Macroporous Aligned Hydrogel Microstrands for 3D Cell Guidance

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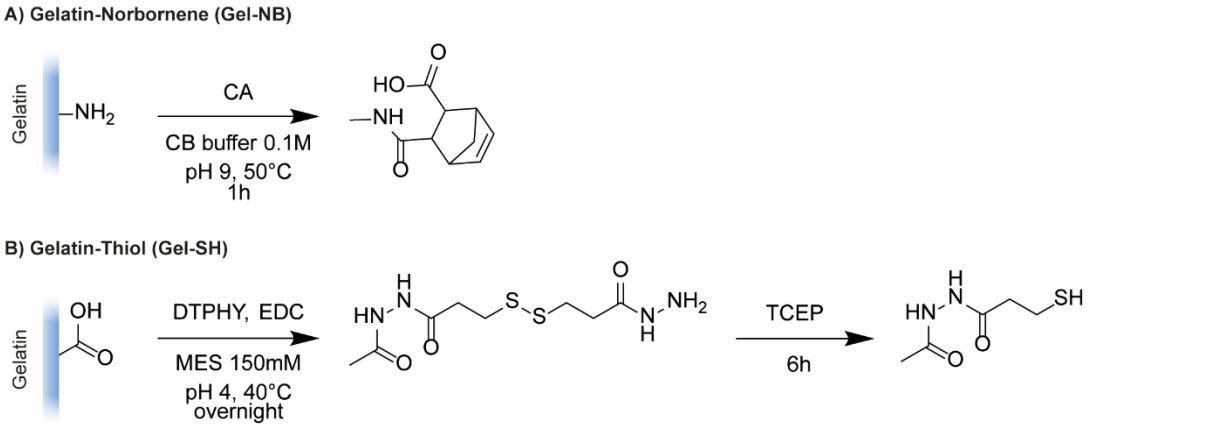

Figure S1. Synthesis of A) Gel-NB and B) Gel-SH. Abbreviations: CA: cis-5-norbornene-endo-2,3-dicarboxylic anhydride, CB: carbonate-bicarbonate, DTPHY: 3,3'-dithiobis(proprionohydrazide), EDC: 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide

hydrochloride, MES: 2-(N-morpholino)ethanesulfonic acid , TCEP: Tris(2-carboxyethyl)phosphine.

