Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

Dynameric G-quadruplex – Dextran hydrogels for cell growth applications

Monica-Cornelia Sardaru, Simona Morariu, Oana-Elena Carp, Elena-Laura Ursu, Alexandru Rotaru and Mihail Barboiu

Materials

Dextran (Mw= ca. 40000) was purchased from TCI (Tokyo, Japan). Guanosine, benzene-1.4-diboronic acid, $LiOHxH_2O$ and KCl were purchased from Sigma (Schnelldorf, Germany) and used as received. For all the performed experiments and stock solution preparation, ultrapure water was used.

Experimental section

1. Hydrogel synthesis

Three hydrogels (DGH1-3) with different degrees of guanosine functionalization were synthesized adapting the previously reported protocol.[1]

Synthesis protocol DGH1 (100% guanosine content): In a glass vial, 50 mg of guanosine (1 eq) and 29.3 mg benzene-1.4-diboronic acid (1 eq) were suspended in 1.6 mL water. The vial was placed for 1 minute in an ultrasonication bath to homogenize the reaction mixture, followed by addition of LiOH solution (200 µl, 14.82 mg, 2 eq) and heated on an oil bath (120 ºC) for 5 minutes until the solution became completely transparent. Next, 28 mg of dextran (0,004 eq) was added and the resulting suspension was sonicated and heated until all the dextran was soluble. The solution was cooled down at room temperature, followed by the addition of KCl solution (200 µl, 13.2 mg) with the instant formation of hydrogel.

Synthesis protocol for DGH2 (50% guanosine): Similarly to **DGH1**, with the same amount of dextran, except for the amounts of guanosine (25 mg), benzene-1.4-diboronic acid (14.6 mg), water (1.8 mL), LiOH solution (100 µl, 7.41 mg) and KCl solution (100 µl, 6.6 mg) with the formation of hydrogel after 20 minutes at room temperature.

Synthesis protocol for DGH3 (30% guanosine): Similarly to **DGH1**, with the same amount of dextran, except for the amounts of guanosine (15 mg), benzene-1.4-diboronic acid (8.8 mg), water (1.88 mL), LiOH solution (60 µl, 4.6 mg) and KCl solution (60 µl, 4.0 mg) with the formation of hydrogel after 24 hours at room temperature.

2. Circular Dichroism (CD) spectroscopy

CD measurement for DGH1-3 hydrogels was performed using a Chirascan plus (Applied Photophysics Ltd., Leatherhead, Surrey, UK). Scanning range utilized for all samples was between 230-340 nm. Lamellas (1 mm) were used by adding 100 µL of sample solution, without any dilution. The CD spectrum data were processed by OriginPro 8 software.

