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Supporting Information

Pseudo-Double Network Hydrogels with Unique Properties as Supports for Cell Manipulation

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Network characterization

Regarding the network formation, after the polymerization reaction the material consists of these three major elements (in addition to water): residual monomers (methacrylate M and/or vinylpyrrolidone V, both water soluble), non-crosslinked polymeric chains (sol content, also water soluble) and crosslinked polymeric chains (gel content, non-soluble). A gravimetric study was carried out to determine the gel content (weight percentage, see **Table S1**). The initial weights of the monomers before polymerization were compared to the weight of the dry polymerized network after exhaustive extraction of soluble materials with water over 48 H. All of the samples retained approximately 90 weight % of the initial feed weights, which means that most of the monomeric precursors were incorporated as units into the final polymeric networks. Furthermore, FTIR-ATR qualitative analysis further confirmed that both units are properly incorporated to the network, as shown in **Fig. S1**. The spectra of the homopolymers poly-V and poly-M have been included in the graph for comparative purposes. There are several intense and isolated peaks representative of each unit, as observed in the spectra of the homopolymers. In the case of poly-V the spectrum shows the stretching of C=O at 1664 cm^{-1}, the bending vibrations of cyclic CH_2 groups at 1492/1459/1419/1371 cm^{-1} and a C-N stretch at 1282/1267 cm^{-1}. For poly-M, the

spectrum shows the C=O stretch at 1730 cm^{-1}, a very intense peak assigned to the sulfonate stretch at 1155 cm^{-1}, and the C-O stretch at 1065 cm^{-1}. All these fingerprint peaks are in the spectra of the 'pseudo'-double networks evaluated in this study, with intensities correlative to the composition i.e. the peaks characteristic of the V units are more intense in the M/V 1/6 material than in the 1/2. In conclusion, these analyses show that both units are present in the final material.

Table S1 Gel content of the four samples evaluated in this work.

sample	Gel content (weight %)
Type 1	88.3
Type 2	89.1
Type 3	92.0
Type 4	86.0

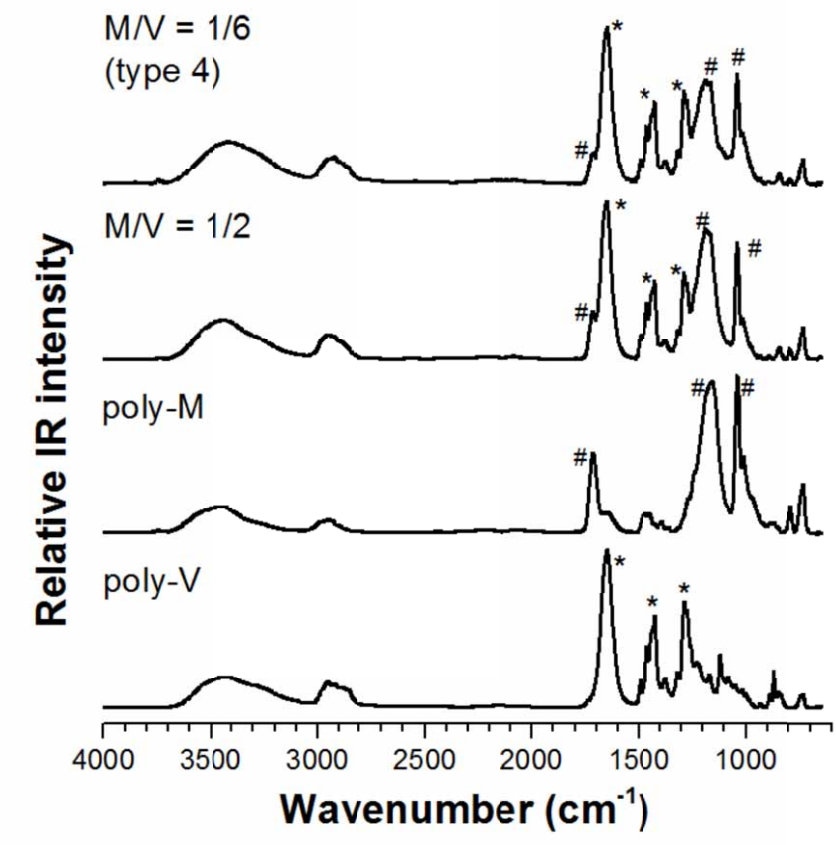

Fig.S1 FTIR-ATR spectra of poly-V, poly-M and two networks with M/V of 1/2 and 1/6. The most representative peaks assigned to the V and M units have been labeled as * and # respectively.

