

SUPPORTING INFORMATION

Extracellular matrix-inspired assembly of glycosaminoglycan-collagen fibers

Agatha Carretero^{1,2}, Diana Soares da Costa^{1,2}, Rui L. Reis^{1,2} and Iva Pashkuleva^{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, 4805-017 Taipas, Guimarães, Portugal

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

Experimental Section

Fibers production: Hyaluronic acid (HA, 25 kDa and 40 kDa, Lifecore), chondroitin sulfate from shark cartilage (CS, 147 kDa, Sigma), heparin from porcine mucosa (HEP, 30 kDa, Sigma) and collagen from rat tail (Col, Gibco, 3g/L in 20mM acetic acid, pH 3.5, 300kDa) were used in this study. The glycosaminoglycans were dissolved in 150mM NaCl at concentration of 3 g/L (pH about 6.5). 10 μ L drop of the respective GAG solution was placed on a Teflon (hydrophobic) plate side by side with 10 μ L drop of Col (**Figure 1A2**). The IPC was formed by putting the two drops in contact via moving the Col drop with the help of a pipette tip (**Figure 1A3**). The fibers were drawn from the formed IPC at a rate of 0.5 mm/sec using the same pipette tip attached to a linear motor.

Fibers characterization: Initial screening about the stability of the fibers was performed under aseptic conditions by immersing them in α -modified Eagle's medium (α MEM, Sigma-Aldrich) for a period up to

14 days and observing them under inverted microscope (Axiovert 40, Zeiss) during this period. The morphology of fibers was evaluated by scanning electron microscopy (SEM, Jeol) after sputter coating with gold. The immunodetection of the GAG component was performed by incubation of the fibers in solution of wheat germ agglutinin Alexa Fluor 633 Conjugate (WGA-AF633, Gibco) in phosphate buffer saline (PBS, 5 μ g/mL) for 10 min at room temperature followed by washing with PBS. A blocking step was then performed with 3% bovine serum albumin (BSA) in PBS for 30 min at room temperature. After washing with PBS, the fibers were incubated with Col type I antibody (AbCam, Ab292, 1:200 in 1% BSA in PBS) for 1h at room temperature, washed again with PBS and incubated with secondary antibody AlexaFluor 488 (Invitrogen) for 1 h at room temperature. The immunostained fibers were washed with PBS and observed in a confocal laser scanning microscope (Leica TCS SP8).

Cell isolation, culture and characterization: Human adipose-derived stem cells (hASCs) were isolated from subcutaneous adipose tissue obtained from lipoaspiration procedures under the scope of a cooperation agreement with Hospital da Prelada (Porto, Portugal).¹⁻² Briefly, the aspirates were washed (PBS with 10% Antibiotic/Antimycotic) and then digested with collagenase from *Clostridium histolyticum* (Sigma-Aldrich, 0.1% in PBS) for 45 min at 37 °C. The digested tissue was centrifuged (1000 g for 10 min) and the obtained cell pellet was incubated in lysis buffer (155 mM NH₄Cl, 5.7 mM K₂HPO₄, 0.1 mM EDTA) for 10 min to remove erythrocytes and then centrifuged at 800 g for 10 min. Cells were expanded in α MEM supplemented with 1% Antibiotic/Antimycotic (Gibco) and 10% Fetal Bovine Serum (FBS, Gibco). Fibers (4 cm) prepared under aseptic conditions were placed in 48 well plates and seeded with 5x10⁴ cells in α MEM supplemented with 10% FBS and 1% antibiotic/antimycotic. Viability was assessed by live/dead assay using calcein/ethidium homodimer staining (Molecular probes, USA/ Sigma Aldrich) and following the supplier's instructions. Cytoskeleton organization and expression of Col I was visualized for cells cultured on the fibers. The substrates with the cultured cells were washed with PBS, fixed with 10% neutral buffered formalin for 30 min at 4 °C, permeabilized with 0.1% Triton X-100 in PBS

for 5 min, and blocked with 3% BSA in PBS for 30 min at room temperature. Cytoskeleton organization was visualized by phalloidin–TRITC conjugate (1:200 in PBS for 30 min, Sigma). Nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI; 1:500 in PBS for 30 min). The Col immunostaining was performed with Col I antibody (AbCam, Ab292, 1:200 in 1% BSA in PBS) for 1 h at room temperature, followed by incubation with secondary antibody AlexaFluor 488 (Invitrogen) for 1 h at room temperature. Cells were imaged by a confocal laser scanning microscope (CLSM, Leica TCS SP8) using 488 and 633 nm lasers.