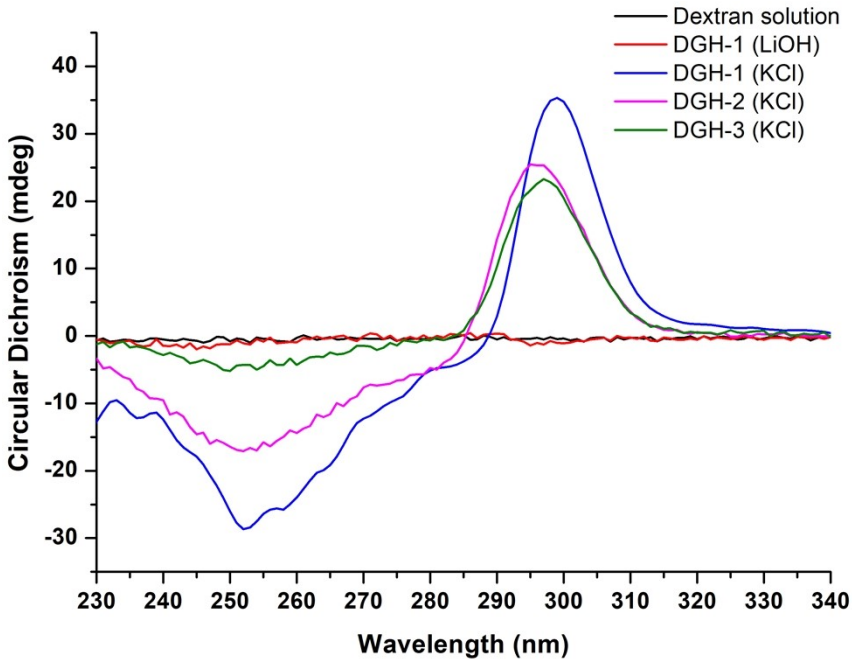

Figure S1. Circular dichroism spectra in the 230-340 nm range for: Dextran solution (black), DGH1 reaction mixture (red), DGH1 reaction mixture after addition of KCl (blue), DGH2 reaction mixture after addition of KCl (purple) and DGH3 reaction mixture after addition of KCl (green).

3. FTIR spectroscopy

The FTIR spectra of DGH1-3 xerogels were registered on an IRTracer-100 FTIR spectrometer (Shimadzu, Kyoto, Japan) with the attenuated total reflection (ATR) module, with 100 scans at resolution of 4 and in the spectral range 4000 - 400 cm^{-1}. FTIR spectra were processed using OriginPro 8 software.

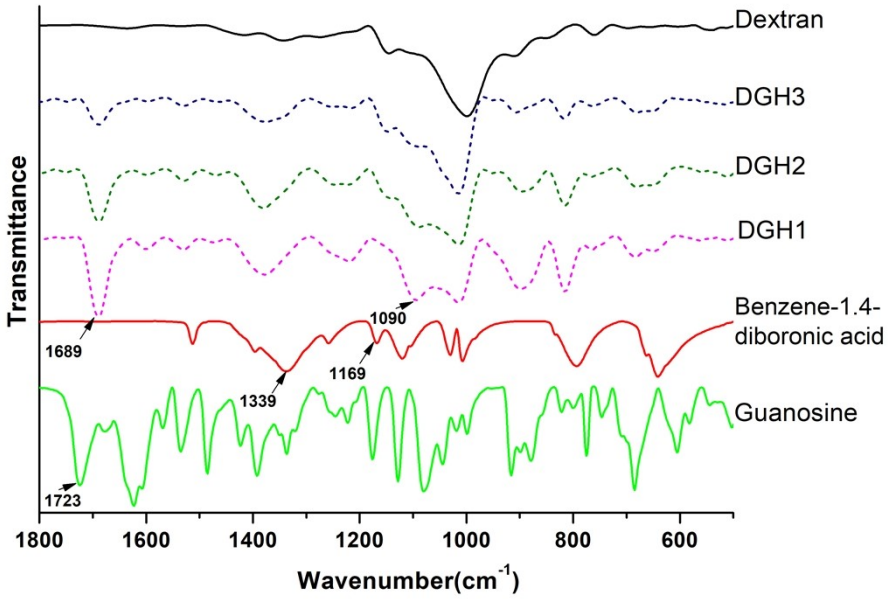

Figure S2. FTIR spectra of DGH1-3 xerogels, and their components (dextran, benzene-1,4-diboronic acid and guanosine).

The amide vibration of free guanosine molecules was observed at 1723 cm^{-1} and the amide vibration in hydrogels was observed at 1689 cm^{-1}. This shift of 34 cm^{-1} to a lower frequency of the amide vibration is typically witnessed at H-bonding between the guanosine molecules in the hydrogel.[2] In the benzene-1,4-dibronic acid spectrum, two vibrations corresponding to the boronic group were found: $\delta_{B\text{-}OH}$ vibration (1169 cm^{-1}) and $\upsilon_{B\text{-}O}$ vibration (1339 cm^{-1}),[2,3] while the hydrogels' FTIR spectra showed a $\upsilon_{B\text{-}OC}$ vibration at 1090 cm^{-1} instead of a $\delta_{B\text{-}OH}$ vibration at 1169 cm^{-1}, confirming that boronate esters formation.[4,5] In the 1080 to 936 cm^{-1} area, both dextran and hydrogels displayed polysaccharide absorption bands produced by vibrational modes such as C-C and C-O stretching and C-H bond bending. The bands in the 936-735 cm^{-1} area correspond to the deformation modes of the glucose ring.[6]

