Supplementary material (ESI) for Journal of Materials Chemistry

Fibroblast encapsulation in a hybrid silica/collagen hydrogel

Martín F. Desimone,[a,b] Christophe Hélary,[a] Gervaise Mosser,[a] Marie-Madeleine Giraud-Guille,[a] Jacques Livage[a] and Thibaud Coradin*[a]

Electronic Supplementary Information (ESI)

ESI-1 : Experimental procedures

ESI-2 : SEM images of the evolution of silica collagen network with time in the presence of fibroblasts with [SiO_2] = 0 (A), 0.5 mM (B), 1 mM (C), 2.5 mM (D), 5 mM (E)

ESI-3: TEM images of the evolution of silica collagen network with time in the presence of fibroblasts with [SiO_2] = 0 (A), 0.5 mM (B), 1 mM (C), 2.5 mM (D), 5 mM (E)

ESI-4: SEM images of the evolution of control (i.e. in the absence of fibroblasts) silica collagen network with time with [SiO_2] = 0 (A), 0.5 mM (B), 1 mM (C), 2.5 mM (D), 5 mM (E)

ESI-5 : ICP-AES data of silica dissolution for hybrid gels in the presence of XX and 5 mM silicate

ESI-1 Experimental procedures

Normal human dermal fibroblasts (Promocell) were propagated in Dubelco's Modified Eagle Culture Medium (Gibco BRL) supplemented with 10% fetal Calf Serum (Gibco BRL), 100 units/ml penicillin, 100 μg/ml streptomycin (Gibco BRL) and 0.25 μg/ml Fungizone (Gibco BRL). Tissue culture flasks (75 cm^2) were kept at 37°C in a 95% air and 5% CO_2 atmosphere. After confluency, fibroblasts were removed from cultured flasks by treatment with 0.1% trypsin and 0.02% EDTA. Cells were rinsed and resuspended in the above culture medium. Fibroblasts were used at passage 7 for the experiments. Collagen type I was purified from rat-tails and the concentration was estimated by the hydroxyproline titration.

Silicified collagen hydrogels with fibroblast were prepared by mixing 0.6ml of collagen solution (2.8 mg ml^{-1}) with 0.8ml of complete culture medium in an ice bath. In parallel a solution of sodium silicate was acidified to pH 3.0 with 1N HCl, in this condition the main specie present in the solution is silicic acid. Precise amounts of the silicic acid solution were added to the collagen solution in order to obtain different final concentrations (0; 0.5; 1; 2.5 and 5 mM) of silicon species in the gels. After that the solution was neutralize with 0.08ml of 0.1M NaOH and finally 0.6ml of a cell suspension in complete medium supplemented with PBS was added. The cell density in each gel at the beginning of the experiments was $1x10^5$ cells per gel.

Cellular activity of entrapped cells was determined by the tetrazolium assay (MTT). This colorimetric assay is based on the ability of mitochondrial dehydrogenase enzymes of living cells to convert 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) into an insoluble formazan. In short, 5mg ml^{-1} solution of MTT in PBS was prepared and added to the immobilized cells, incubated at 37°C in a humidified 5% CO_2 air atmosphere for 4hs. Afterwards, the MTT solution was removed, the gels washed three times with PBS and dimethyl sulphoxide was added. The OD of the purple solution was read at 570 nm with a spectrophotometer.

