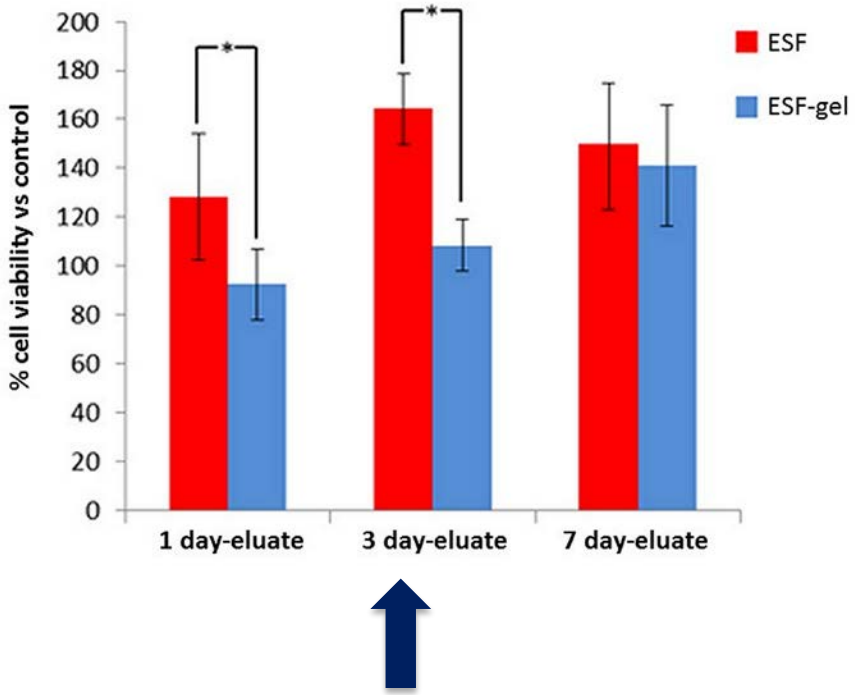


	ESF	ESF-gel
E [MPa]	1.82 ± 0.49*	0.58 ± 0.25*
σ_b [MPa]	2.41 ± 0.69**	0.97 ± 0.57**
ε_b [%]	142 ± 24	153 ± 42

ESF-gel: lower stifness

(*) $p < 0.05$ (**) $p < 0.05$

In vitro cytocompatibility (L929 murine fibroblasts)

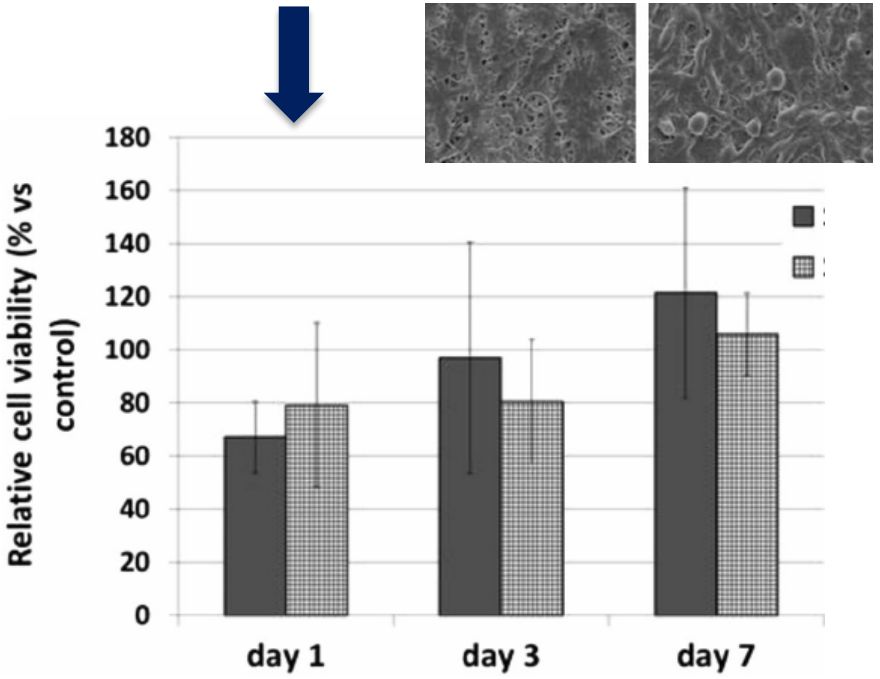


INDIRECT CYTOTOXICITY

Cells cultured for 24h in the presence of DMEM eluates obtained after 1, 3 and 7 days of samples immersion
(MTT assay)