4. Procedure for powder X-Ray diffraction

X-ray diffraction measurements were investigated on a Rigaku Miniflex 600 diffractometer using CuKα-emission in the angular range 2–50º (2θ) with a scanning step of 0.01º and a recording rate of 1 º/min. DGH1-3 samples were frozen and dried to form a white powder. The X-Ray data were processed using OriginPro 8 software.

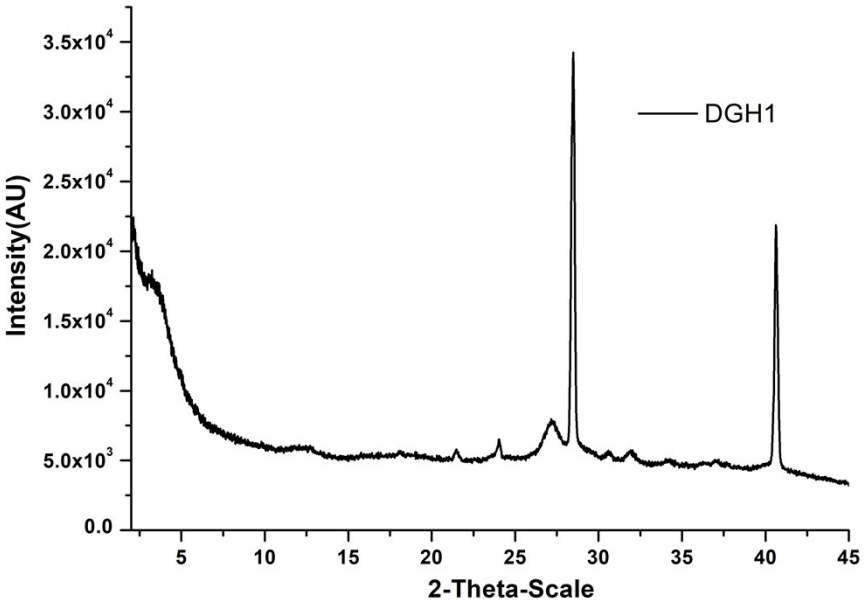

Figure S3. Representative powder X-ray diffraction pattern for DGH1 xerogel; sharp peaks at 28.4° and 40.4° corresponding to the KCl.[7]

5. Scanning Electron Microscopy (SEM)

The Scanning Electron Microscope (FEI NanoSEM 430, FEI Company, Hillsboro, USA, landing E: 10.0 keV) was used to investigate the morphology of samples. Hydrogels DGH1-3 (2 mL) were lyophilized prior to SEM measurements, the xerogel samples were carefully placed onto 25 aluminium plate with double-sided adhesive and investigated at an accelerating voltage of 20 kV.

6. Atomic Force Microscopy (AFM)

The Ntegra Spectra Atomic Force Microscope was used for the AFM examination (NT-MDT Spectrum Instruments, Zelenograd, Moscow, Russia). Silicon cantilever tips (NSG 10, NT-MDT Spectrum Instruments, Zelenograd, Moscow, Russia) with gold reflecting coating were utilized, with a resonance frequency of 140-390 kHz, a force constant of 3.1-37.6 N m^{-1}, and a tip curvature radius of 10 nm. Before scanning, aliquots of 10 uL gel solution were equally applied onto freshly cleaved mica substrates and dried in air at room temperature. Gwyddion 2.59 software[8] was used to analyze AFM images and to calculate the corresponding mean widths.

