Engineered 3D Microporous Gelatin Scaffolds to Study Cell Migration

Liesbeth J. De Cock,^a Olivier De Wever,^b Hamida Hammad,^c Bart N. Lambrecht^c, Els Vanderleyden,^d Peter Dubruel,^d Filip De Vos,^e Chris Vervaet,^a Jean Paul Remon^a and Bruno G. De Geest^a*

^{*a*} Laboratory of Pharmaceutical Technology, Ghent University, Ghent, Belgium. +32 9 264

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- ^b Laboratory of Experimental Cancer Research, Ghent University, Ghent, Belgium
- ^c Laboratory of Immunoregulation, Ghent University, Ghent, Belgium
- ^{*d*} Polymer Chemistry and Biomaterials Research Group, Ghent University, Ghent, Belgium
- ^e Laboratory of Radiopharmacy, Ghent University, Ghent, Belgium

corresponding author's e-mail: <u>br.degeest@ugent.be</u>

Supporting Information

Materials and methods

Materials

Gelatin type B, methacrylic anhydride, Atto 647 NHS ester, potassium peroxo disulfate (KPS), tetramethylethylenediamine (TEMED), DAPI and DMSO were purchased from Sigma-Aldrich (St. Louis, MO). 24-well transwell inserts were obtained from Trevigen (Gaithersburg, MD). Alexa Fluor 647 (i.e. Cy5) NHS ester, Phosphate buffered saline (PBS), Dulbecco's Modified Eagle Medium (DMEM), L-glutamine, sodium pyruvate, penicillin and streptomycin were purchased from Invitrogen (Carlsbad, CA). Poly(vinyl pyrrolidone) K90 (PVP) and Irgacure 2959 were obtained from respectively BASF (Ludwigshafen, Germany) and Ciba (Basel, Switzerland). Pronase was purchased from Roche (Mannheim, Germany). Cy5 labeled streptavidin was purchased from Zymed laboratories (San Francisco, CA). PDGF-BB was purchased from Pepro Tech EC (London, UK). HCA2-hTERT human fibroblasts were kindly provided by C. Jones (Cardiff University, UK). Primary PDGF-BB antibody (sc-127) and a secondary biotin labeled goat anti-rabbit antibody (sc-2040) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Cy5 labeled poly-L-arginine was synthesized by dissolving 100 mg poly-L-arginine at room temperature in 20 ml demineralized water with the pH adjusted to 7.5 (no buffer was used to avoid complexation of the poly-L-arginine amine residues) with 0.1 M NaOH. 1 mg Alexa Fluor 657 (i.e; Cy5) NHS ester was dissolved in 0.5 ml DMSO and added drop wise to the stirring poly-L-arginine solution. The solution was vigorously stirred at room temperature for 1 hour followed by dialysis of the reaction mixture against distilled water to remove unreacted dye.

Synthesis of methacrylamide-modified gelatin

Methacrylamide-modified gelatin with a DS of 65 % was synthesized according to the method described by Van Den Bulcke et al.^[1] 30 g gelatin type B was dissolved in 300 ml phosphate buffered saline (PBS, pH 7.4) at 50°C followed by slow addition of 3 ml methacrylic anhydride. After 1 hour reaction, the mixture was diluted with 300 ml water and dialysed against distilled water during 4 days at 37°C. Finally the methacrylamide-modified gelatin was obtained as a dry powder after lyophilization.

Fluorescently labeled methacrylamide-modified gelatin was synthesized by dissolving 100 mg methacrylamide-modified gelatin in 50 ml bicarbonate buffer (0.1 M; pH 7.8) at 37°C followed by drop-wise adding 1 mg Atto 647 NHS which was dissolved in 0.5 ml DMSO. The solution was vigorously stirred at room temperature for 1 hour followed by dialysis of the reaction mixture against distilled water to remove unreacted Atto 647 NHS.

Fabrication of microporous 3D gelatin hydrogels

A microporous 3D hydrogel of methacrylamide-modified gelatin was obtained through a water-in-water emulsion of methacrylamide-modified gelatin and PVP. PVP (6.5 % (w/v)) and methacrylamide-modified gelatin (18.56 % (w/v)) were mixed and sonicated at 10 % power output using a tip-sonicator. Radical polymerization of the methacrylamide functions was obtained by adding KPS (50 mg/ml) and TEMED (pH neutralized with 4N HCl). The reaction was carried out during 1 hour at room temperature followed by incubating the scaffolds in 1 l water during 3 days to leach out the PVP. Gels were frozen in liquid nitrogen followed by lyophilization to obtain dry scaffolds.

Microscopy

Scanning electron microscopy (SEM) images of lyophilized gelatin scaffolds were recorded with a Quanta 200 FEG FEI scanning electron microscope operating at an acceleration voltage of 5 kV. Fluorescence microscopy images were recorded with a Leica DM2500P microscope equipped with a DFC360FX camera. Two-photon confocal microscopy was performed with a LSM710 microscope (Carl Zeiss, Inc.) equipped with a tunable Mai-Tai DeepSee multiphoton laser (Coherent).

Enzymatic degradation of microporous gelatin hydrogels

A disc shaped gelatin hydrogel with dimensions 7 mm x 3 mm (diameter x height) was incubated in an aqueous pronase solution (0.5 mg/ml) at 37°C. After several time intervals the hydrogel was transferred to a glass cover slip and optical microscopy images were recorded with an Olympus SZX9 stereomicroscope equipped with a digital camera. Afterwards, the hydrogel was replaced to the aqueous pronase solution.