A quantitative determination of the composition of the hydrogels has been obtained indirectly by the ^{1}H-NMR analysis of the extractable soluble components. Fresh samples (freshly polymerized) were extracted with an excess of deuterated water (D_2O) for 48 H, and an aliquot of this solution was directly analyzed by ^{1}H-NMR spectroscopy. **Fig. S2** shows a representative spectrum of sample type 4. This analysis has shown that the extractable components are V-containing compounds, that is, residual V monomer or poly-V soluble chains. Most (or all) of the extractable content is actually poly-V sol content, as can be observed in Fig. S2 for sample type 4, which is in agreement with high monomer conversion in the polymerization. Thus, all of the anionic methacrylates have therefore been incorporated into the network. This is in agreement with previous studies, (ref. 22 of the manuscript), and with the theoretical prediction of the reaction (**Fig. 1** of the manuscript), which shows that the methacrylates (M and its homologous and more populated crosslinker C1) are exhausted in the middle stages of the reaction and that poly-V chains, very slightly crosslinked with the homologous crosslinker C2, are formed towards the end of the polymerization reaction. From this NMR analysis, a mass balance has been carried out to correct the nominal M//V molar ratio to the actual value (considering this loss of V). The corrected M/V molar ratios, which are close to 1/5, are quoted in Table 1, and have been determined according to:

$$\frac{M}{V} = \frac{1}{\frac{111x6-(111x6+230)\left(\frac{1-GC}{100}\right)}{111}} \qquad \text{Eq. S1}$$

Where the M/V feed molar ratio is 1/6, GC is the gel content (weight percentage, see Table S1), and the molecular weights of V and M are 111 g/mol and 230 g/mol.

