Modular bioink for 3D printing of biocompatible hydrogels: sol-gel polymerization of hybrid peptides and polymers

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Abbreviations

3D, three dimensional; CAD, computer-aided design; DMEM, Dulbecco's modified eagle medium; DPBS, Dulbecco's phosphate buffered saline; ECM, extracellular matrix; EthD-III, ethidium homodimer III; FBS, fetal bovine serum; ICPTES, 3-isocyanatopropyltriethoxysilane; mMSC, mouse mesenchymal stem cell; PA, polyacrylamide; PEG, polyethylene glycol; PHEMA, polyhydroxyethylmethacrylate; PLA, poly(lactic acid); RGD, ArgGlyAsp cell adhesion peptide sequence; RT, room temperature; TC-PS, tissue culture polystyrene; UV, ultra-violet. Other abbreviations used were those recommended by the IUPAC-IUB Commission (Eur. J. Biochem. 1984, 138, 9-37).

Preparation of the bioink

The syntheses of the hybrid blocks **1** and **2** were achieved following a previously described procedure (Echalier,C. et al. Easy Synthesis of Tunable Hybrid Bioactive Hydrogels. *Chem. Mater.* **2016**,*28*, 1261–1265). Hybrid PEG **1** (10 wt%, 300 mg) and hybrid GRGDSP peptide **2** (1 wt%, 30 mg) were dissolved in DPBS (3 mL, Gibco ref. 14190) containing sodium fluoride (0.3 wt%, 9 mg, Acros). The term bioink refers to this solution.

Viscometry

Viscosity measurements were performed using a SV-10 sine-wave vibro viscometer (A&D) equipped with a 10 mL sample cup (polycarbonate). The sample cup was filled with 10 mL of the bioink and fitted with a water jacket connected to a water tank. The temperature was set at 37°C. Viscosity was recorded automatically every 30 seconds. Full sets of data are presented in Figure S1.

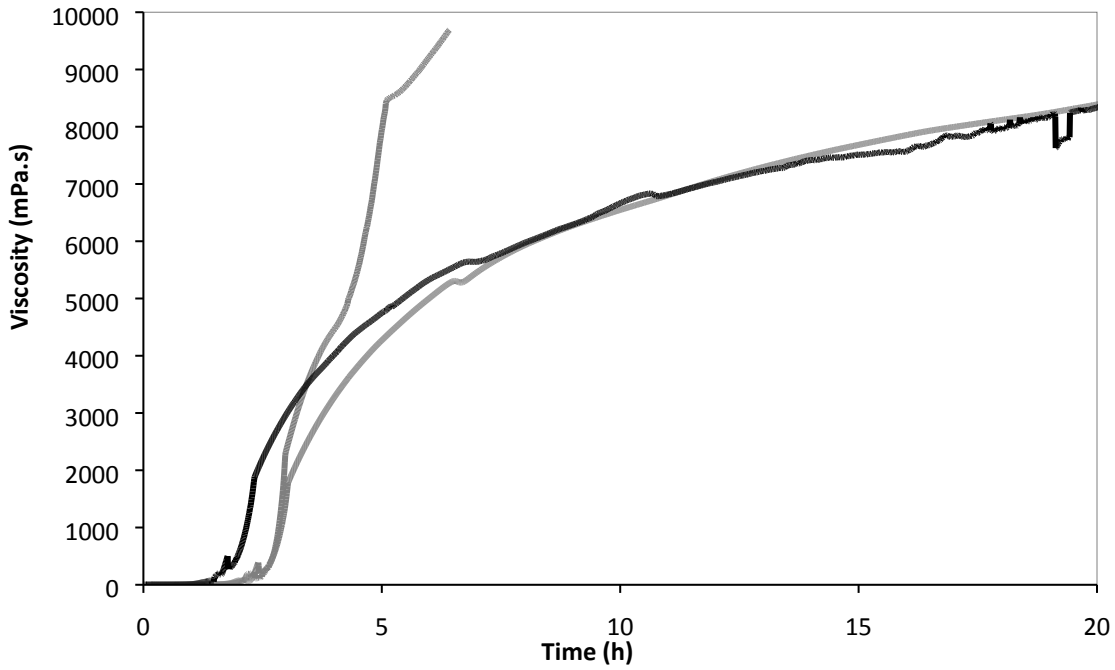

Figure S1. Viscosity of the hybrid solutions recorded as a function of time. Dashed grey line: hybrid PEG solution at 37°C; solid grey line: hybrid PEG solution at 37°C until gel point and then at 25°C; black line: hybrid PEG-RGD solution at 37°C until gel point and then at 25°C

Gel points can be read from the viscosity measurements. Indeed, gel point triggers a small decrease in viscosity after the initial increase (Figure S2). Gel points were also confirmed by the tilting method.