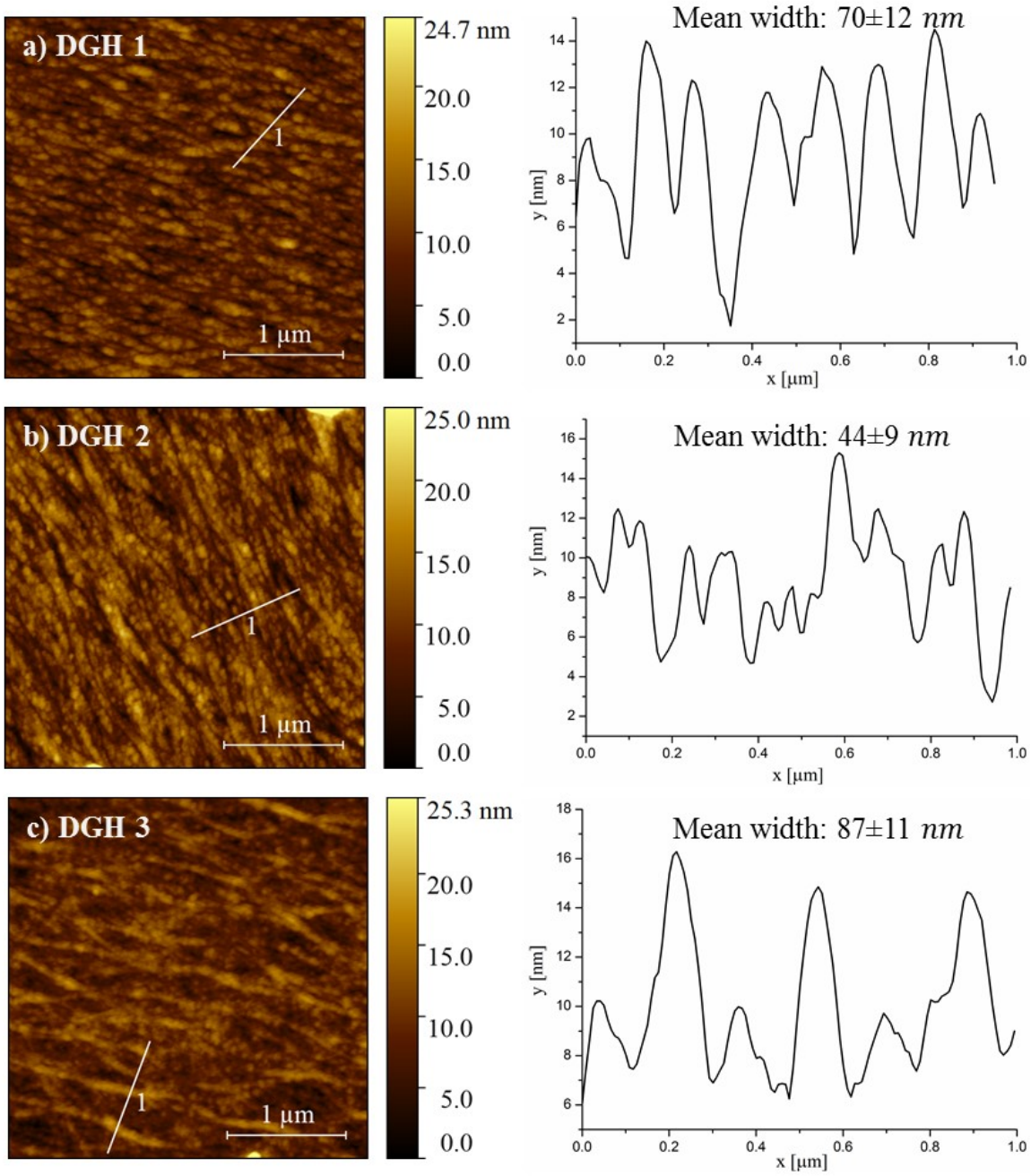

Figure S4. AFM images and corresponding cross section profiles (lines traced in the profile measured positions) with calculated mean width of fibrils for: (a) DGH1, (b) DGH2 and (c) DGH3.