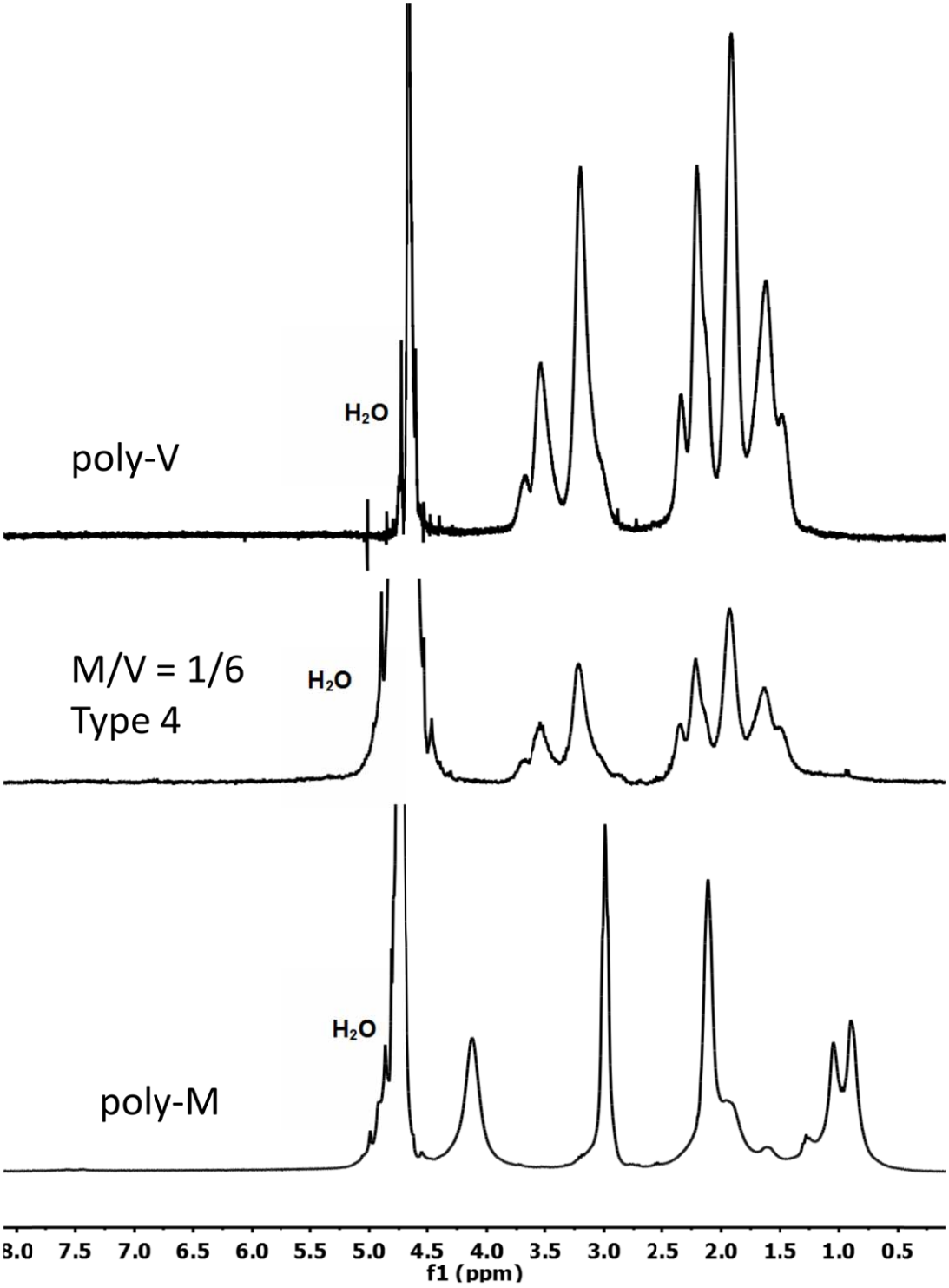

Fig. S2 ^{1}H-NMR spectrum of the extractable components in D_2O (signal of 4.79 assigned to H_2O) for sample type 4, central spectrum. Spectra of pure poly-*V* and poly-*M* are included for comparative purposes, top and bottom spectra respectively.

Mechanical properties of the networks.

Due to the sample characteristics, the stress-strain analysis was carried out in a tensile mode for the photoinitiated samples and in a compressive mode for the thermoinitiated samples. Fig. S3 shows the fracture stress under compression of networks with 1/4 and 1/6 M/V ratio compared to a V-based single network (type SN_{V1}).

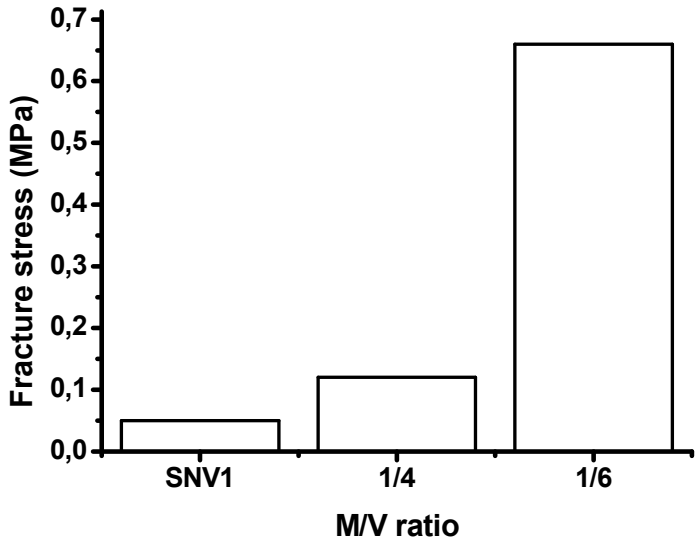

Fig. S3 Fracture stress of control SNV1 and hydrogels with 1/4 and 1/6 M/V ratio.