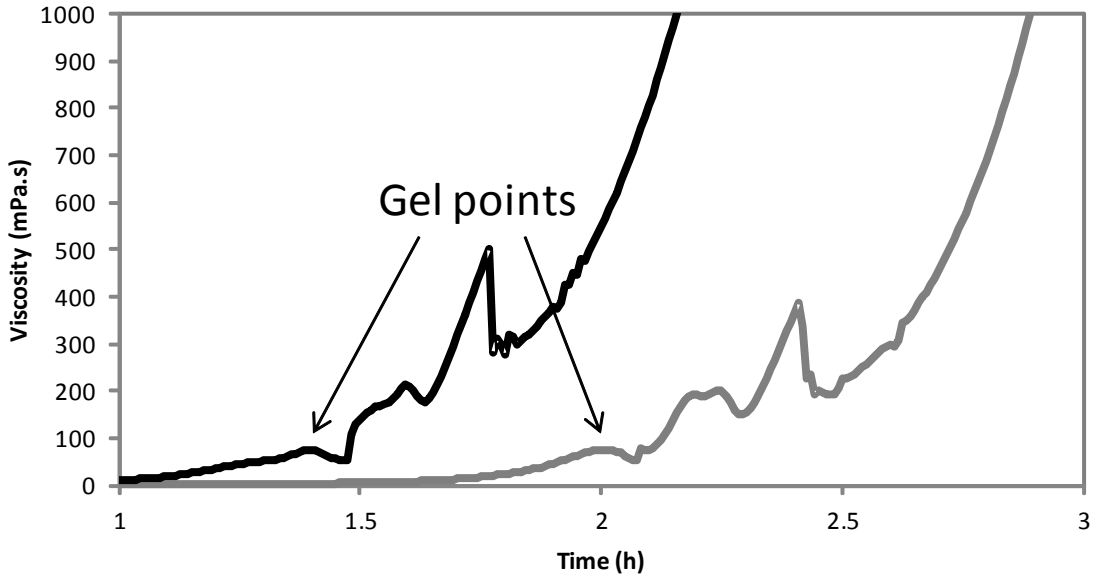

Figure S2. Zoom in the curves of viscosity. Solid grey line: hybrid PEG solution at 37°C until gel point and then at 25°C; black line: hybrid PEG-RGD solution at 37°C until gel point and then at 25°C.

Hydrogel printing

All the printing assays were performed at RT on a nScrypt 3Dn-300 rapid prototyping machine (nScrypt, Orlando, FL). The bioink was placed in a clear 3 mL syringe barrel (optimum fluid dispensing system Nordson EFD 3 cc) equipped with a piston (Nordson EFD) and a 27G conical dispensing tip (Nordson EFD, ref. 7018417). Scaffolds were printed on microscope glass slides (Thermo Scientific, Superfrost). The script was written and visualized with Path2D software. The printer was controlled using the Machine tool software.