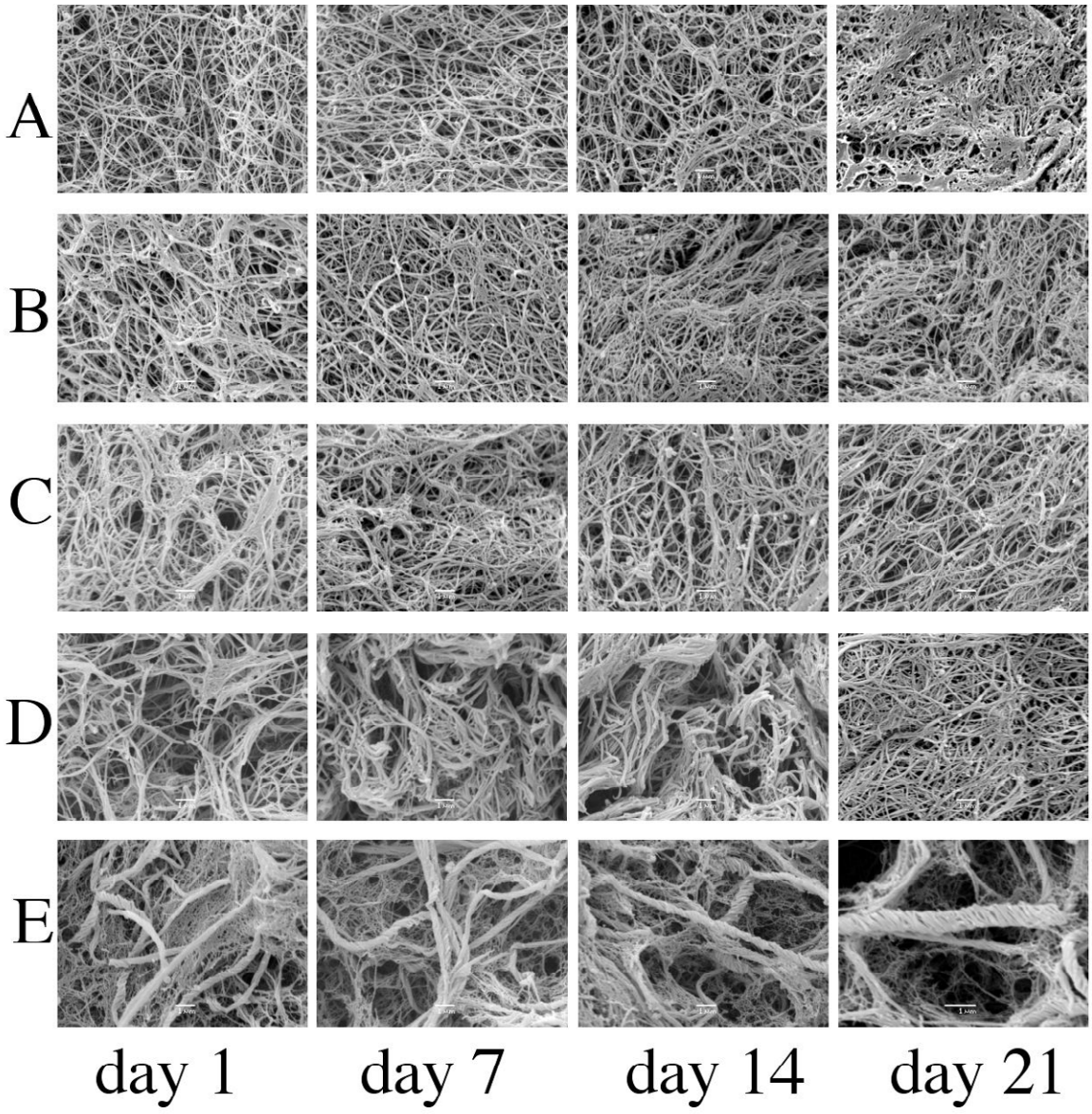
Samples for scanning electron microscopy were fixed using 3.63% glutaraldehyde in 0.05M sodium cacodylate buffer (pH 7.4) with 0.3M saccharose for 1 hour at 4°C. Following fixation, samples were washed three times in the same buffer and then dehydrated in a graded series of ethanol (70%, 95% and two changes of alcohol 100%). Finally the samples were

subjected to supercritical dried and gold sputter-coated for analysis using a Jeol JSM 5510LV SEM operating at 10kV.

