SUPPORTING INFORMATION

HYBRID FLUORESCEIN GEL.

Experiments with HRGD6 fluorescent polymers were carried out in order to verify the presence of the ELRs in the hybrid gels along time.

A solution of fibrinogen 10 mg/mL and 10mg/mL of HRGD6-fluorescein (60% substitution, only 40% of the lysine remains free) in TBS was prepared. A solution of 30% of thrombin and 30% of $CaCl_2$ in TBS was prepared.

0.5 mL of each solution were placed in 1 mL separate syringes, these syringes were fixed to a double syringe holder with a mixer tip.

Three gels were prepared in a 24 well-plate (0,3 mL per gel). The gels were left to react for 45 minutes. The final polymer concentration was 10 mg/mL.

Two gels of fibrin (10 mg/mL) were prepared as control.

The absorption of the HRGD6-fluorescein as solution was measured at 494 nm. A calibration line with HRGD6-fluorescein was prepared as shown in fig 1

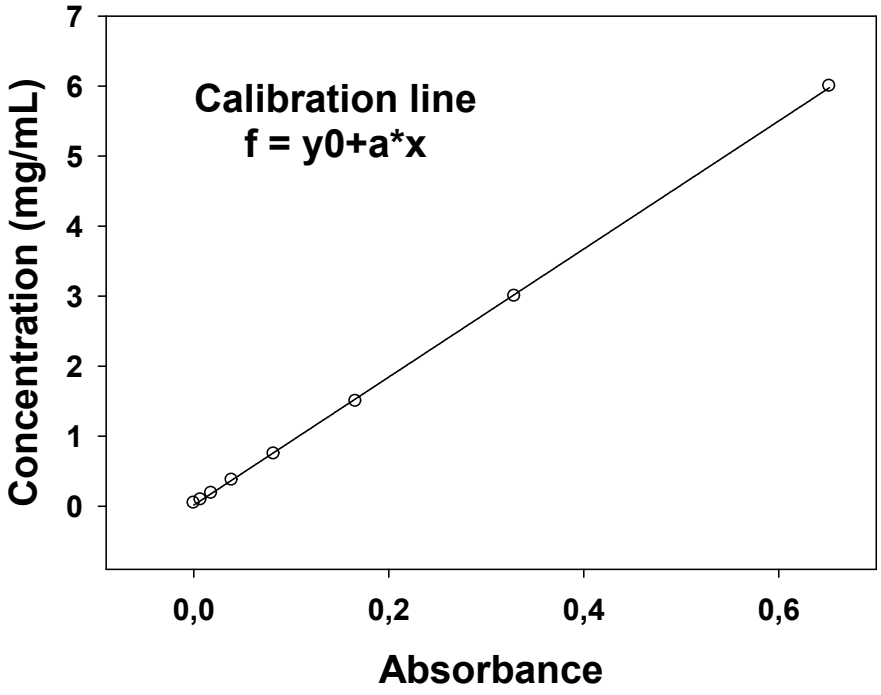


Fig 1. Calibration curve for eluting experiments.

Parameters of the linear fitting of the curve in fig 1 (Sigmaplot x11 software)

Data Source: Data 1 in Notebook1
Equation: Standard Curves; Linear Curve
f = y0+a*x

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0,9999	0,9999	0,9999	0,0248

	Coefficient	Std. Error	t	P
y0	0,0165	0,0110	1,4979	0,1848
a	9,1425	0,0412	221,7604	<0,0001

All gels were washed with TBS (1 mL) for 24 h and the absorbance of the wash solution was measure (the absorbance of the wash solution of the fibrin gels was taken as background signal). Then, the gels were washed with more TBS (1 mL) for another 24 h and the absorbance was measured. The washing process was repeated once more for another 48 h and the absorbance was measured. No HRGD6-fluorescein in the liquid could be detected after the last washing step.