7. Rheological Investigations

The rheological measurements were carried out at 25 °C using a MCR302 Anton-Paar rheometer (Graz, Austria) equipped with Peltier device for a rigorous control of the temperature. Plane-plane

geometry with the diameter of the upper plate of 25 mm was used and, the solvent evaporation was limited by using a solvent trap cover (Malvern Instruments Ltd., Worcestershire, UK). The linear range of viscoelasticity (LVR) of the investigated samples was determined by stress sweep experiment at a constant oscillation frequency (ω) of 10 rad·s^{-1}, in the shear stress (τ) range of 10^{-3} Pa - 10^{2} Pa (corresponding to the deformation (γ) between 10^{-2} % and 10^{3} %). Rotational measurements were carried out at shear rates ($\dot{\gamma}$) from 10^{-3} s^{-1} to 2 x 10^{2} s^{-1} to establish the flow behavior and zero shear viscosity (η_0) of the samples. Frequency sweep tests were performed between 10^{-1} rad·s^{-1} and 10^{2} rad·s^{-1}, setting a constant shear stress of 1 Pa from LVR, to determine the storage (G') and loss (G") moduli of the samples. The thixotropic behavior and structure recovery after deformation were evaluated by a steps test with three intervals of time, oscillation-rotation-oscillation (O-R-O). In the first and the third interval were performed oscillatory tests at ω = 10 rad·s^{-1} and γ = 1% (from LVR) for 60 s and 145 s, respectively. In the second interval, a high shear of 100 s^{-1} was applied for 15 s. The rheological measurements were performed in duplicate using refresh sample for each measurement and, the relative standard errors were lower than 15%.

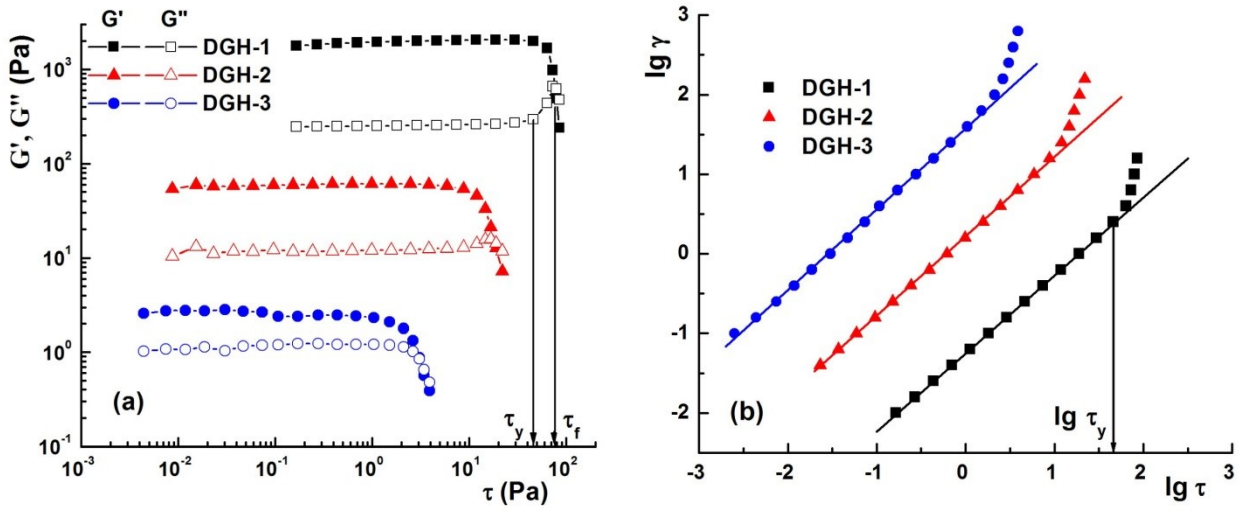

Figure S5. (a) Storage (G') and loss (G") moduli as a function of τ and (b) γ dependence of τ for the investigated samples at 10 rad·s^{-1} and 25°C.