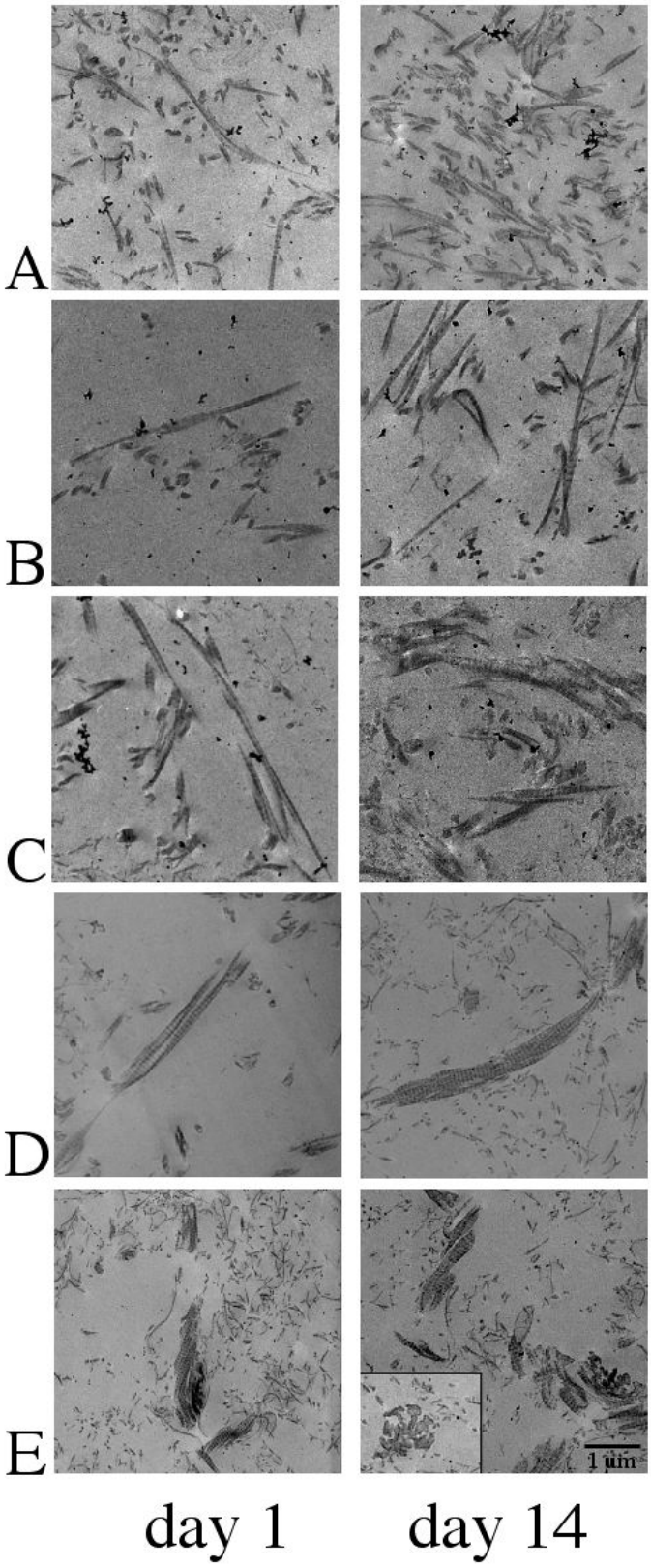
For transmission electron microscopy, following fixation and wash as described above, the samples were post-fixated using 2% osmium tetra-oxyde in 0.05M sodium cacodylate buffer (pH 7.4) with 0.3M saccharose for 1 hour at 4°C. Samples were then washed three times in the same buffer, dehydratated with ethanol and embedded in araldite. Thin araldite transverse sections (70-80nm) were performed by an Ultracut ultramicrotome (Reichert, France) and contrasted by phosphotungstic acid. Slides were then analyzed with Philips CM12 electron microscope operating at 120kV.

Gelatin zymography was carried out using the Miniprotean III system (Bio-Rad), using a previously reported procedure. Gelatinase activity was expressed as arbitrary units. A control was performed with culture medium collected on a collagen hydrogel without fibroblast. Results were normalized by the MTT assay and expressed as the average value $\pm$ SD for each culture time point and compared to the results of collagen hydrogels at day 7.