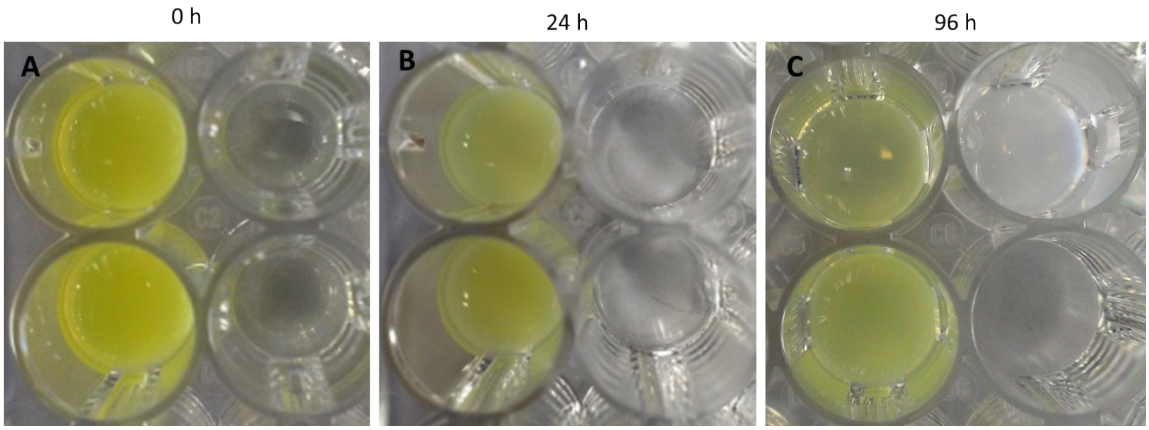
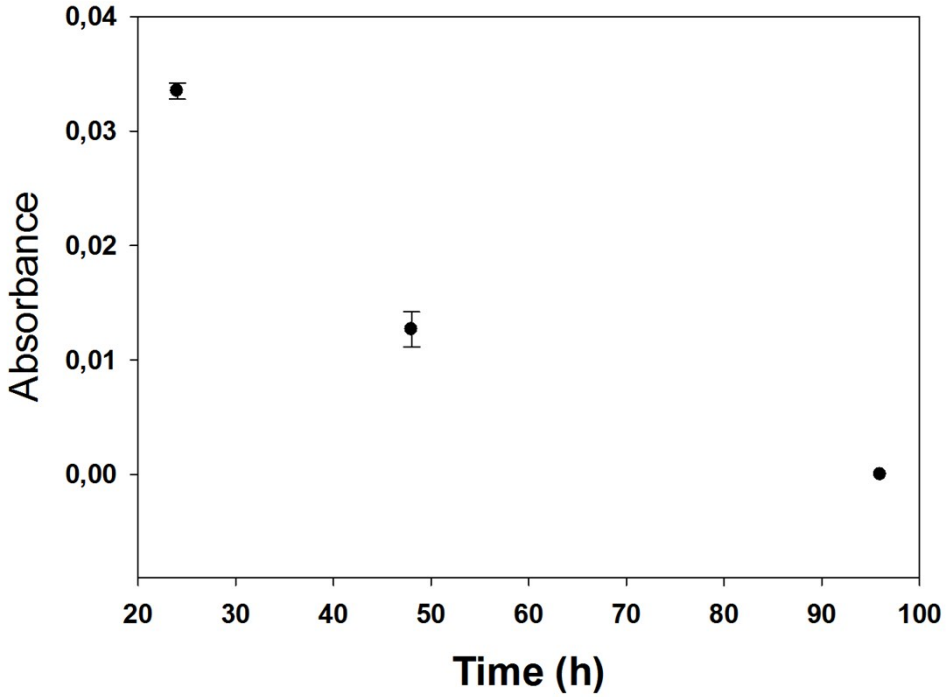


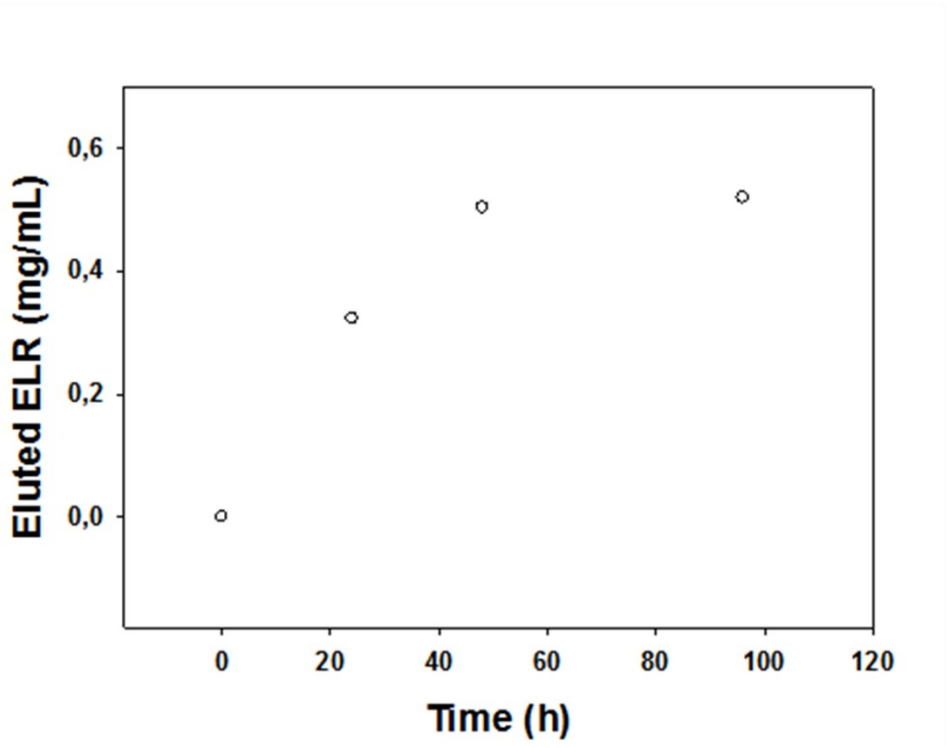
Fig 2. General appearance of the fluorescent hybrid gels after 24 and 96h.

