Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information

Priming the Cells for Their Final Destination: Microenvironment Controlled Cell Culture by a Modular ECM-mimicking Feeder Film

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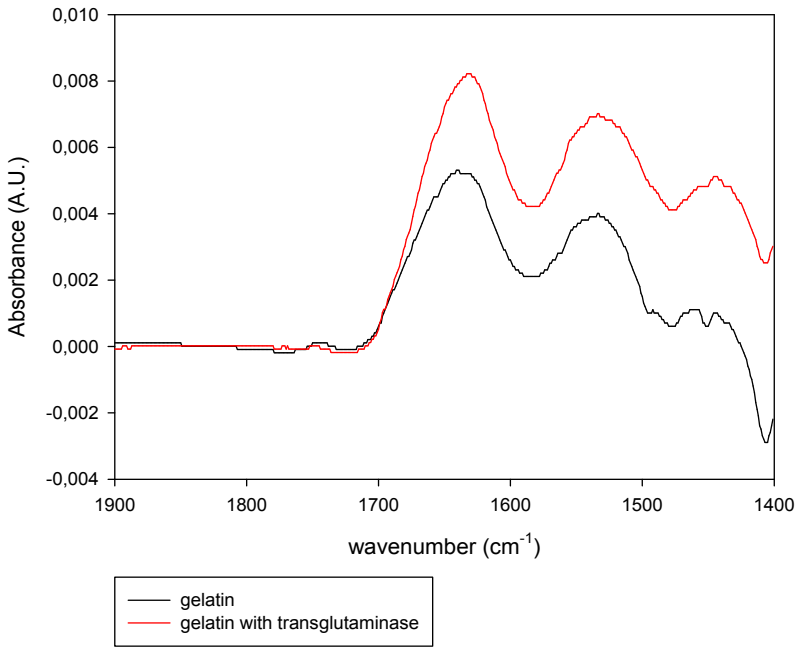


Figure S1. FT-IR spectra of gelatin films with and without transglutaminase treatment.

The intra red spectra were acquired in the attenuated total reflection mode (ATR) using a Spectrum Two spectrometer (Perkin Elmer). 16 interferograms were averaged with a spectral resolution of 4 cm^{-1} between 700 and 4000 cm^{-1}. Particular emphasis was made on the amide I (1600 -1700 cm^{-1}) and amide II bands (1450-1600 cm^{-1}) of the proteins present in the gels deposited on the ZnSe reflection element. The ethanol cleaned ZnSe was taken as the reference.

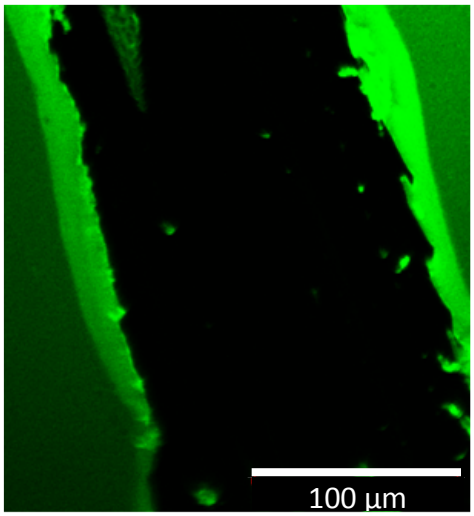


Figure S2. Observation by CLSM of the crosslinked gelatin film labeled with PLL^{FITC}. A scratch has been performed with a tip in the imaged area.