Table S1. Rheological parameters of the studied samples

Sample	G'[a] (Pa)	G''[a] (Pa)	tan δ[α]	τ_y[b] (Pa)	τ_f[b] (Pa)	η_0[c] (Pa·s)	Structure recovery[d] (%)
DGH1	1990	252.5	0.13	45.9	76	15406.8	100
DGH2	61	11.9	0.20	8.7	17.9	5.4	100
DGH3	2.8	1.1	0.39	1.5	3.1	0.7	42

[a] values from LVR determined by amplitude sweep test at ω of 10 rad·s^{-1} and γ of 1%;
[b] τ_y established by one tangent method in dependence of lg τ on lg γ; τ_f is the point where G'= G'' in τ dependence of viscoelastic moduli;
[c] estimated with the simplified Carreau equation;[9]
[d] determined at the end of the O-R-O test from the values of G'.

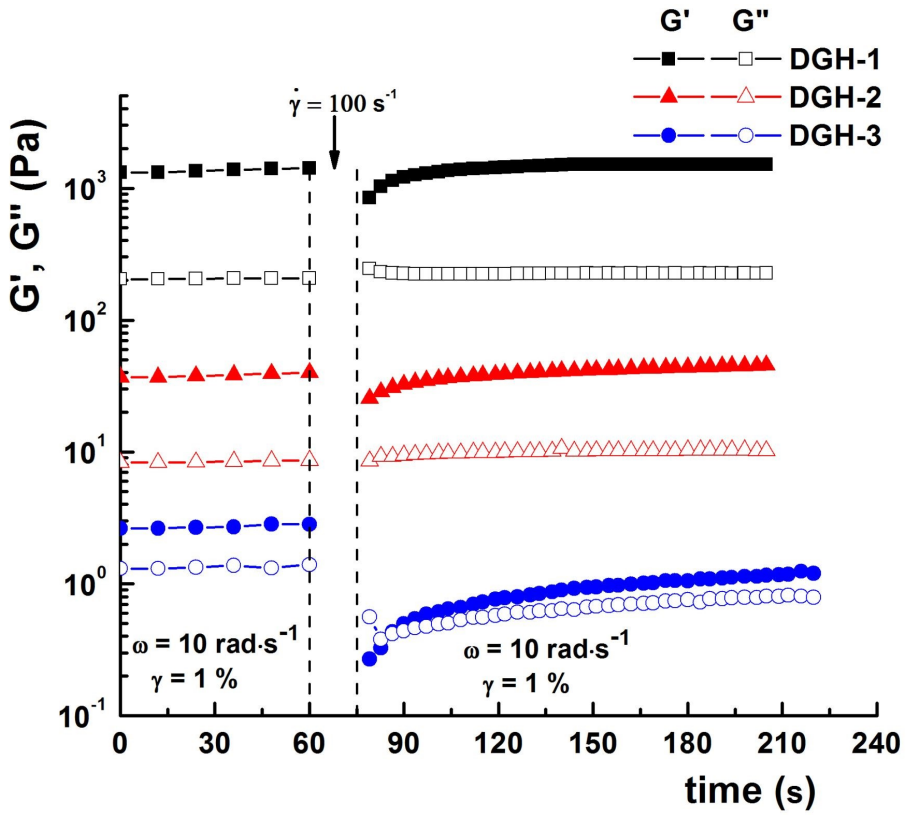

Figure S6. Structure recovery ability determined by O-R-O test with three intervals of time (1%, 10 rad·s^{-1} - 100 s^{-1} - 1%, 10 rad·s^{-1}) of the studied samples at 25 °C.

8. Cytotoxicity assay

Human Gingival Fibroblast (hGF) cell (from) were cultivated in tissue culture flasks with alpha-MEM medium (Lonza, Basel, Switzerland) supplemented with 10% fetal bovine serum (FBS, Biochrom GmbH, Germany) and a 1% penicillin-streptomycin amphotericin B mixture (10 K/10 K/25 µg in 100 mL, Lonza). The medium was changed with a fresh one, once in every 2 or 3 days. Once confluence was reached, the cells were washed with phosphate buffered saline (PBS, Invitrogen, Paisley, Scotland, UK), detached with a 1× Trypsin EDTA mixture (Lonza) followed by the addition of complete growth medium, centrifuged at 200× g for 3 min and subcultured into new tissue culture flasks.