Next, quantitative results of the absorbance, eluted and remaining ELRs as a function of time are shown in fig 3

A)


Absorbance
0,04
0,03
0,02
0,01
0,00
20
30
40
50
60
70
80
90
100
Time (h)

B)


Eluted ELR (mg/mL)
0,6
0,4
0,2
0,0
0
20
40
60
80
100
120
Time (h)

C)

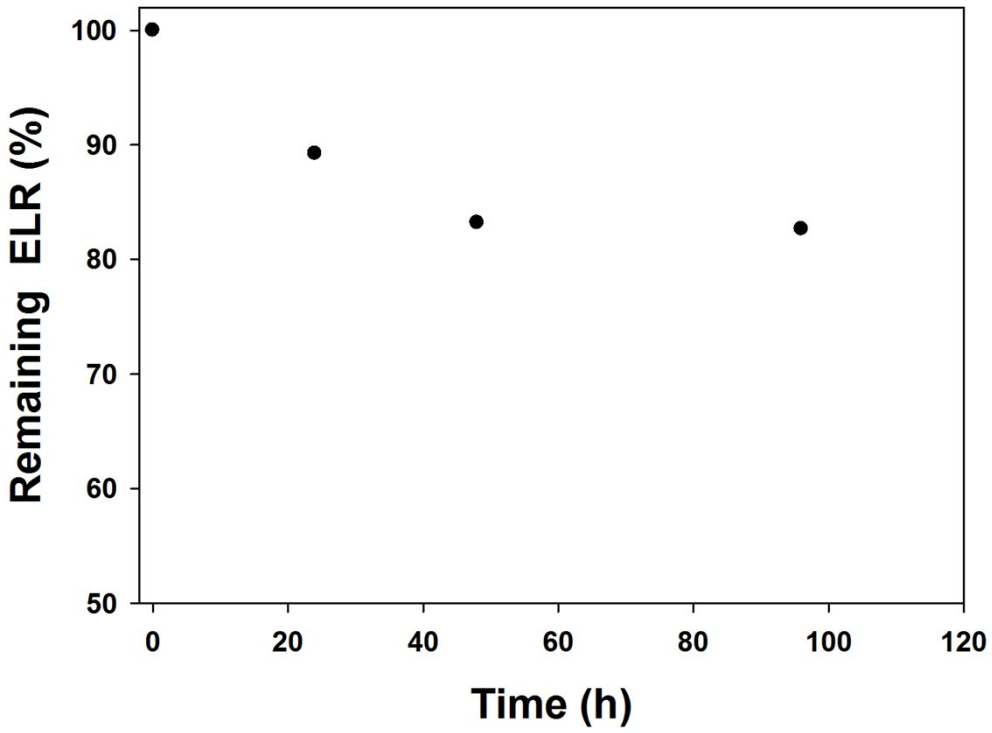


Figure 3, Absorbance (A), eluted (B) and remaining amounts (C) of ELRs in hybrid gels after the three washing points (24, 48 and 96 h).

Therefore, more than 80% of the ELR-fluorescein remains into the gels after washing the samples at time= 96 h.

CELL VIABILITY IN HYBRID GELS.