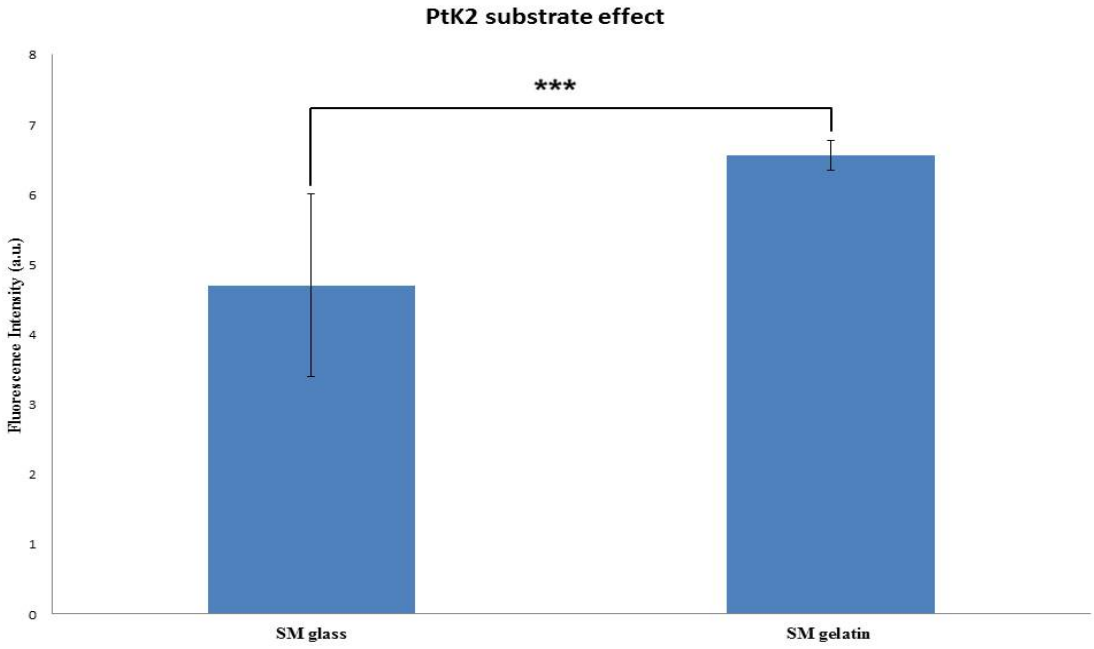


Figure S3. Substrate effect (glass or gelatin crosslinked with transglutaminase) on metabolic activity of PtK2 cells (epithelial line) cultivated for 3 days in starvation medium having the gelatin substrate only improved the metabolic activity of cells. This was determined on three different experiments and the results were was statically different with $p=0.024$ ($p<0.05$).

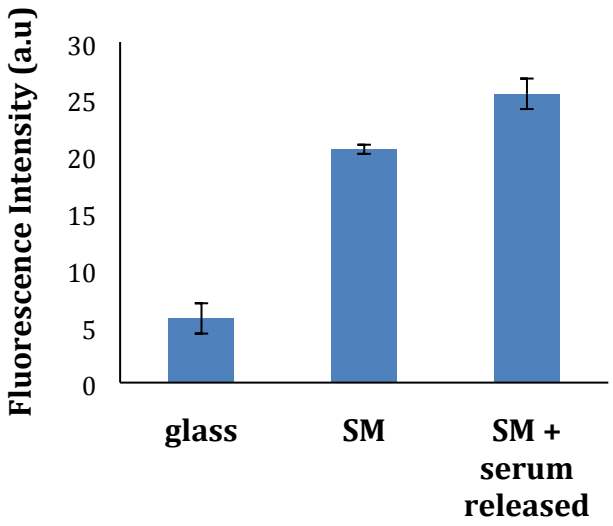


Figure S4. Metabolic activity of HUVEC cells seeded 3 days on a glass slide with starvation conditions (glass), or on gelatin films in starvation conditions (SM), or on the gelatin film pre-incubated with growth factors ("SM+serum released" condition).

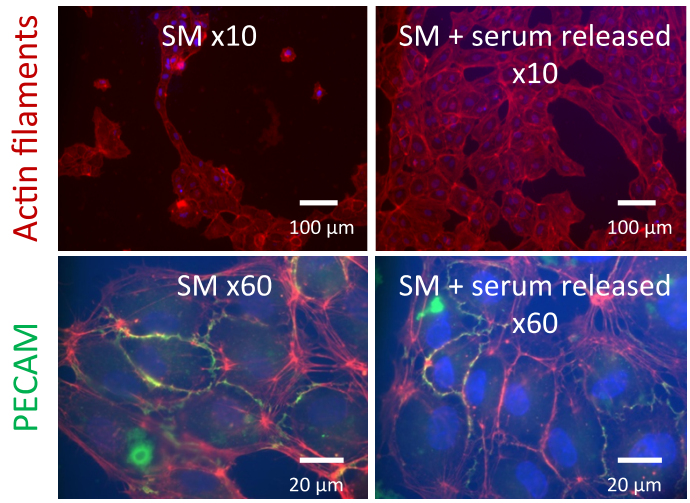


Figure S5. F-actin and PECAM labeling of HUVEC cells seeded 3 days on gelatin films in starvation conditions (SM), or on the gelatin film pre-incubated with growth factors ("SM+serum released"). For SM condition, occasional islands of cells were observed.

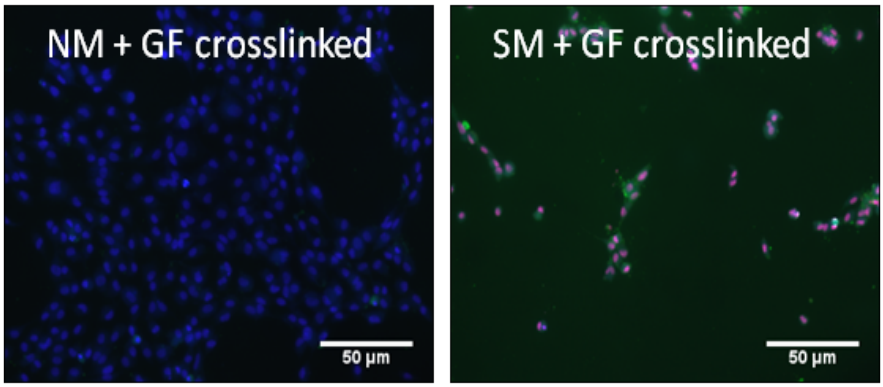


Figure S6. Observation of HUVEC cells (alive in blue, necrotic in red, apoptotic in green) after 1 day of seeding on gelatin films first loaded with growth factors and then crosslinked with transglutaminase. Normal medium (left image) or starvation medium (right image) were used. The release of loaded growth factors is necessary for cell survival.