DIRECT CONTACT

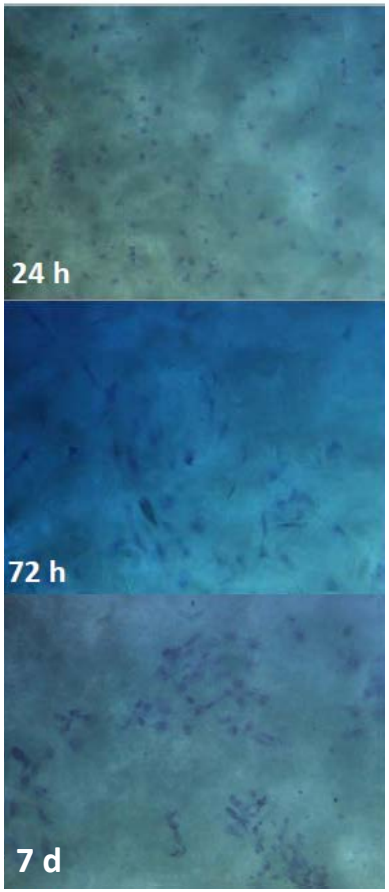
density of seeding= $5x10^3$ cells/sample
control: tissue culture plastic
(Alamar Blue assay)



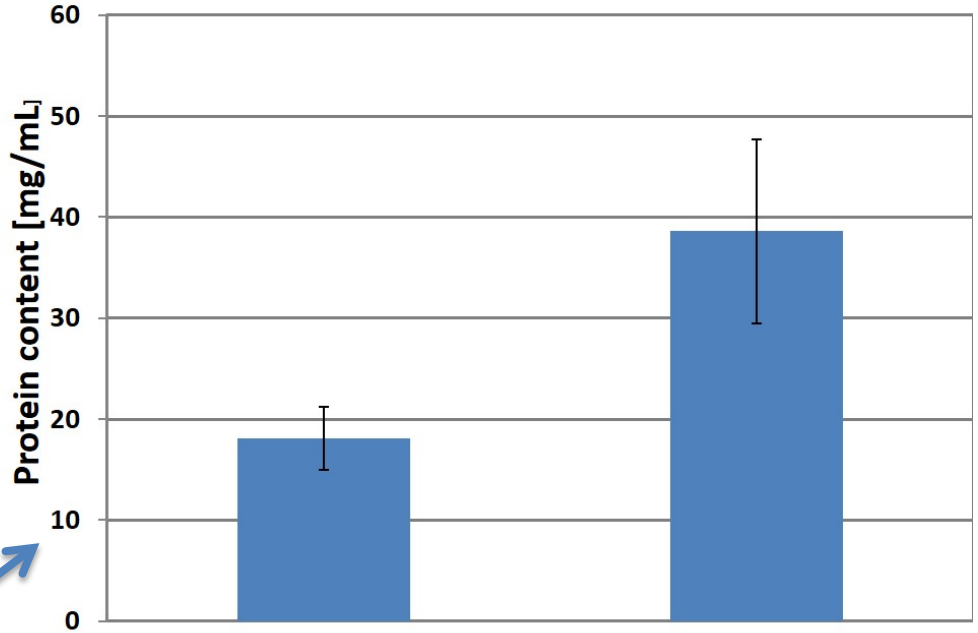
[Marcolin et Al, J. Mat. Sci.: Materials in Medicine, 2017, 28 (5), art. no. 80]

In vitro cytocompatibility: primary HUVEC

Primary cultures of HUVEC obtained from Human Umbilical cord Vein at Cittadella Hospital (Verona), seeded on gelatin gels (OM images) or 2D on silk fibroin-based samples (n=3) and cultured up to 7 days



On crosslinked gelatin matrix many viable cells both rounded and adherent were visible (24h), forming organized colonies (72h, 7d)



By quantitative determination of the total proteins in cell lysates gelatin-coated ES fibroin displayed a better cell growth than uncoated ES fibroin ($p < 0.05$)

Conclusions

The gelatin-coated structures here described demonstrated adequate morphological properties to support cell colonization, combined with good stability in an aqueous environment and acceptable mechanical properties, with an increase in flexibility compared to the structure of electrospun fibroin

- in wet conditions gelatin-coated samples showed a stable and homogeneous coating crossing the whole sample section
- a good cytocompatibility was demonstrated with L929 cells
- from the interaction tests with HUVEC, gelatin MBA-crosslinked proved to be a favourable environment for endothelial cells
- It is also expected that the stability of the gelatin coating on the surface of fibroin substrate is increased by the participation of fibroin amino groups in the crosslinking reaction with MBA

In summary, the per se remarkable biocompatibility of silk fibroin is here combined with a coating that acts as a sealant and is capable to increase cell adhesion and proliferation

Many thanks for your attention!