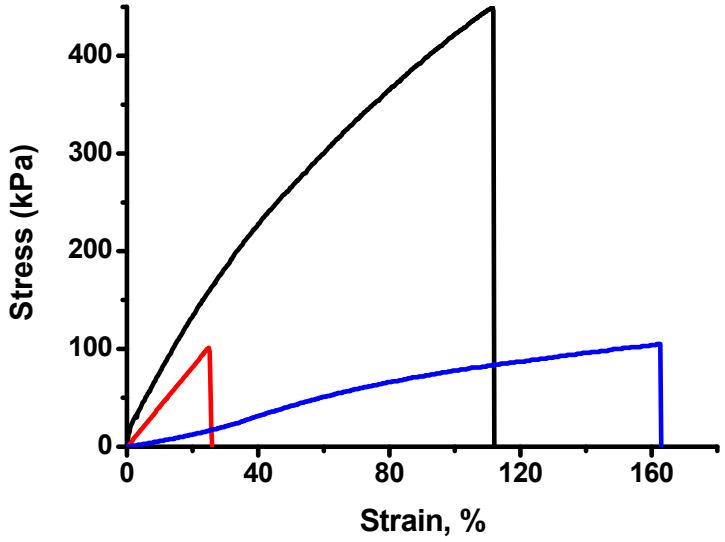

Fig. S4 Tensile stress-strain curve for the samples type 3 (line red), sample type 4 (black line) and reference sample SN_{V2} (blue line).

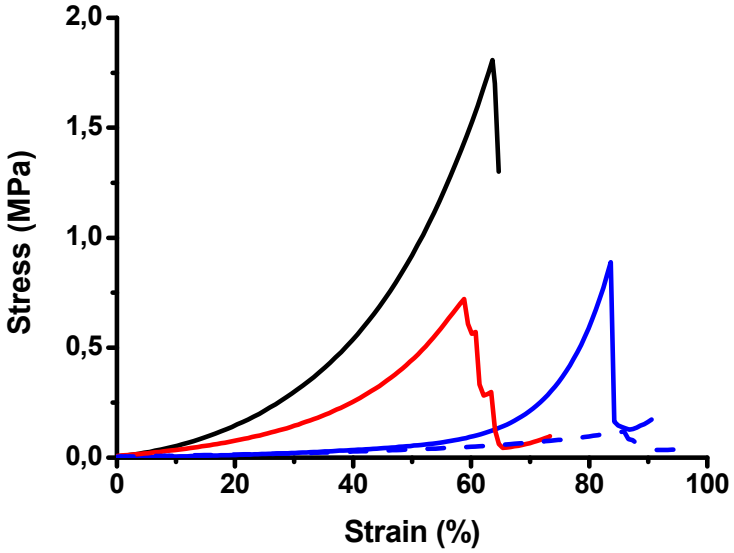

Fig.S5 Compressive stress-strain curve for the samples type 1 (red line), type 2 (black line), and the two V-based single networks SN_{V1} (dashed blue line) and SN_{V2} (solid blue line). The SN_{V2} sample used for this study was obtained by thermoinitiation.

Video S1 Video displaying the easy handling of the prepared pseudo DNs (see separate supplementary movie file)