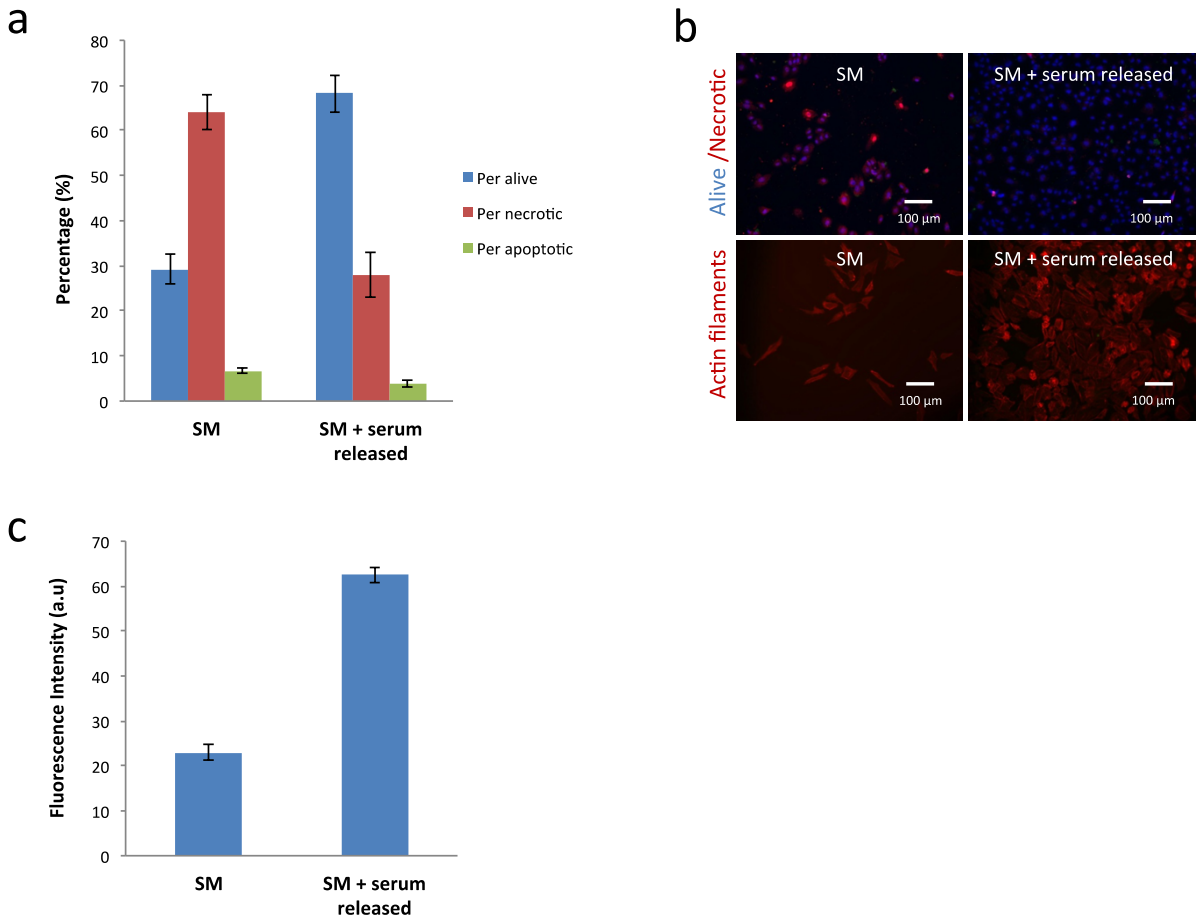


Figure S7. Effectiveness of the gel-feeder substrate loaded with growth factors after storage in frozen conditions. a) Significantly more HUVECs attached at day 3 to the gelatin films pre-incubated with growth factors ("SM+serum released" condition) compared to non-loaded films (SM). Error bars correspond to standard deviations. b) HUVECs were more spread and established more cell-to-cell contacts in the growth factor containing gelatin films after 3 days of culture. Top images: alive (blue), necrotic (red) and apoptotic (green) cells. Bottom images: actin labeling (red). c) Metabolic activity of HUVEC cells seeded 3 days on ?, or on gelatin films in starvation conditions (SM) or on the gelatin film pre-incubated with growth factors ("SM+serum released" condition). Before cell seeding, films used were dried and stored at -20°C for a week.