Table S1. IPC fiber formation as a function of the polyions concentration (% v/v). Different drawing speeds were tested for each combination.

	Col 0.50	Col 0.75	Col 1.00	Col 1.50	Col 3.00
HA 0.50	X	X	X	X	X
HA 0.75	X	✓**	X	X	X
HA 1.00	X	✓**	✓*	X	X
HA 1.50	X	✓**	X	X	✓*
HA 3.00	X	✓*	X	X	✓
HEP 0.50	X	✓	✓*	✓	X
HEP 0.75	X	✓*	X	X	X
HEP 1.00	X	✓*	✓*	X	X
HEP 3.00	X	X	X	X	✓

(X) no fiber formation; (✓) fiber formation; (*) the formed fiber is not stable during the drawing process (return to the drop); (**) the process is not reproducible

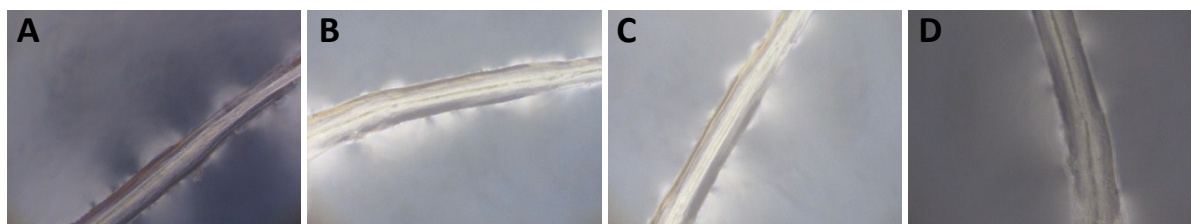


Figure S1. Macroscopic stability of HEP/Col fibers in cell culture medium after 0h (A), 7h (B), 24h (C) and 7 days (D) at 37°C.

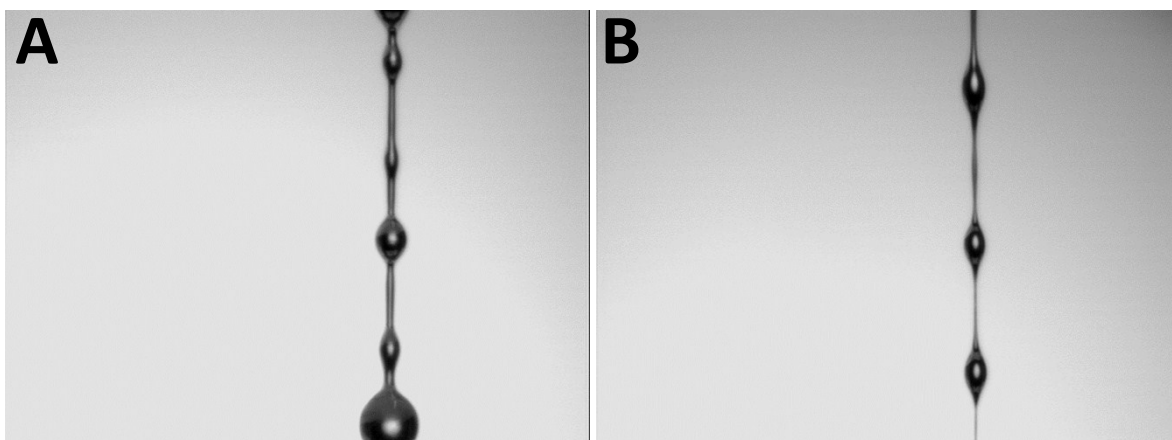


Figure S2. Examples of IPC fibers with beads: CS/Col (A) and HEP/Col (B) fibers obtained at drawing speed of 1.3 mm/sec. The concentration of all polymers was 3 g/L.