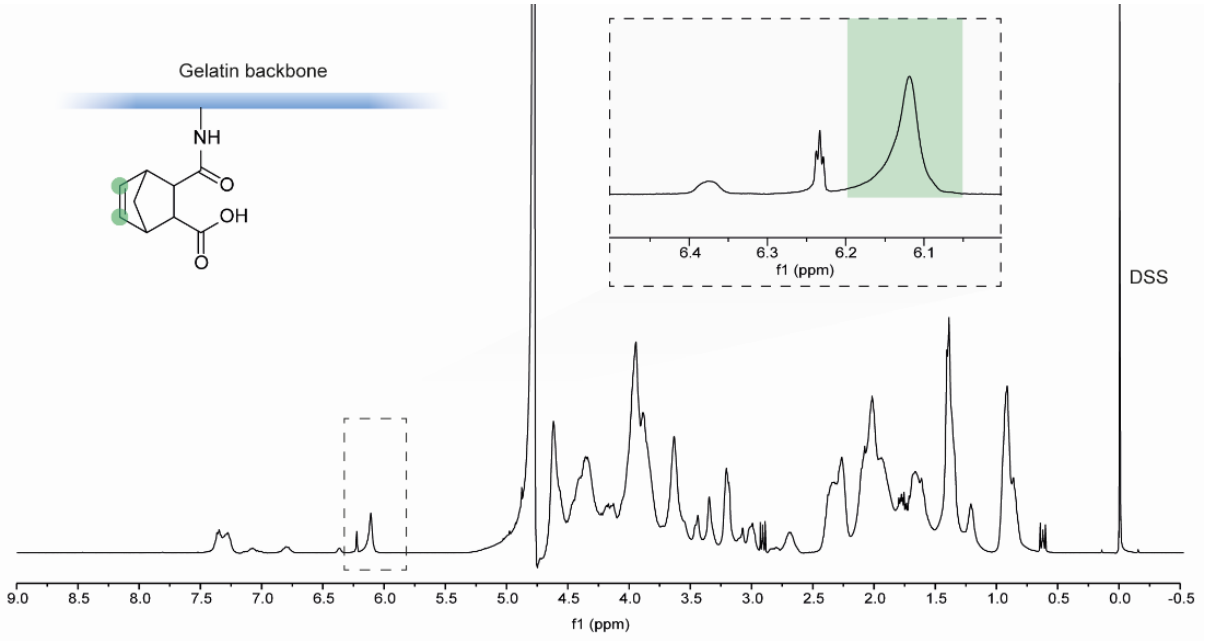

Figure S2. NMR spectrum of Gel-NB. Alkene protons (6.2 to 6.05 ppm) used to determine the DS are highlighted in green in the norbornene bearing moiety illustration (left) and NMR spectrum close-up (right). The integrals of the –ene peaks are compared to the methyl protons of the DSS internal standard (0.05 to –0.05) on the right end side of the spectrum.

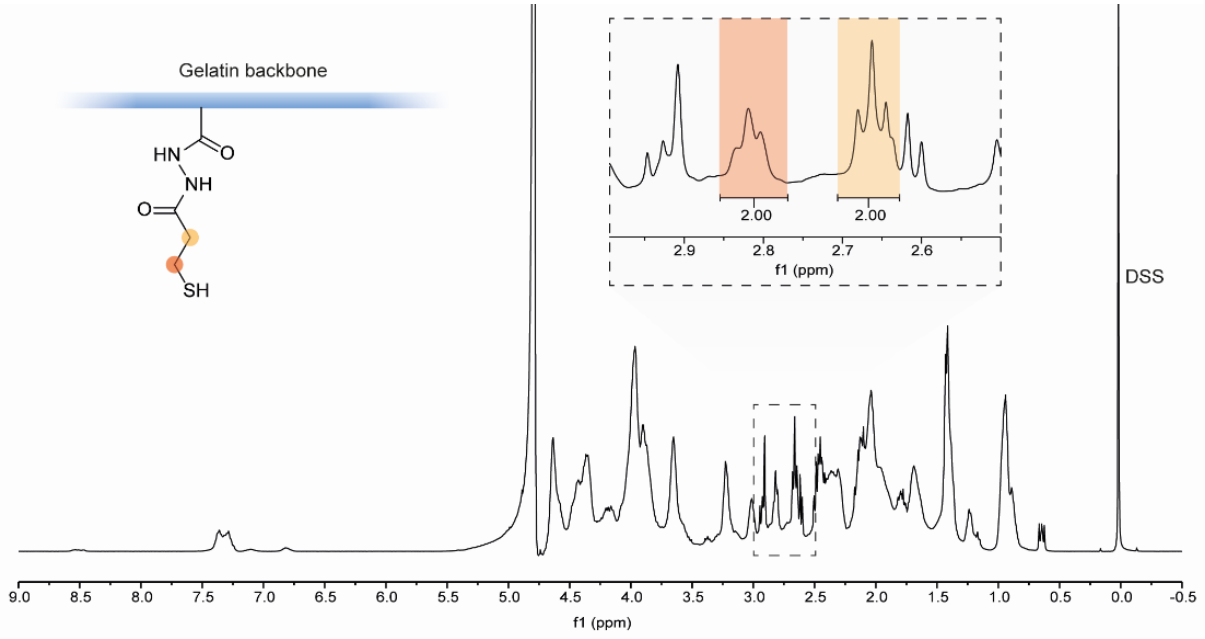

Figure S3. NMR spectrum of Gel-SH. Triplets of methylene groups (2.82 and 2.66 ppm) used to determine the DS are highlighted in shades of orange in the thiol-bearing moiety illustration (left) and NMR spectrum close-up (right). The integrals of the methylene peaks are compared to the methyl protons of the DSS internal standard (0.05 to –0.05) on the right end side of the spectrum.