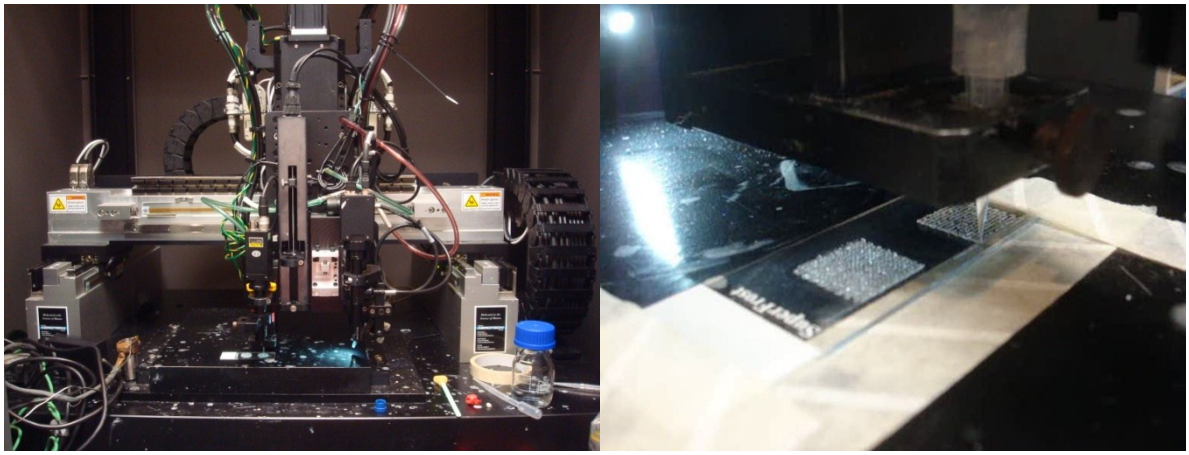

Figure S3. nScrypt 3Dn-300-TE rapid prototyping machine

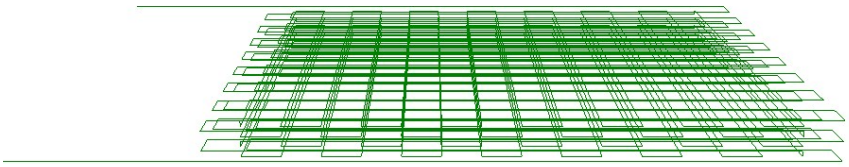

Figure S4. Scaffold design

PLA scaffold printing

PLA scaffolds were 3D printed to be used as control for biological assessment of the hybrid hydrogel scaffolds. Printing was performed on a 3D Volumic Stream 20Pro printer. A black PLA filament (1.75 mm in diameter) was forced through the nozzle at 200°C. As the molten material was extruded, the nozzle followed a path designed to yield grid-patterned scaffolds similar to hydrogel scaffolds.

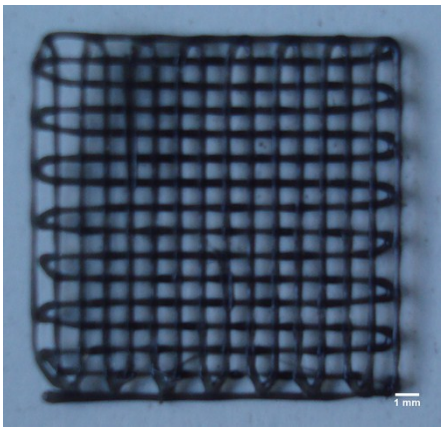

Figure S5. PLA scaffold

Biological assessment

Cell culture reagents were purchased from Gibco and Sigma-Aldrich. Mouse mesenchymal stem cells (mMSC) from Black 6 mice were cultured in high glucose DMEM containing phenol red and GlutaMAX (Gibco ref. 31966) with 10% FBS and 1% penicillin-streptomicin at 37°C with a humidified 5% CO_2 atmosphere.

Hydrogel scaffolds were sterilized by autoclaving (120°C for 20 min) on the glass slides used for printing. Then, they were detached with a blade, allowed to swell in cell culture medium and cut into discs of 7 mm in diameter with a punch. PLA scaffolds were sterilized in 70% ethanol baths, washed in cell culture medium and cut into discs of 7 mm in diameter with a punch.

Cells (passage n°18) were detached with trypsin (Trypsine-EDTA solution 0.25%) after washing with DPBS without calcium and magnesium. They were centrifugated, resuspended in culture medium and counted with a hemocytometer. The suspension was diluted to a concentration of 200,000 cells per mL. For each type of scaffolds, 4 samples were placed in a 1.5 mL Eppendorf tube with 1.5 mL of the cell suspension. The tubes were continuously rotated on a MACSmix tube rotator at 37°C for 4h. Then samples were put in 2 mL polypropylene tubes (Sarstedt) filled with cell culture medium. Non-adherent cells were removed by pipeting the media and the tubes were filled again with 400 µL of cell culture media. Tubes were incubated at 37°C for 4 days with loose caps to allow gas exchange. After 4 days of proliferation, scaffolds were placed in a 24 well cell culture plate (non treated PS) and 400 µL of the Live/Dead staining solution were added to each well (0.8 µL of calcein-AM solution and 3.2 µL of EthD-III solution in 1.6 mL of DPBS). Samples were observed 30 minutes later.

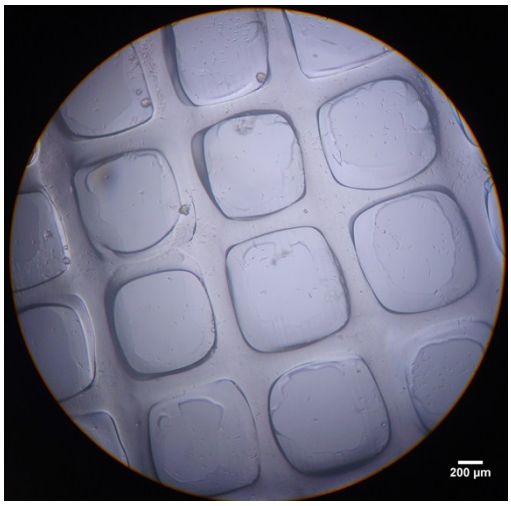

Figure S6. Hybrid hydrogel scaffold in cell culture medium after autoclaving, swelling and cutting

Microscopy

Stereomicroscopy images of the hydrogel scaffolds were taken on a Leica MZ16F stereomicroscope 24 hours after printing. Scaffolds were still on glass slides.