The possible cytotoxic effects of the obtained hydrogels based on dextran cross-linked with different amounts of guanosine (100% guanosine (DGH1), 50% guanosine (DGH2), and respectively 30% guanosine (DGH3)) was assessed using MTS and Live/Dead assays. The hydrogels were synthesized at a temperature of 90 ºC, thereby obtaining transparent solutions that gels at room temperature. In order to obtain hydrogels with the same size and weight for in vitro tests, 50 µL of sample in solution state were transferred in 1 mL syringes after cutting off their tips. Resulting in the fabrication of hydrogels in the shape of tiny tablets.

MTS was performed using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA) following the manufacturer's protocol. hGF cells were seeded at a density of 1x105 cells/well in 24 well plates in complete medium (alpha-MEM medium (Lonza) supplemented with 10% fetal bovine serum (FBS) and a 1% penicillin-streptomycin-amphotericin B mixture (10 K/10 K/25 µg in 100 mL, Lonza)). After 24 h, with the help of syringes, the obtained hydrogels were placed in the 24 well plate, and left for incubation for 48 h. Next, 20 µL of CellTiter 96® Aqueous One Solution reagent were added to each well, and the plates were incubated for 2 h. After incubation, 100 µL of medium of each well was transferred to a 96-well plate and absorbance at 490 nm was recorded by a microplate reader (BMG LABTECH) (Ortenberg, Germany). A blank absorbance value from wells without cells but treated with MTS and with or without the inclusion complex was subtracted from the corresponding absorbance values. Cell viability was calculated and expressed as a percentage relative to the viability of untreated cells which served as the negative control for the inclusion complex. Experiments were performed in three replicates and repeated three times. Data are presented as mean ± S.D.

Living and dead cells around and under the tested hydrogels were distinguished using fluorescence microscopy according to the procedure provided by LIVE/DEAD Cell Imaging Kit (488/570, Thermo

Fisher). The final concentration of the calcein AM and the EthD-1 reagents was 1 μM. The stained cells were observed under an inverted microscope Leica DMI 3000 B equipped with GFP and N2.1 filters.

Notes and references

1 E.-L. Ursu, G. Gavril, S. Morariu, M. Pinteala, M. Barboiu and A. Rotaru, *Mater Sci Eng C Mater Biol Appl*, 2020, **111**, 110800.

2 J. Ye, L. Zhu, M. Yan, T. Xiao, L. Fan, Y. Xue, J. Huang and X. Yang, *Talanta*, 2021, **230**, 122351.

3 G. Srinivas, J. W. Burress, J. Ford and T. Yildirim, *J. Mater. Chem.*, 2011, **21**, 11323–11329.

4 Y. Li, Y. Liu, R. Ma, Y. Xu, Y. Zhang, B. Li, Y. An and L. Shi, *ACS Appl. Mater. Interfaces*, 2017, **9**, 13056–13067.

5 C. Arnal-Hérault, A. Pasc, M. Michau, D. Cot, E. Petit and M. Barboiu, *Angewandte Chemie International Edition*, 2007, **46**, 8409–8413.

6 S. S. Rohiwal, Z. Ellederova, A. P. Tiwari, M. Alqarni, S. T. Elazab, G. E.-S. Batiha, S. H. Pawar and N. D. Thorat, *RSC Adv.*, 2021, **11**, 4308–4317.

7 Y. Li, Y. Liu, R. Ma, Y. Xu, Y. Zhang, B. Li, Y. An and L. Shi, *ACS Appl. Mater. Interfaces*, 2017, **9**, 13056–13067.

8 Gwyddion 2.59, Available online: http://gwyddion.net/.

9 P.J. Carreau, *J. Rheol*, 1972, **16**, 99-127.