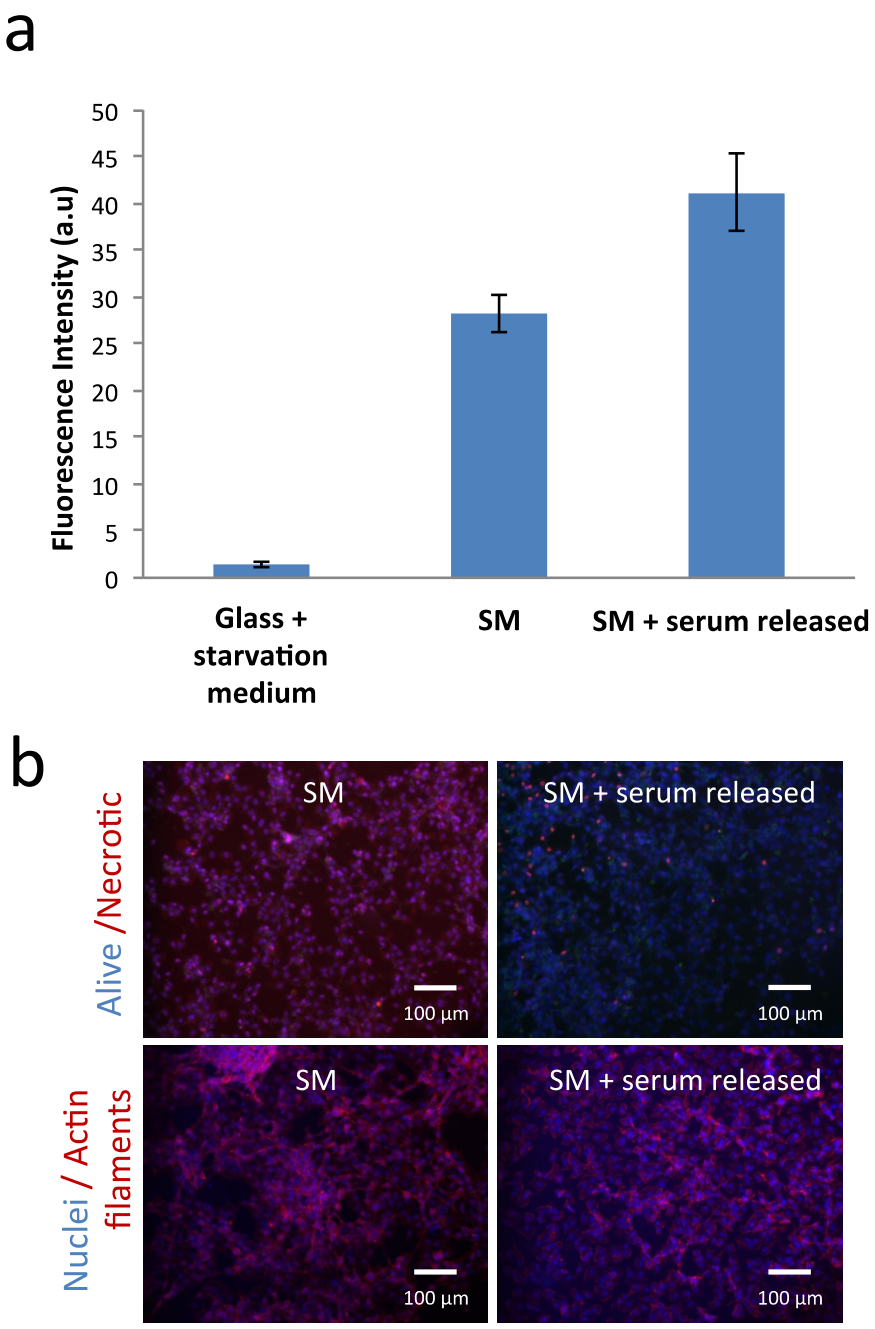


Figure S8. 3T3 fibroblast cells seeded 3 days on gelatin films in starvation conditions (SM) or on the gelatin film pre-incubated with growth factors ("SM+serum released" condition). a) Metabolic activity, b) 3T3 labeling. Top images: alive (blue), necrotic (red) and apoptotic (green) cells. Bottom images: nucleus (blue), actin labeling (red).

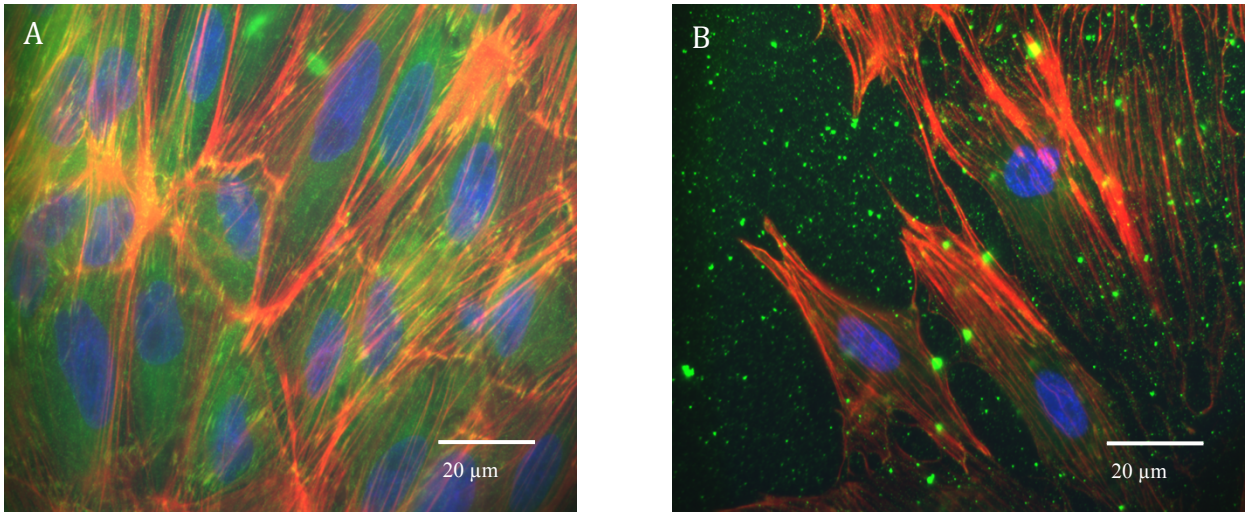


Figure S9. Focal adhesion on PTK2 cells labelled with vinculin immunostaining (green) for cells cultivated on gelatin substrate for 3 days with supplement released (A) and without supplement released (B). Blue and red staining represents respectively DAPI and Phalloidin staining. Images were acquired with a fluorescent microscope (60x objective).