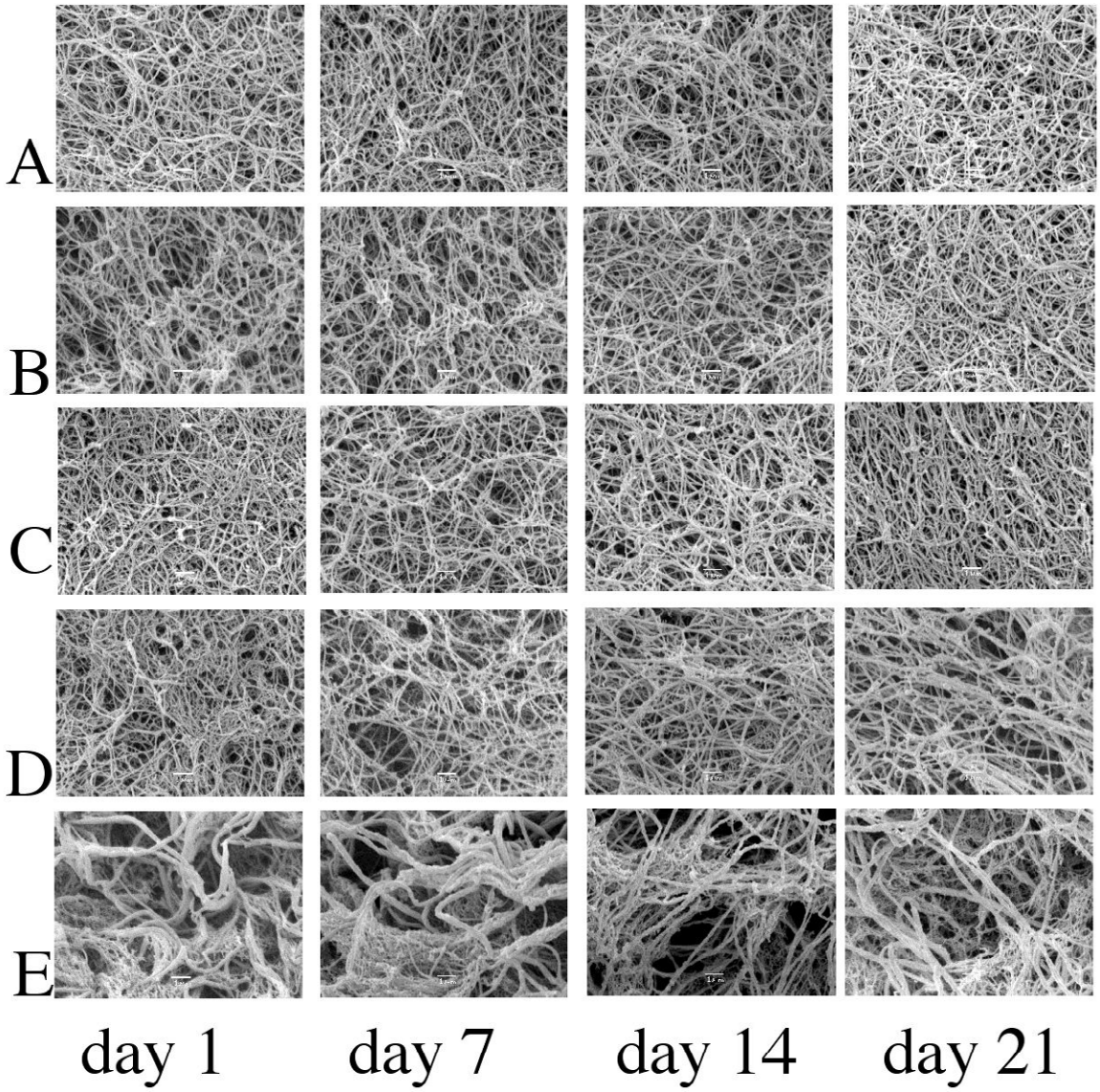
ESI-2: SEM images of the evolution of silica collagen network with time in the presence of fibroblasts with [SiO_2] = 0 (A), 0.5 mM (B), 1 mM (C), 2.5 mM (D), 5 mM (E)



ESI-3: TEM images of the evolution of silica collagen network with time in the presence of fibroblasts with [SiO_2] = 0 (A), 0.5 mM (B), 1 mM (C), 2.5 mM (D), 5 mM (E)



ESI-4: SEM images of the evolution of control (i.e. in the absence of fibroblasts) silica collagen network with time with [SiO_2] = 0 (A), 0.5 mM (B), 1 mM (C), 2.5 mM (D), 5 mM (E)



ESI-5 : ICP-AES data of silica dissolution for hybrid gels in the presence of silicates

At each point of measurement, the gel supernatant was withdrawn and analyzed by ICP-AES and replaced by fresh culture medium.