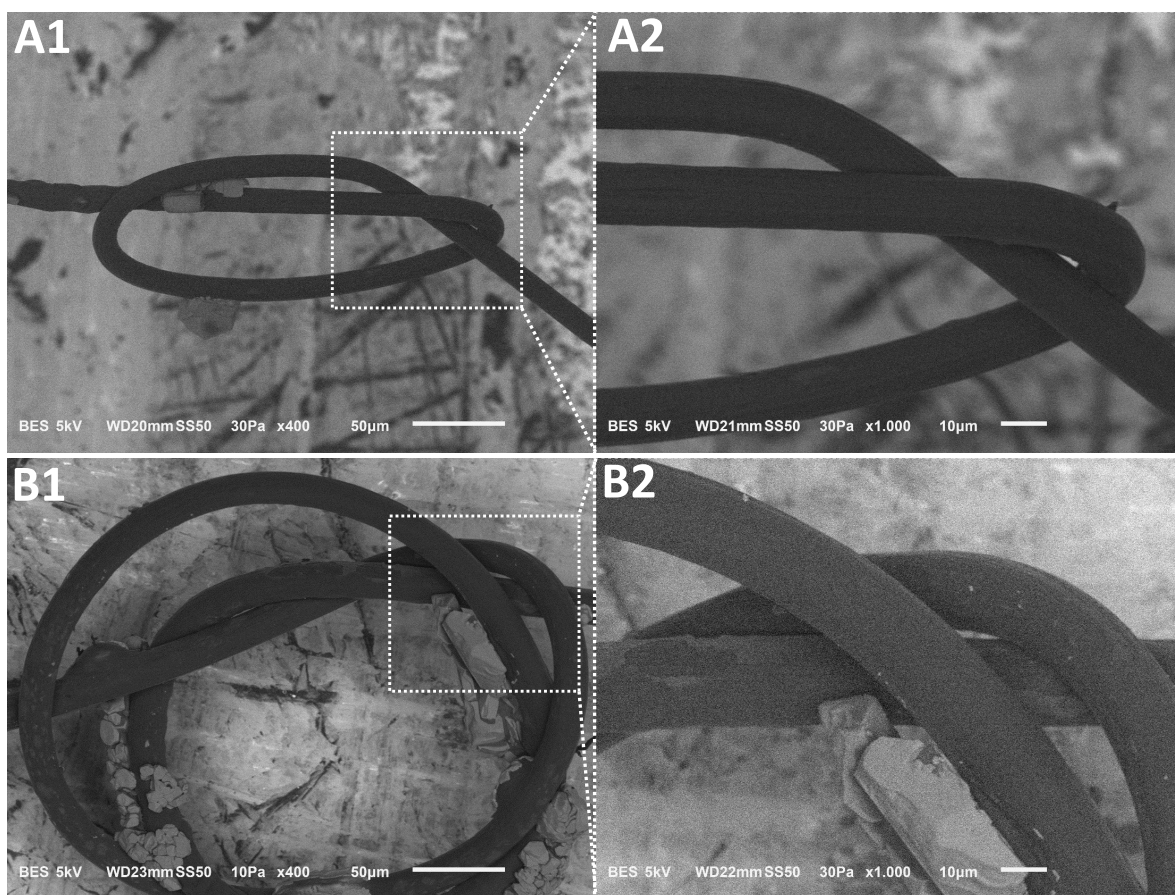


Figure S3. Flexibility of the CS/Col (A) and HEP/Col fibers in dry state.

REFERENCES

1. Araujo, A. R.; da Costa, D. S.; Amorim, S.; Reis, R. L.; Pires, R. A.; Pashkuleva, I., Surfaces Mimicking Glycosaminoglycans Trigger Different Response of Stem Cells via Distinct Fibronectin Adsorption and Reorganization. *Acs Appl Mater Inter* **2016**, 8 (42), 28428-28436.
2. da Costa, D. S.; Pires, R. A.; Frias, A. M.; Reis, R. L.; Pashkuleva, I., Sulfonic groups induce formation of filopodia in mesenchymal stem cells. *Journal of Materials Chemistry* **2012**, 22 (15), 7172-7178.