Cell Proliferation on all pseudo DN hydrogel types

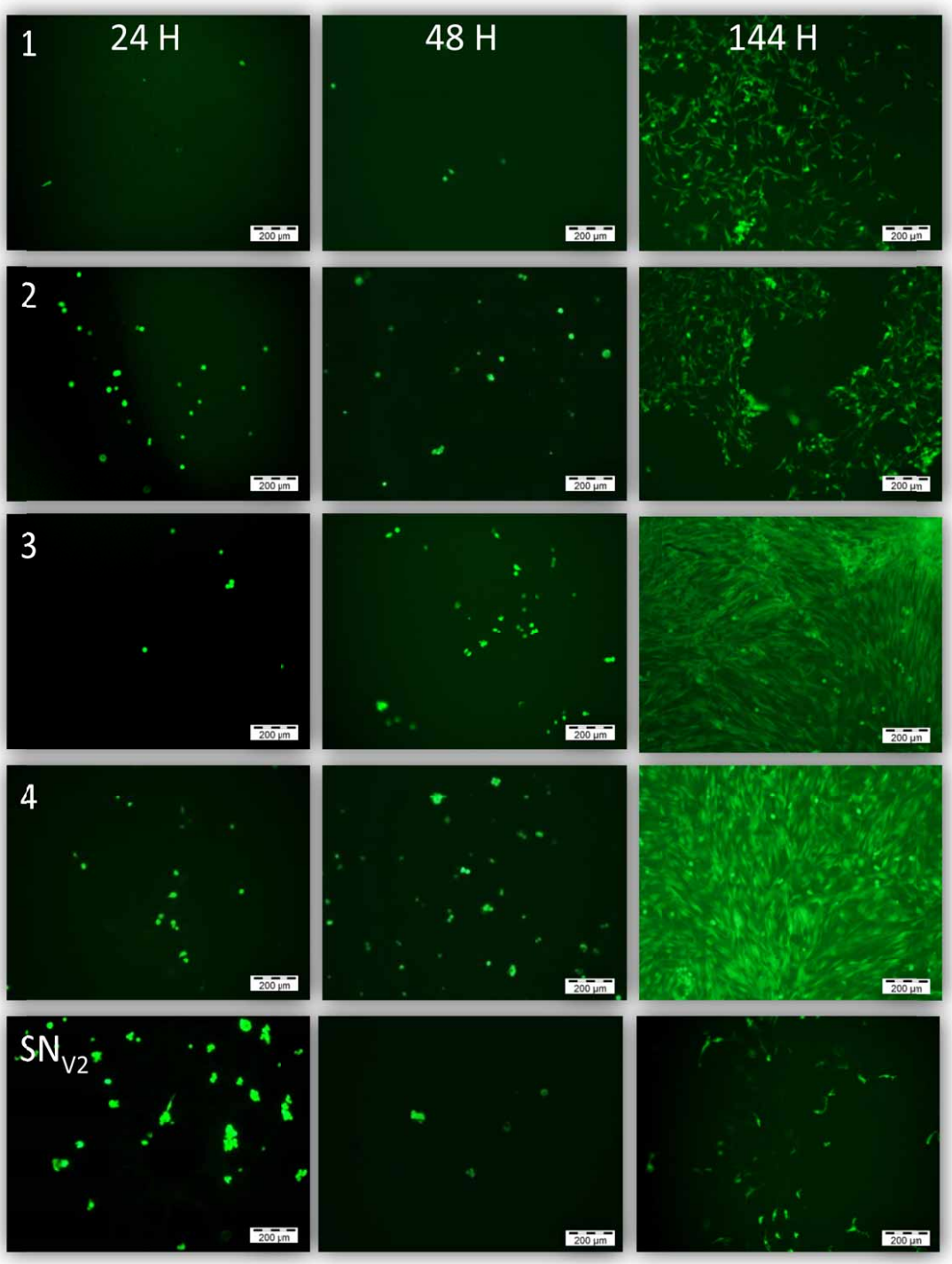

Fig. S6 Cell proliferation on all hydrogel types 24, 48 and 144 H after initial seeding. Initially cells resisted adhering/proliferating as evidenced in the micrographs on the left and the middle. After this cells proliferated until confluent on hydrogels types 4 and 5 and to a lesser extent on types 1 and 2. Comparably, very little proliferation was observed on the single network V based hydrogel SN_{V2}. Scale bar denotes a distance of 200 µm.

Flux-based cell detachment

Applying a controlled flux of medium over the hydrogel, cell detachment was induced from hydrogel type 4 (the best hydrogel in terms of ability to host cells to confluence). The hydrogel was seeded with C2C12 cells and incubated as normal over 6 days after which the cell layer reached confluence. The confluent cells and hydrogels were placed inside the sterile methacrylate chamber. Using an inflow and a syringe pump, a controlled flux of medium replenished the medium, while the outflow port ensured that the volume of medium remained approximately constant at all times, Fig. S7, A and B. In this way, the disruption to the cell layer was controlled. The outflow collection allowed for cells to be collected after detachment. Flux flows of less than 10 ml/min yielded no cells. Flux flows of 10, 12.5 and 15 ml/min yielded small amounts of cells. A flux of 20 ml/min was the minimum flux needed to detach a confluent cell sheet. The harvested cells were reseeded on TCP to assess their ability to proliferate as compared to cells detached through conventional means. Cells attached and proliferated to confluence and their assumed morphology was typical of this type of adherent dependent cell line, Fig. S7, C and C'. Additionally, Trypan blue viability assays confirmed the harvested cells retained viability in excess of 95 % on each occasion.