To assess the cell viability, live and dead test and fluorescent staining (Phalloidin/DAPI) were carried out. For that, 5000 HHF-1 cells (SCRC-1041, form ATCC, USA) were embedded in 50 µL of hybrid gel (10mg/mL) and cultured for five days. 6 different gels of each type (fibrin and hybrid) were prepared for each time point, four of them were used for the live/dead test and other 2 for the Phalloidin/DAPI staining. Live/Dead staining was performed according to the manufacturer's instruction (Molecular Probes™) and fluorescence intensity was measured at 530 nm and 645 nm (Tecan Infinite 200 micro plate reader). The fluorescence intensity at 530 nm was converted into cell number by using calibration curves obtained with known-numbers of HFF-1 cells seeded on 96 well-gelatin-precoated plate 24 hours before the measurement (1.000 to 15.000 cells/well in 100 µL in the medium used for HFF-1 cells culture). The results showed a good proliferation with a very similar values for fibrin and hybrid gels. So, it is easy to conclude that the cell viability in the hybrid gels is as high as in the fibrin controls

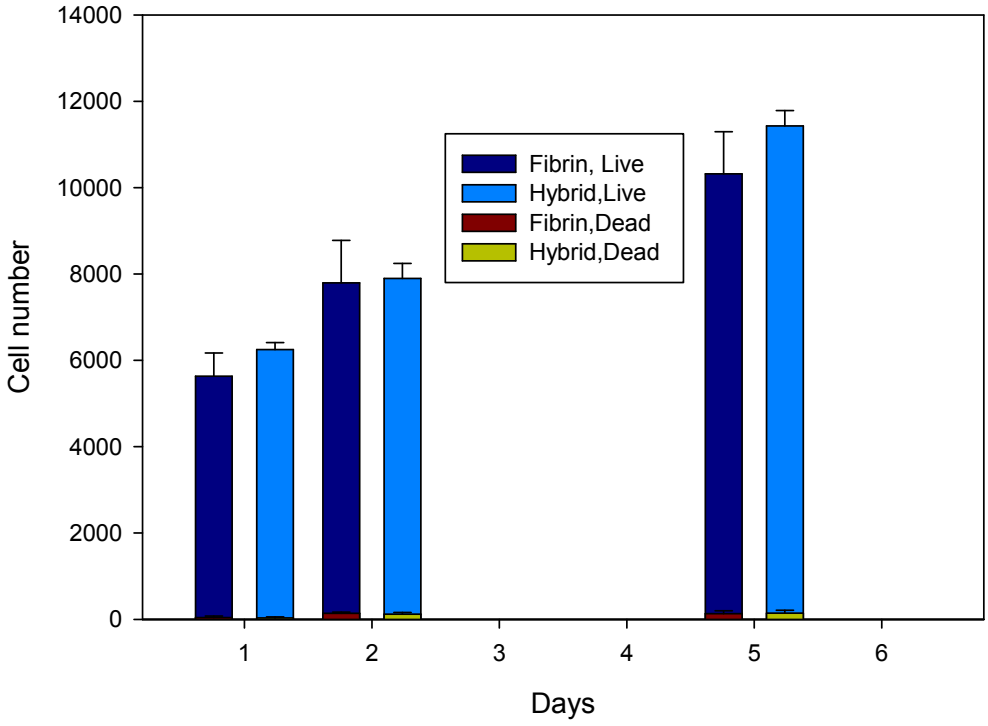


Fig 4 Comparison of cell proliferation in fibrin and hybrid gels along time.