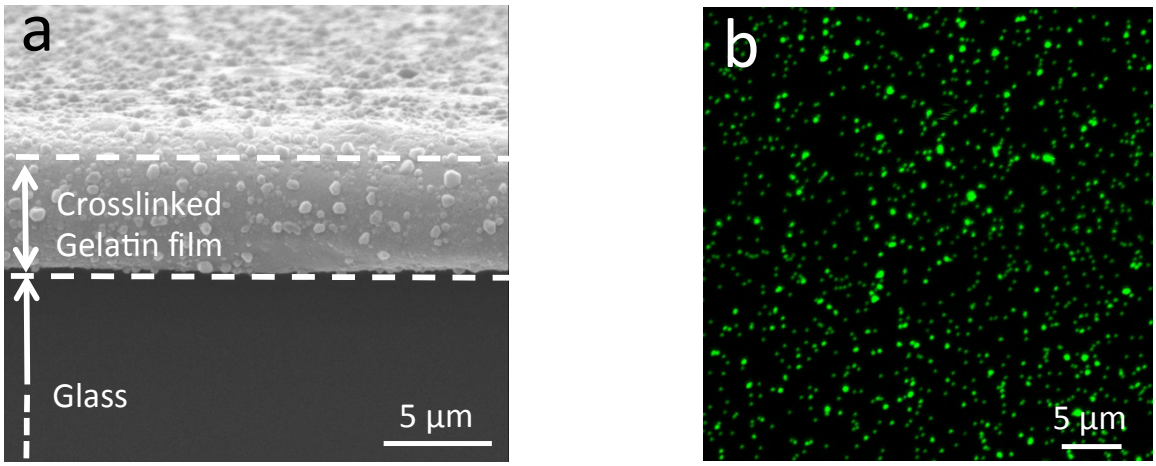


Figure S10. a) 3D ESEM image in semi-hydrated conditions of the spin-coated crosslinked gelatin film loaded with 100 nm nanoparticles (dilution 1/500). b) CLSM (x,y) images inside the structure of a spin-coated crosslinked gelatin film loaded with 100 nm green labeled nanoparticles.

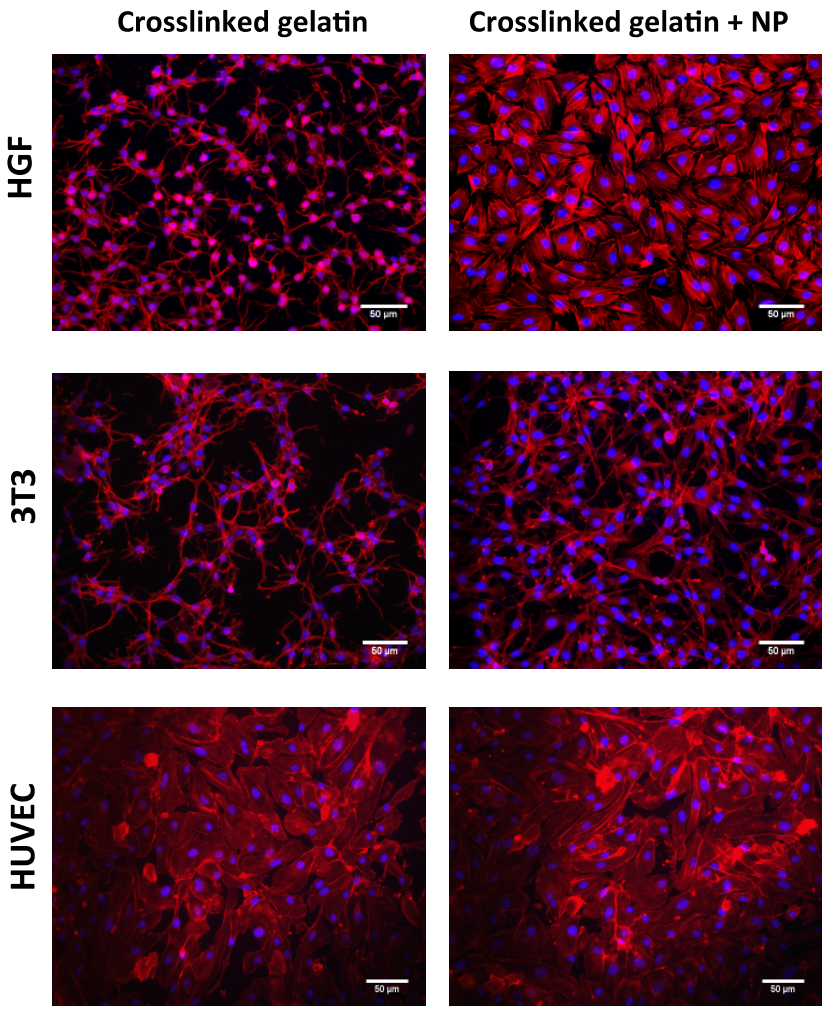


Figure S11. Effect of nanoparticle loading (dilution 1/500) in the gelatin film on spreading of HGF, 3T3 and HUVEC cells. Actin filaments have been labeled in red and nuclei in blue.