Interaction between PDGF-BB and gelatin hydrogels

Interaction between PDGF-BB and gelatin was investigated using surface plasmon resonance (SPR; Biacore-X). The sensor surface (gold-coated sensor chip) was spincoated with a 5 % (w/v) gelatin solution containing Irgacure 2959 (2 mol %) as photoinitiator and exposed to UV light to crosslink the methacrylamide moieties.^[29] After inserting the chip in the SPR instrument, 50 µl of the growth factor dissolved in Tris buffer (pH 7.4) containing 0.1 % Tween 20 was injected (flow rate of 5 µl/min) and the signal was monitored. Three concentrations, namely 2, 1 and 0.5 µg/ml PDGF-BB were evaluated.

Functionalization of 3D gelatin scaffolds with a solid-phase PDGF-BB gradient

Disc shaped gelatin hydrogels with dimensions 5 mm x 3 mm (diameter x height) were fixed in a transwell insert followed by the incubation in a 500 ng/ml PDGF-BB solution (in DMEM containing 1 % FBS) during 7 days. After the incubation period, the hydrogels were rinsed extensively in DMEM containing 1 % FBS. These gelatin hydrogels are referred to as PDGF-BB-functionalized gelatin hydrogels in the text. As control samples, disc shaped gelatin hydrogels with dimensions 5 mm x 3 mm (diameter x height) were fixed in a transwell insert followed by the incubation in DMEM containing 1 % FBS during 7 days. After the incubation period, the hydrogels with dimensions 5 mm x 3 mm (diameter x height) were fixed in a transwell insert followed by the incubation in DMEM containing 1 % FBS during 7 days. After the incubation period, the hydrogels were also rinsed in DMEM containing 1 % FBS. These gelatin hydrogels are referred to as non-functionalized gelatin hydrogels in the text.

Cryosections of Tissue Tek (Sakura) embedded PDGF-BB-functionalized hydrogels were cut at 10 µm thickness and stained for PDGF-BB by initially blocking the samples with PBS containing 1 % BSA. Subsequently, the samples were incubated with a 1/100 dilution (in PBS containing 1 % BSA) of respectively a primary PDGF-BB antibody and a secondary biotin labeled goat anti-rabbit antibody. Fluorescent analysis was obtained through incubation of the sections with CY5 labeled streptavidin (1/50 dilution in PBS containing 1 % BSA).

PDGF-BB was labeled with iodine-131 (¹³¹I) by incubating 10 µg growth factor with ¹³¹I in a vial coated with 70 µg iodogen in PBS containing 0.1 % Tween 20 during 20 min at room temperature. After the incubation period, residual ¹³¹I was separated from ¹³¹I-PDGF-BB on a PD-10 column by size exclusion chromatography using PBS containing 0.1 % Tween 20 as eluent. The concentration of the radioactive growth factor was calculated via the percentage of nonspecific

adsorption of growth factor on the PD-10 column and the original amount of growth factor, and the concentrated ¹³¹I-PDGF-BB solution was further diluted in PBS containing 0.1 % Tween 20. Microporous 3D gelatin hydrogels were functionalized with a solid-phase gradient of ¹³¹I-PDGF-BB as described previously. The amount of bound PDGF-BB was measured in a Packard cobra automated γ -counter. The experiment was performed in triplicate.

Migration assay

HCA2-hTERT human fibroblasts were cultured in Dulbecco's Minimum Essential Medium supplemented with 10 % FBS, L-glutamine, sodium pyruvate and antibiotics (penicillin and streptomycin). The cells were kept in a humidified environment with control of CO₂ (5 %).

Prior to the migration assay, fibroblasts were starved in DMEM containing 1 % FBS for 1 day. Subsequently, fibroblasts were harvested and drop-seeded on top of a PDGF-BB-functionalized or non-functionalized gelatin hydrogel. After an incubation period of 1 day, the construct was transferred to a new dish. At specific time points, a quarter of the gelatin hydrogel was isolated and fixed in phosphate buffered formaldehyde followed by staining the cell nuclei with Hoechst (1/5000 dilution in PBS). The construct was immersed in TissueTek and frozen in liquid nitrogen. Cryoslices having a thickness of 10 µm were cut and incubated with RITC-labeled poly-L-arginine to stain the gelatin red fluorescent. The samples were analyzed on a Leica fluorescent microscope.

Quantification of cell position was performed by manual counting the cell nuclei on crosssections of the 3D hydrogels at several areas ranging from a depth of 0 μ m to 1.05 mm. The migration index was calculated as the ratio (%) of the amount of cell nuclei in a specific area to the total amount of cell nuclei on the cryosection. 5 microscopy fields were counted for each data point.

References

 Van den Bulcke, A.I.; Bogdanov, B.; Schacht, E.H.; Cornelissen, M.; Berghmans, H. Biomacromolecules 2000, 1, 31.

Supplementary Figures







Figure S2. SEM image of a macroporous gelatin hydrogel (i.e. cryogel) obtained by cryogenic treatment of an aqueous gelatin solution.



Figure S3. Optical microscopy images of a gelatin hydrogel exposed to pronase at 37°C after incubation periods of (D1) 0 min, (D2) 20 min, (D3) 30 min and (D4) 40 min.



Figure S4. Interaction between PDGF-BB and gelatin through surface plasmon resonance.