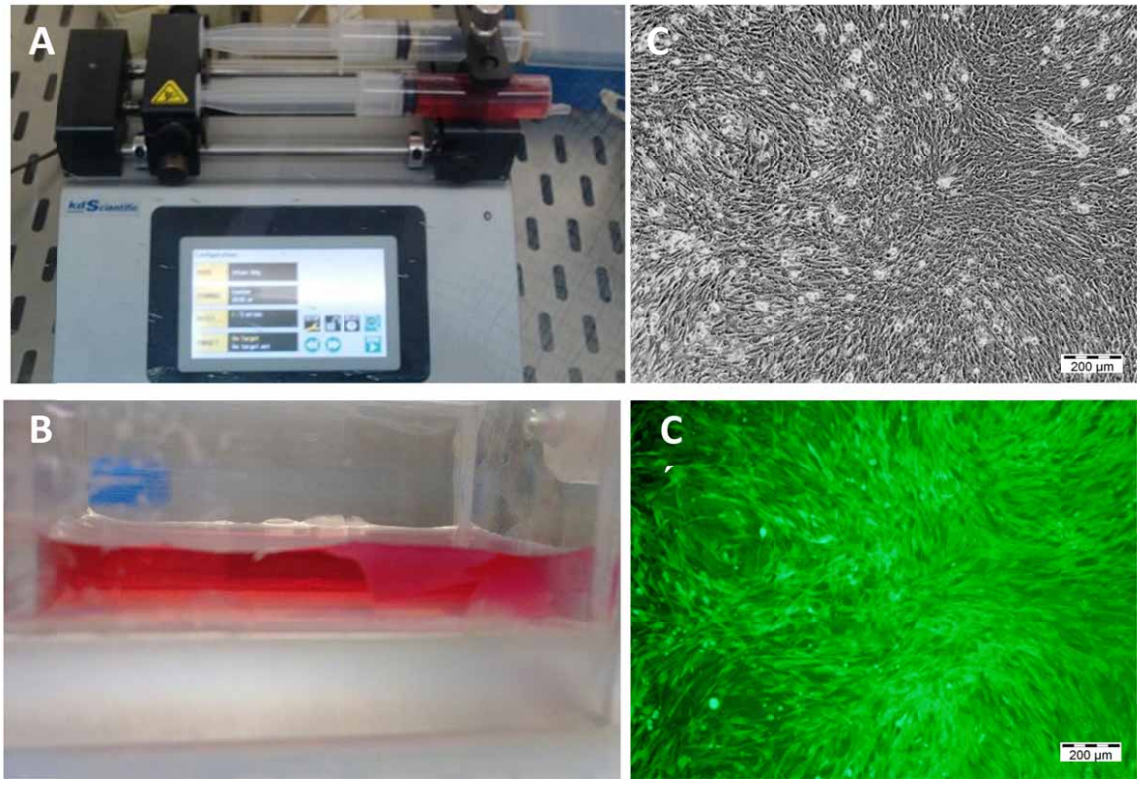

Fig. S7 A. Syringe pump. B. Methacrylate chamber which houses a section of a pseudo-DN on which a confluent layer of C2C12 GFP cells have been cultured. They were used to facilitate the forced detachment of a cell sheet by the controlled application of a cell media flow which was passed over the grown cells. After the cells had been detached via mechanical agitation the cells were reseeded on TCP and incubated as normal to assess subsequent cell behavior, growth and morphology. Brightfield (C) and fluorescent (C') images of reseeded cells on TCP after 144 H incubation, post mechanically stimulated cell detachment.