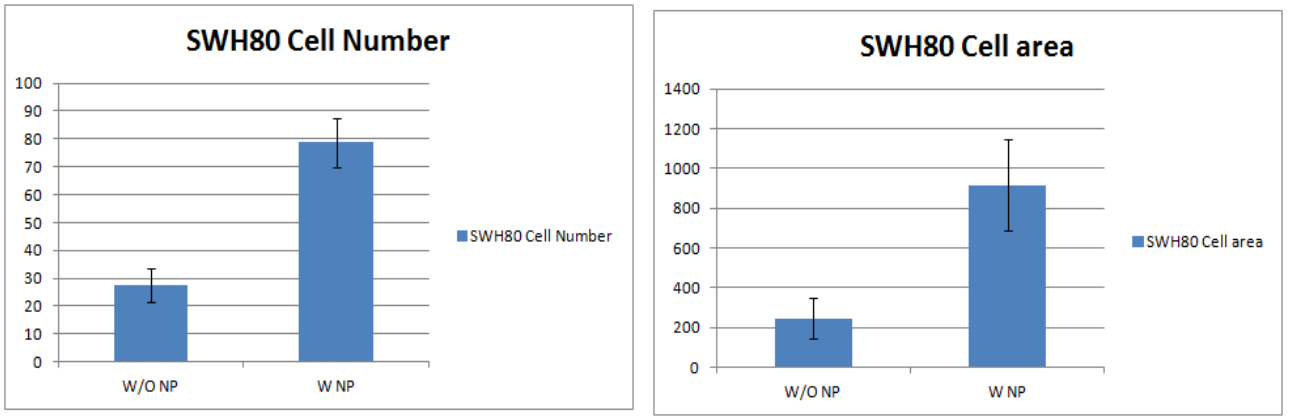
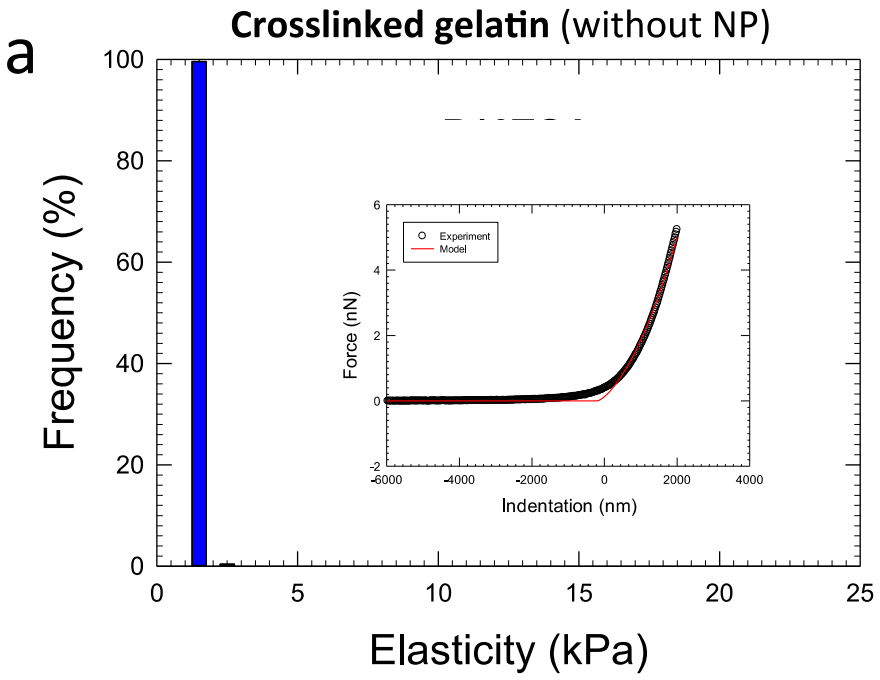
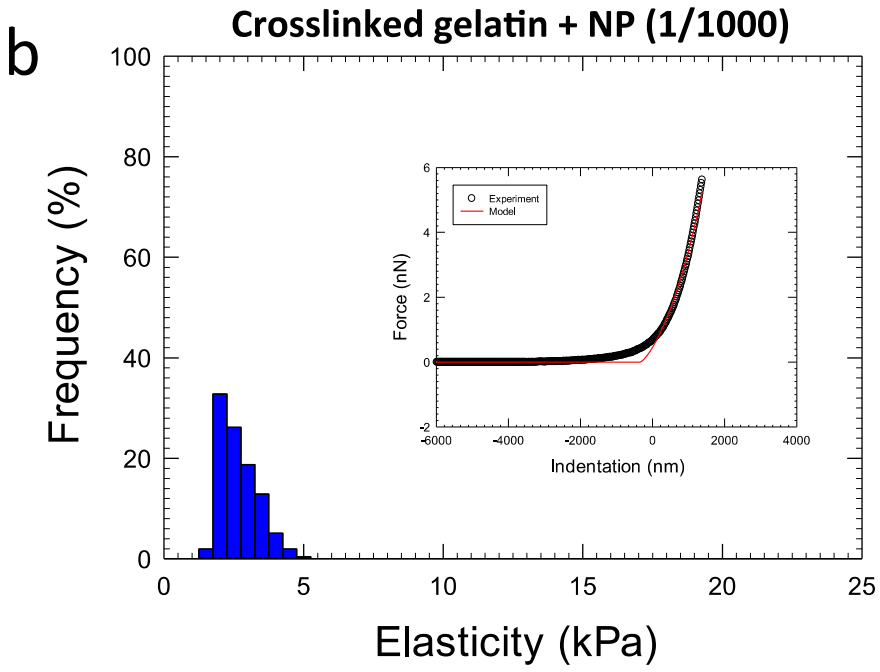


