

Supplementary information

Tumor Growth Suppression Induced by Biomimetic Silk Fibroin Hydrogels

Le-Ping Yan^{1,2}, Joana Silva-Correia^{1,2}, Viviana P. Ribeiro^{1,2}, Vera Miranda-Gonçalves^{2,3},

Cristina Correia^{1,2}, Alain da Silva Morais^{1,2}, Rui A. Sousa^{1,2}, Rui M. Reis^{2,3,4}, Ana L.

Oliveira^{1,2,5}, Joaquim M. Oliveira^{1,2,*}, and Rui L. Reis^{1,2}

¹3B's Research Group–Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal.

²ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal.

³Life and Health Science Research Institute (ICVS), School of Health Sciences, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal.

⁴Molecular Oncology Research Center, Barretos Cancer Hospital, Barretos, São Paulo, Brazil.

⁵CBQF–Center for Biotechnology and Fine Chemistry, School of Biotechnology, Portuguese Catholic University, Porto, 4200 – 072, Portugal.

*Corresponding author: Joaquim M. Oliveira

Tel: +351-253-510931 (Direct) or +351-253-510900 (General)

Fax: +351-253-510909

E-mail: miguel.oliveira@dep.uminho.pt

Supplementary Figures

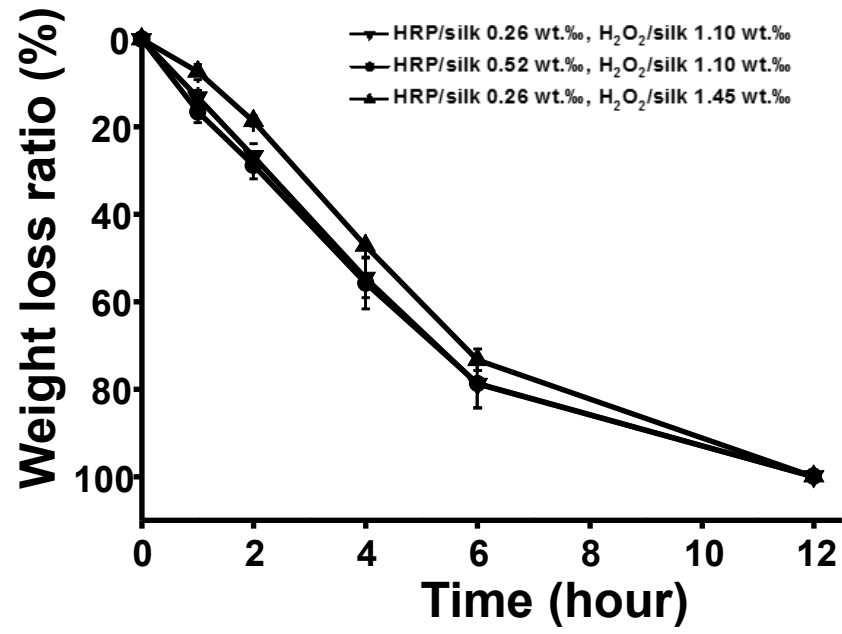


Figure S1. Enzymatic degradation for the SF hydrogels (n=5).

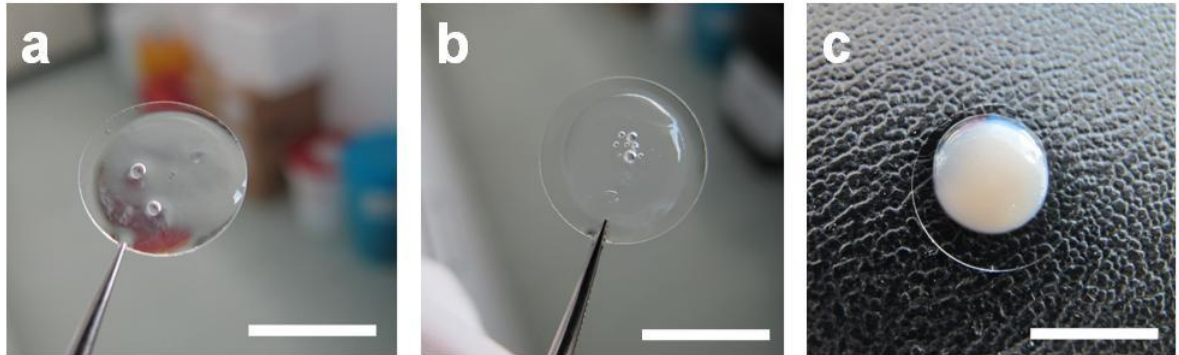


Figure S2. Macroscopic images for the SF hydrogels with ATDC-5 cells encapsulated. (a), (b) and (c): at day 1, day 6 and day 10, respectively. Scale bar: 1 cm.