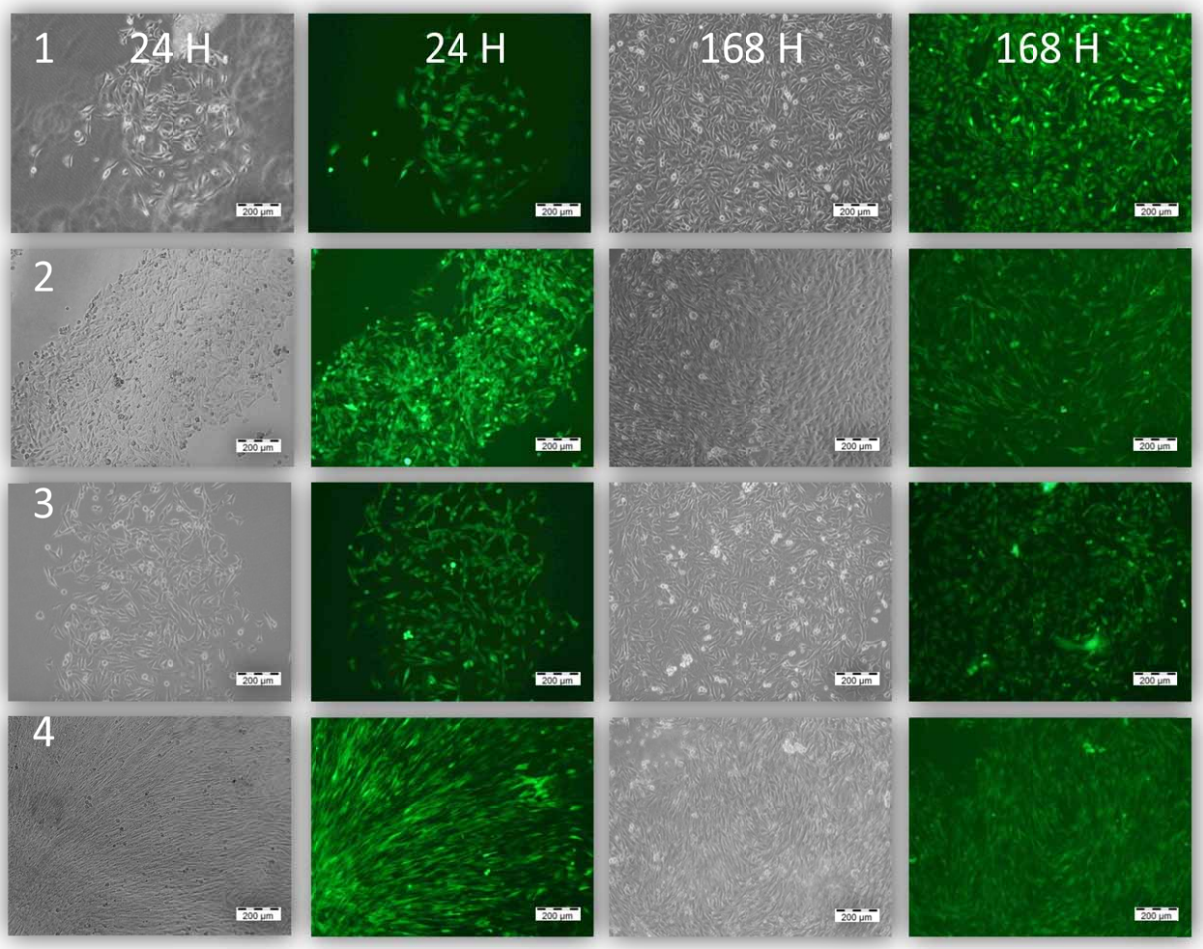

Fig. S8 Cell proliferation post transplantation. Cell transplantation to TCP was successful in the cases of all 4 pseudo-DNs (hydrogels types 1 to 4 seen from the top downwards), but due to better proliferation on the hydrogels types 3 and 4, more cells were naturally transplanted in these cases. In all cases, cell proliferated on TCP and the morphology assumed was typical of this type of cell line. The 2 columns of images to the left show cell attachment and proliferation on TCP after 24 H, the left most

column shows the brightfield view and the 2nd column the fluorescent equivalent. The right 2 columns show proliferation of the same transplants after 168 H. Again, the brightfield images and fluorescent equivalents are shown. Scale bar 200 μm.