Fluorescence microscopy images were recorded on a Leica DM IRB inverted fluorescence microscope (objective lens ×4, NA 0.1) equipped with a MicroMAX RS camera (Princeton instruments). Images were analyzed with ImageJ.

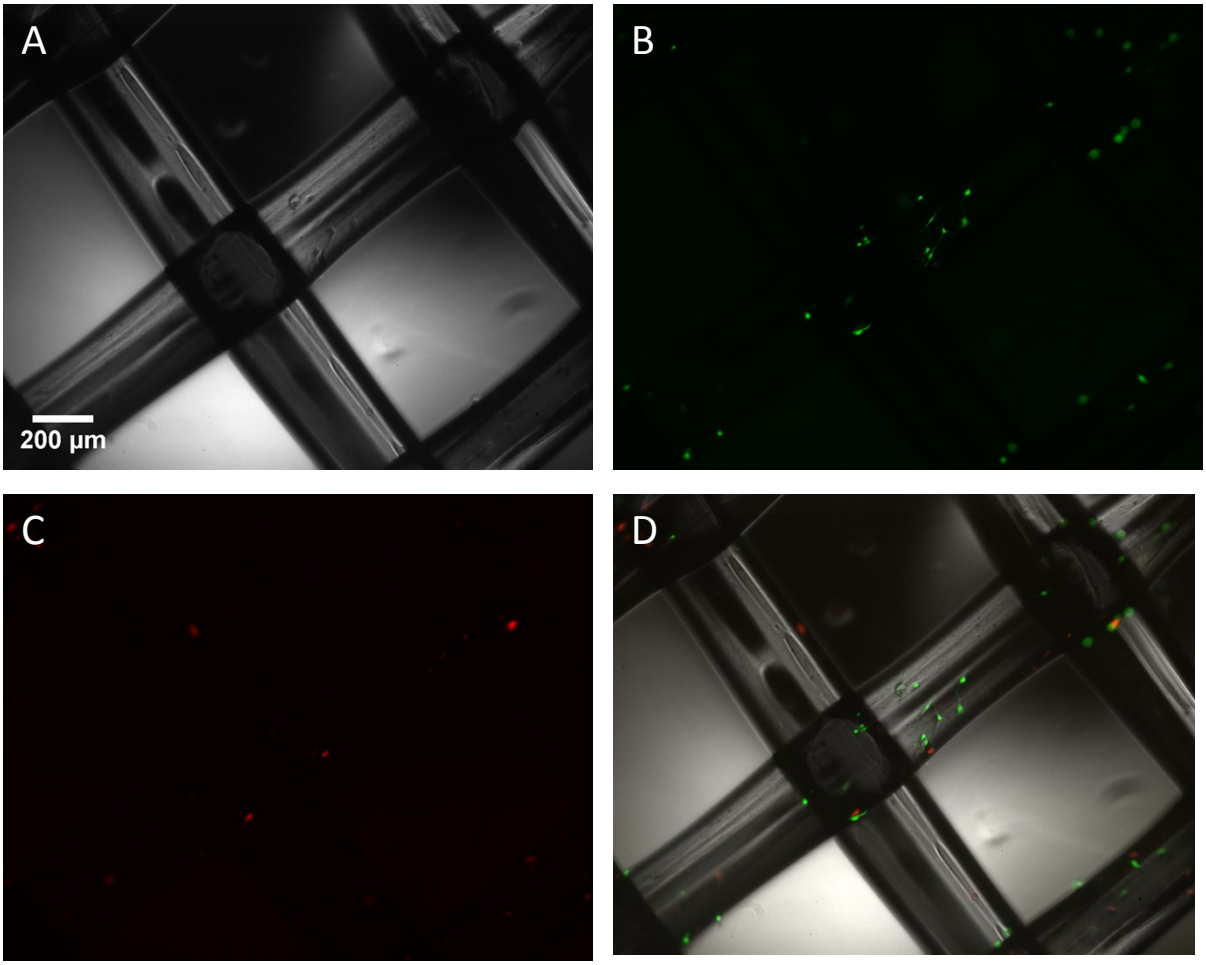

Figure S7. Fluorescent microscopy images of mMSC after 4 days of culture on a PLA 3D printed scaffold. (A) transmitted light image; (B) calcein-AM stain showing live cells in green; (C) EthD-III stain showing dead cells in red; (D) merged images.