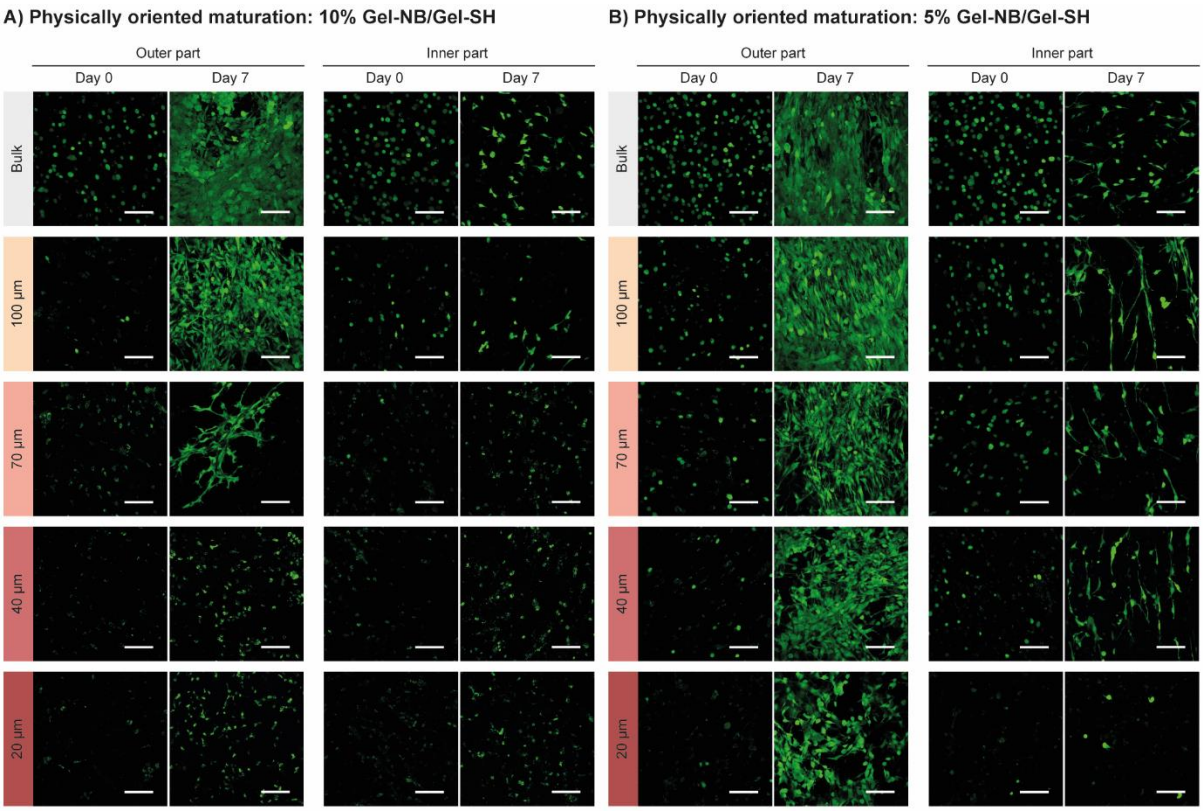

Figure S4. Calcein AM staining (green) of the outer part (left) and inner part (right) of bulk and sized constructs at day 0 and day 7 for A) 10% Gel-NB/Gel-SH and B) 5% Gel-NB/Gel-SH formulations. Scale bars: 100 µm.