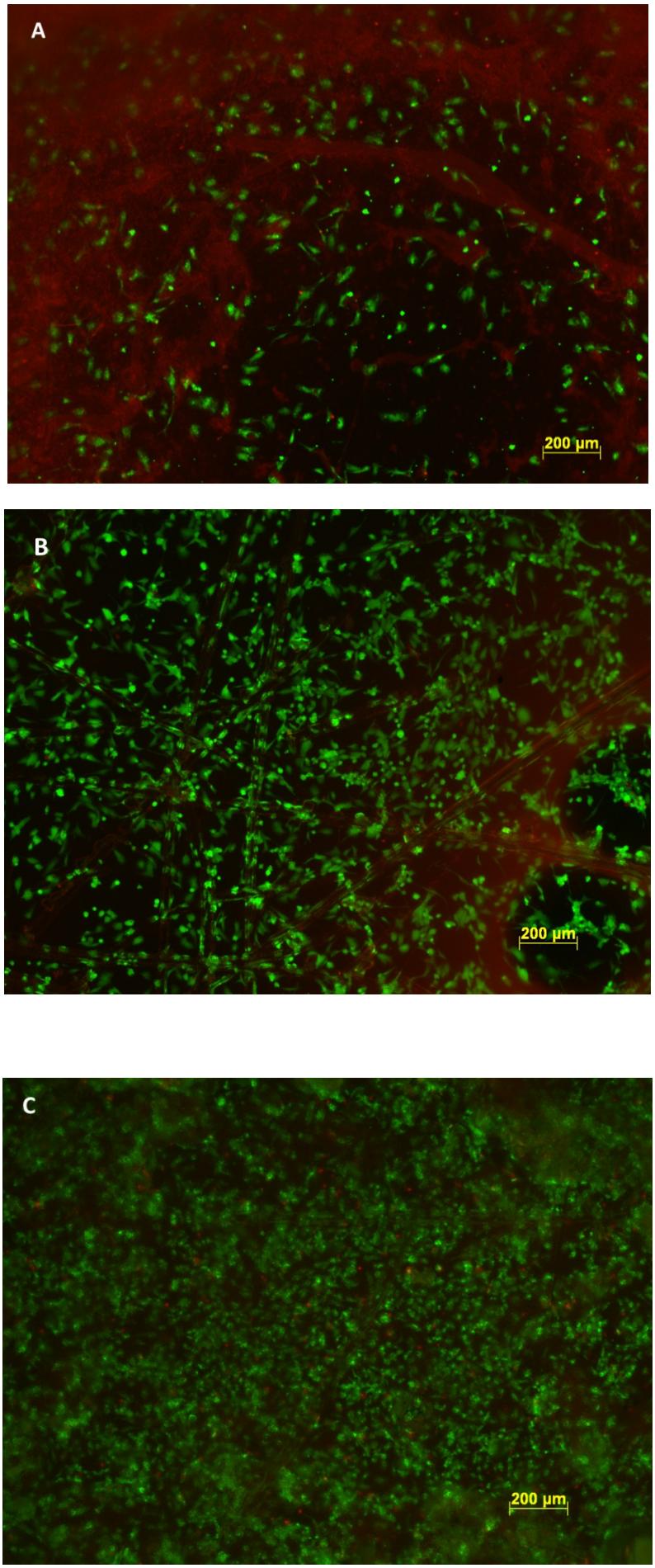


Fig. 5. Live and dead staining in the hybrid gels after 1 day (A), 2 days (B) and 5 days (C)

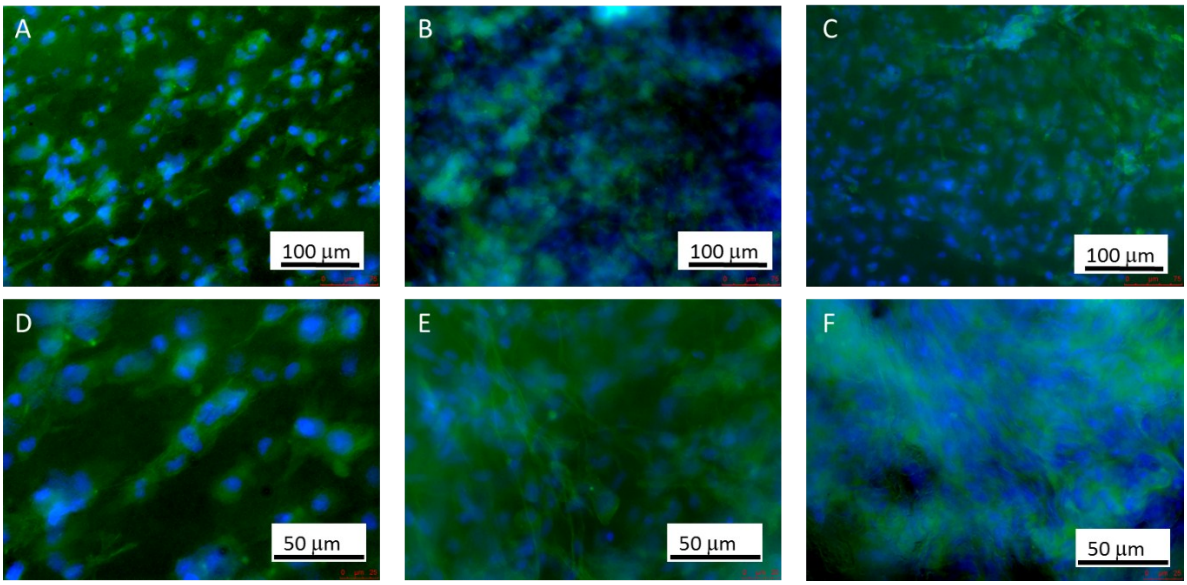


Fig 6. Phalloidin/DAPI staining of the HFF-1 cells inside the hybrid gels at different magnifications after 1 day (A, D), 2 days (B, E) and 5 days (C, F).