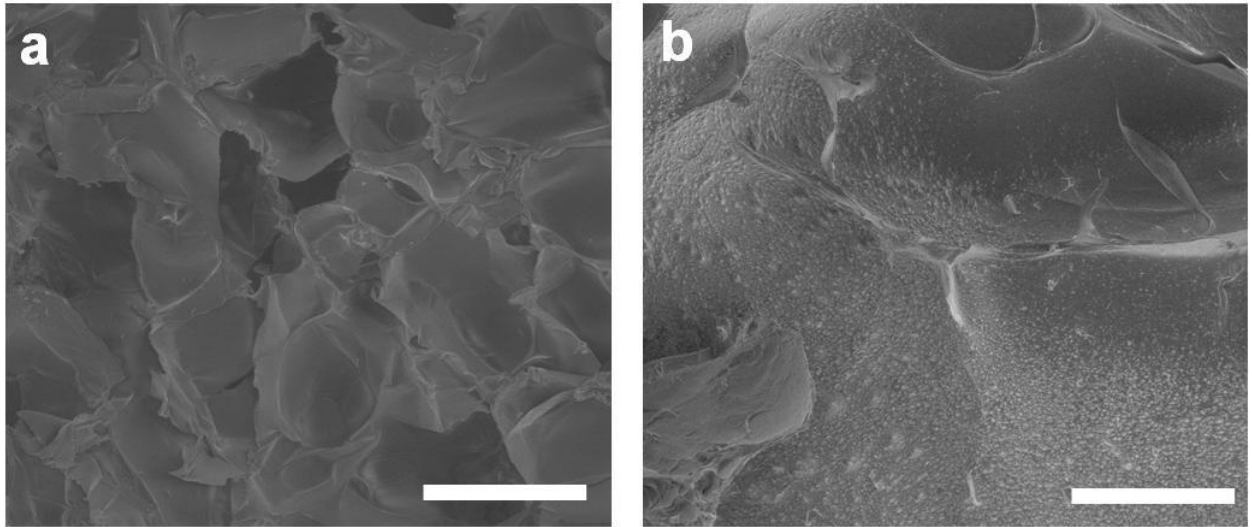


Figure S3. SEM images of the cell encapsulated SF hydrogels. (a), (b): at day 6 and day 10, respectively. Scale bar: 200 μm .

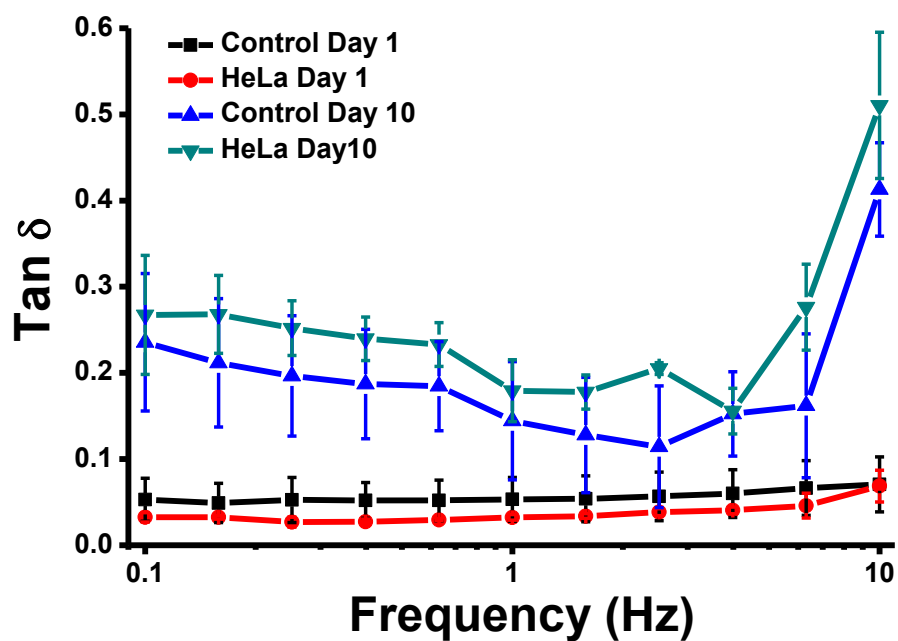


Figure S4. HeLa cells-laden hydrogel loss factor measured by dynamic mechanical analysis. Control Day 1 and Control Day 10: hydrogels without cells encapsulated freshly prepared and ten days later, respectively; HeLa Day 1 and HeLa Day 10: hydrogels encapsulated with HeLa cells for one day and ten days, respectively. (n=3).