Figure S12. Influence on particles addition on gelatin substrate on cell number and area for SW480 cells (epithelial cancer line). These cells were cultivated for 3 days on gelatin substrate and the cells were counted for each picture taken with fluorescent microscope using DAPI staining.


a
Crosslinked gelatin (without NP)
Frequency (%)
Elasticity (kPa)
Force (nN)
Indentation (nm)
Experiment
Model


b
Crosslinked gelatin + NP (1/1000)
Frequency (%)
Elasticity (kPa)
Force (nN)
Indentation (nm)
Experiment
Model

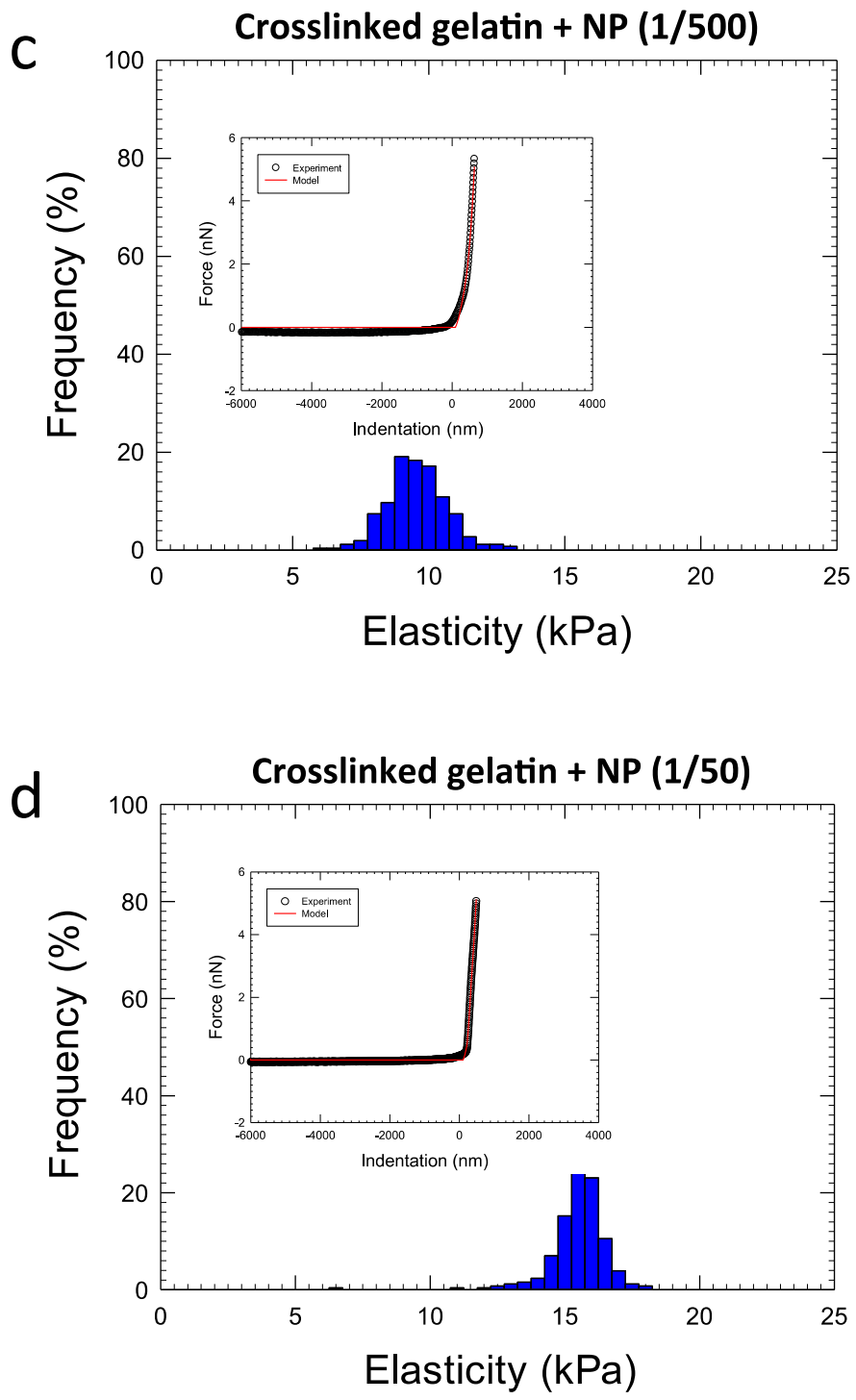


Figure S13. AFM nanoindentation experiments to determine the Young modulus of gelatin films loaded with various concentrations of nanoparticles: a) without nanoparticles; b) with nanoparticles loaded with a solution diluted at 1/1000; c) with nanoparticles loaded with a

solution diluted at 1/500; d) with nanoparticles loaded with a solution diluted at 1/50. Stock particle solution was concentrated at 3.6×10^{10} particles.mL^{-1}.