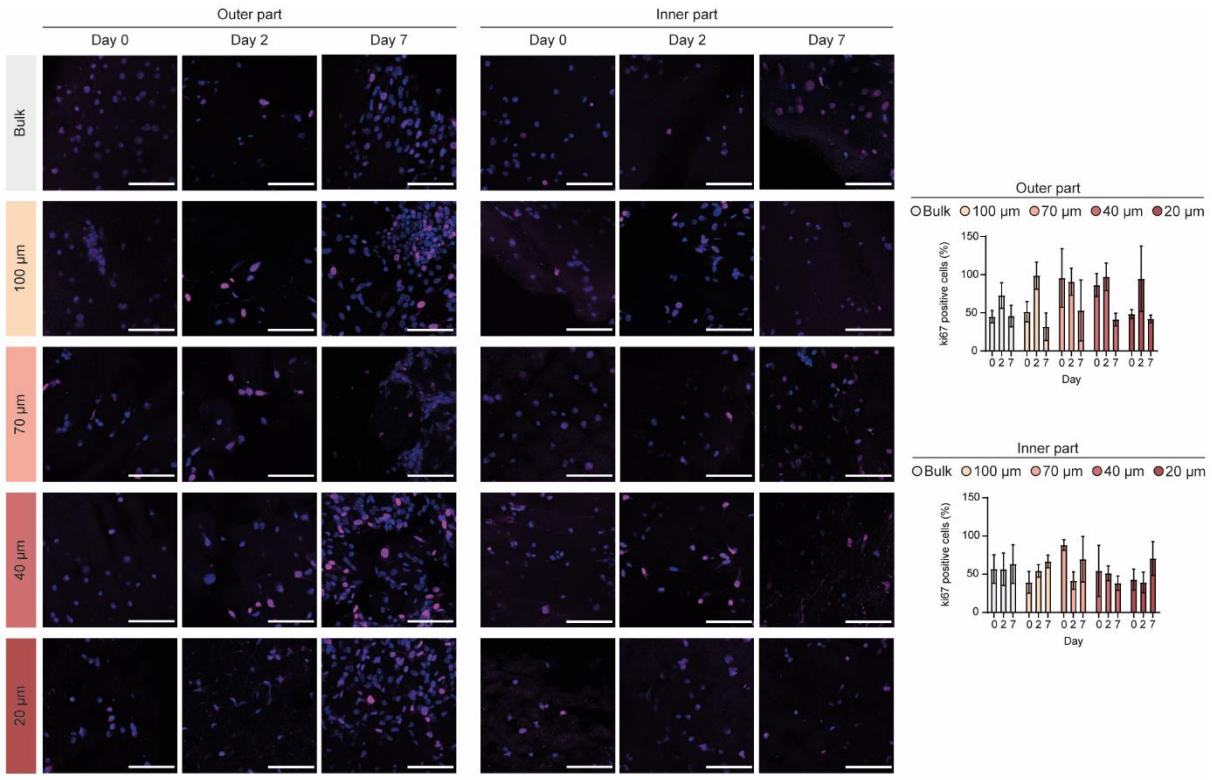

Figure S5. Immunofluorescence images of ki67 (magenta) and HOECHST (blue) stained nuclei for 5% Gel-NB/Gel-SH sized gels in outer and inner parts at day 0, 2 and 7. Scale bar: 100 µm. On the right, bar graphs evidencing the presence of proliferating cells (ki-67 positive) during the first week of culture.

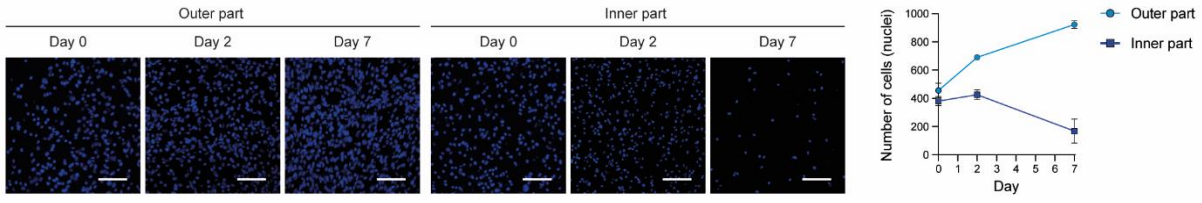

Figure S6. Images showing the changes in number of cells (HOECHST-stained nuclei in blue) for bulk gel during the first week of culture in outer and inner parts (left). Scale bar: 100 µm. On

the right, trendline reporting the increase in cell number in the outer part of the bulk gel and decrease in cell number in the inner part due to cell death.