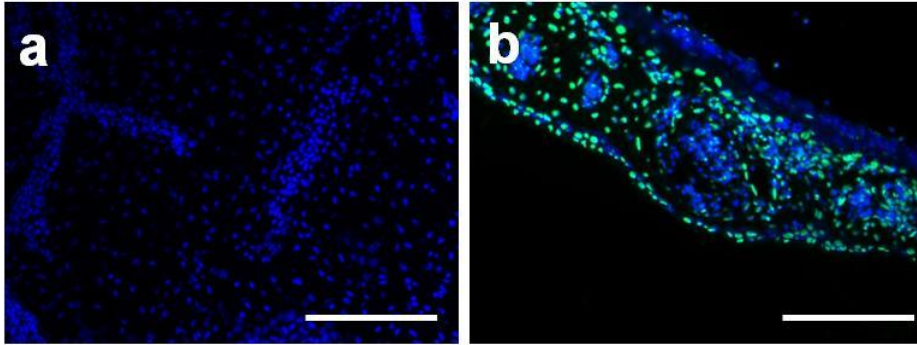
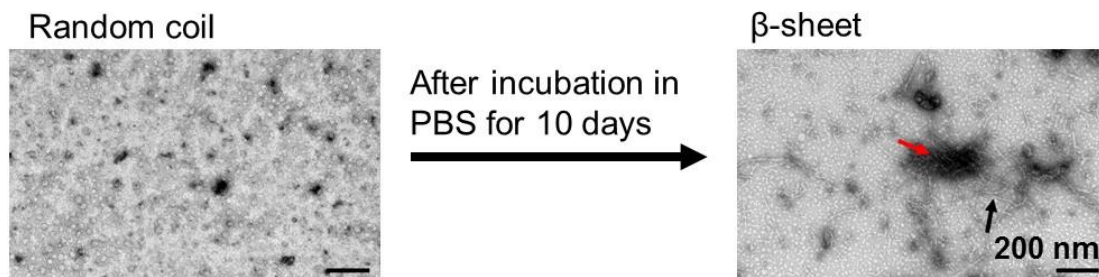


Figure S5. Control for TUNEL assay. (a) Negative (without terminal transferase) and (b) positive controls (with recombinant DNase I). Scale bar: 200 μ m.

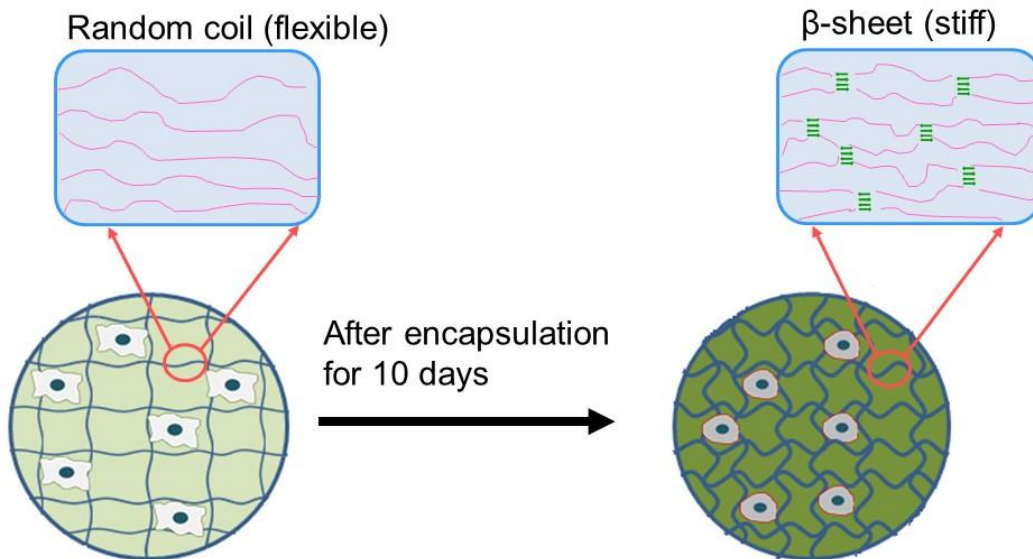
Table of Content

Silk fibroin hydrogels prepared via peroxidase mediated crosslinking present dominant random coil conformation. These hydrogels irreversibly change to β -sheet conformation after a few days *in vitro* and *in vivo*. HeLa cells can be encapsulated in these hydrogels in random coil status, while the later β -sheet transition of the hydrogel induce cell apoptosis and therefore suppress the tumor formation.

Mechanism of apoptosis of cells in the silk fibroin hydrogels



TEM images of silk fibroin hydrogel



Cells encapsulated in silk fibroin hydrogel