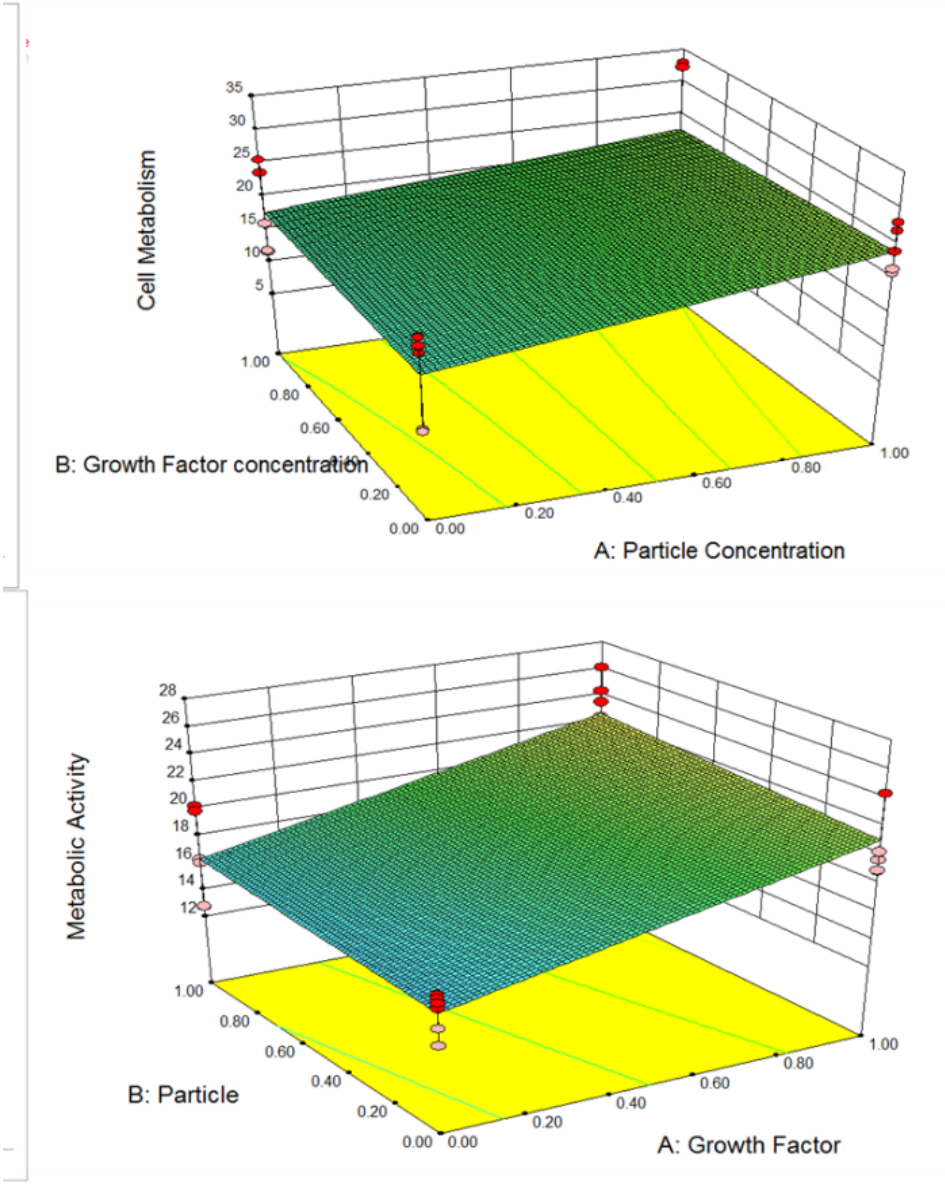


Figure S14. In order to see whether concomitant utilization of nanoparticles and growth factors has a synergistic or antagonistic effect to cell behavior, 2^2 factorial experimental systems were used to determine the interaction between these two parameters by Design Expert program (StatEase, USA). From the model, it could be seen that initially the presence of the particles governed the cell behavior (presence of the particles had a positive effect on day 1), whereas the later behavior was mainly governed by growth factor presence with a small synergistic effect between the particles and the growth factor.

Serum		Release at day 1		Release at day 3	
Protein	amino acid	Protein	amino acid	Protein	amino acid
serum albumin	9	serum albumin	42	serum albumin	23
alpha-2-HS-	16	alpha-2-HS-	11	alpha-2-HS-	6
serotransferrin	14	serotransferrin	12	serotransferrin	4
human FGF	10	human FGF	6	human FGF	6
human EGF	8	human EGF	2	human EGF	4
		collagen	13	collagen	12
		collagen	8	collagen	8

Table 1. Monitoring the loading and release of proteins from gelatin films pre-incubated with endothelial cell growth supplement. Capillary electrophoresis (CE) coupled to electrospray ionization mass spectrometry (ESI-MS) is used to determine the release of proteins at day 1 and day 3. First the serum components were separated and each peak was characterized with protein sequencing. The protein content at day 1 and day 3 was monitored by the collection of the supernatant and analyzed by comparison with the protein contents of the original endothelial cell growth medium. The number of the identified amino acid for each component detected is also mentioned.