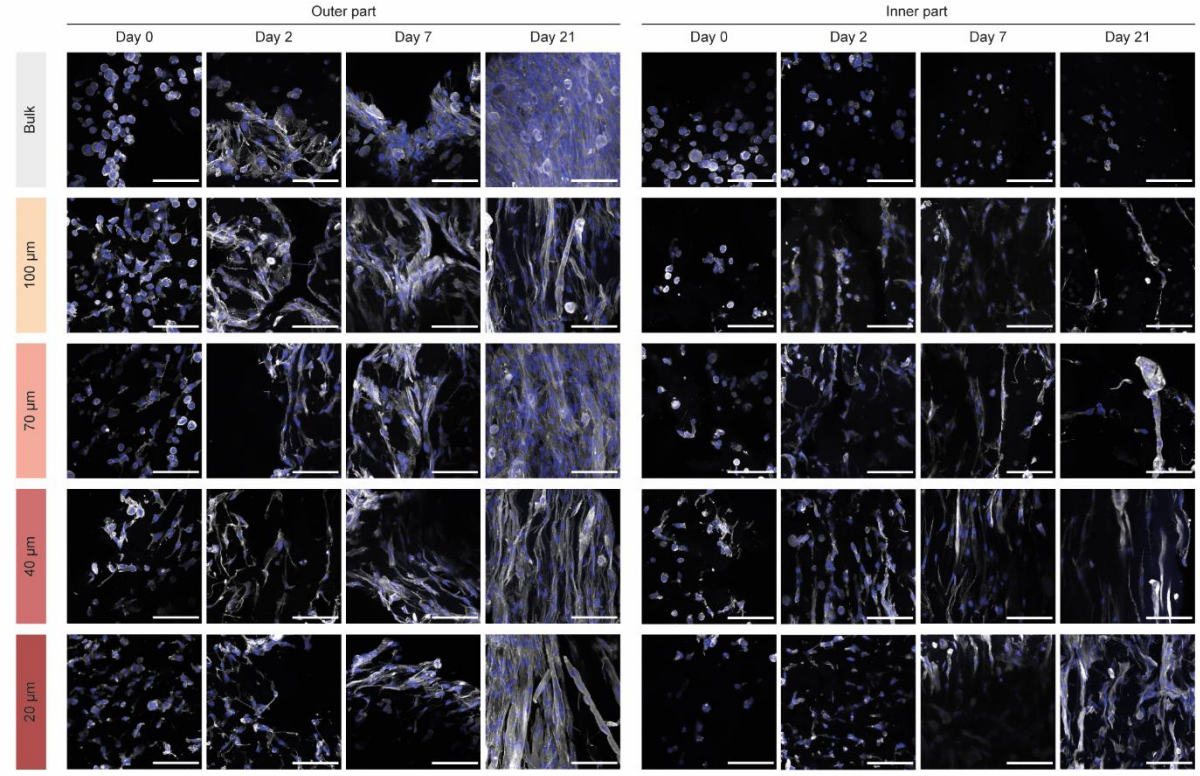

Figure S7. Change in cell morphology over 21 days of culture for the outer part (left) and inner part (right) of bulk and sized constructs. In grey, immunofluorescence staining of cytoskeletal actin shows significant difference between the spread, elongated morphology of cells in the inner part of sized constructs and the round morphology observable in monolithic (bulk) gels. This panel re-enforces the finding that bulk gel did not provide a suitable 3D environment for myoblast maturation and alignment. Scale bar: 100 µm

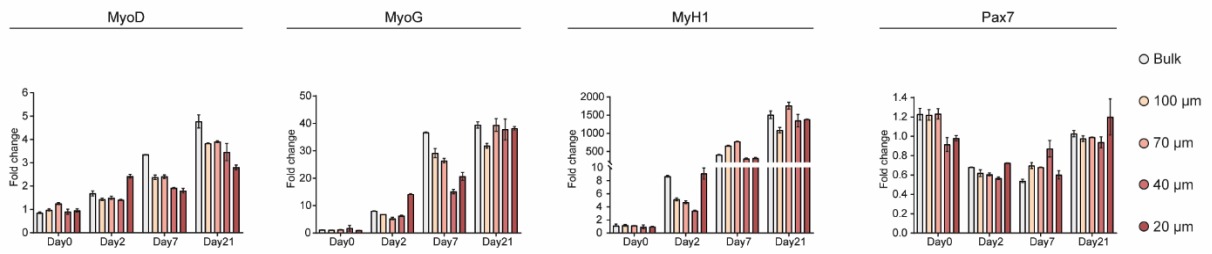

Figure S8. Gene expression analysis of myoblasts differentiation markers. Samples were collected at day 0, 2, 7 and 21 and expression of MyoD, Myog, MyH1 and Pax7 genes was compared to day 0 of differentiation. In this case, gels were analysed as a whole, without separating inner and outer part. For bulk constructs, for which the inner part was shown to not induce formation of myotubes (Figure 6, Figure S7), high expression of differentiation markers can be referred to the myotubes formed in the outer part of the gel.

Video S1. Sizing process, from assembly of sizing device to extrusion of the aligned construct.

Video S2. Brightfield view of bulk and sized constructs after 3 weeks of culture. Muscle twitching is appreciable for the sized constructs, while it appears negligible or absent for the bulk gel (top row). The twitching contribution of the myotubes found in the inner part (bottom row) and the presence of the microarchitecture (aligned microstrands and macropores) enhance the displacement of the whole gel during the contractions. Scale bars: 100 µm