

Supplemental Information

Nanoengineered myogenic scaffolds for skeletal muscle tissue engineering

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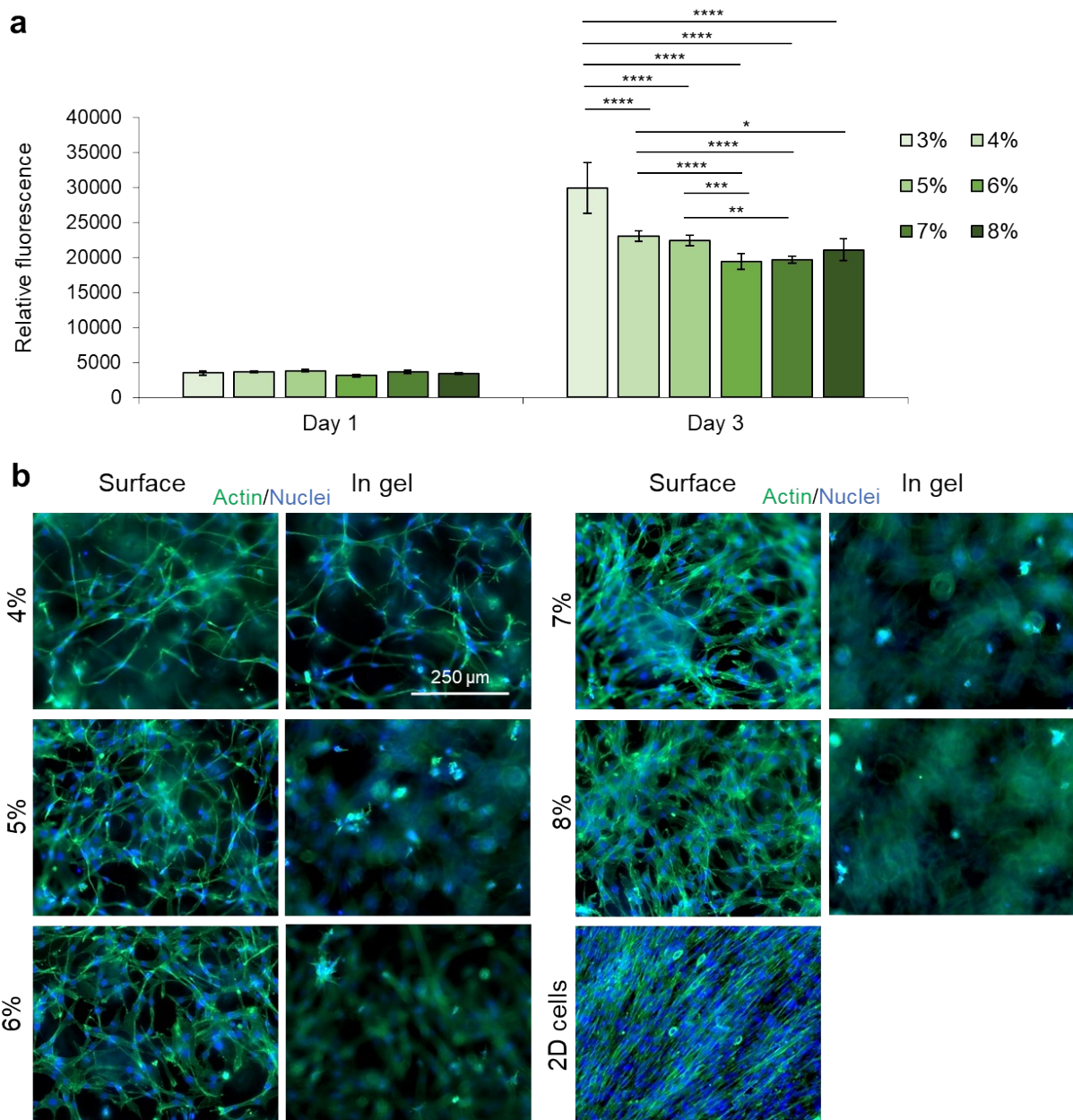


Figure S1. GelMA hydrogel optimization of C2C12 myoblast encapsulation measured for cell proliferation and morphology. a) PrestoBlue™ viability assay displays the proliferation of the C2C12 myoblasts over 3 days of culture. No significant difference is observed on day 1. On day 3, the 3% and 4% groups resulted in the highest proliferation rate, but the 3% samples were not robust enough to survive culture conditions into the differentiation phase. b) Phalloidin 488 staining of actin filaments (green) and DAPI staining for the nucleus (blue) of C2C12s in varying concentrations of GelMA hydrogel at day 6 of culture. The left figures were captured on the surface of the gel and the right figures were captured inside of the hydrogel structure. The 4% (w/v) GelMA hydrogel was the only condition that exhibited cell ingrowth with similar morphologies inside and on the surface of the gel. No 3% (w/v) images were captured because the samples detached and broke apart after 3 days of culture. Scale bars 250 μ m for each micrograph.

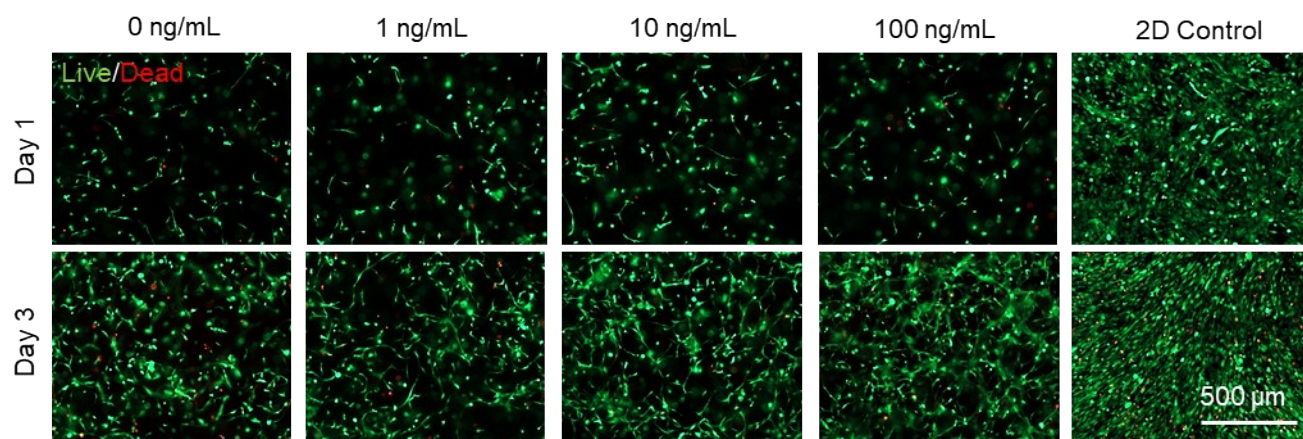


Figure S2. IGF-1 biocompatibility in GelMA and control samples. A Live/Dead assay demonstrated that a concentration of IGF-1 up to 100 ng/mL in GelMA hydrogels did not induce cytotoxicity compared to lower concentrations of IGF-1 or 2D control cells. Live cells are shown in green and dead cells are shown in red. Scale bars 500 μm for all micrographs.

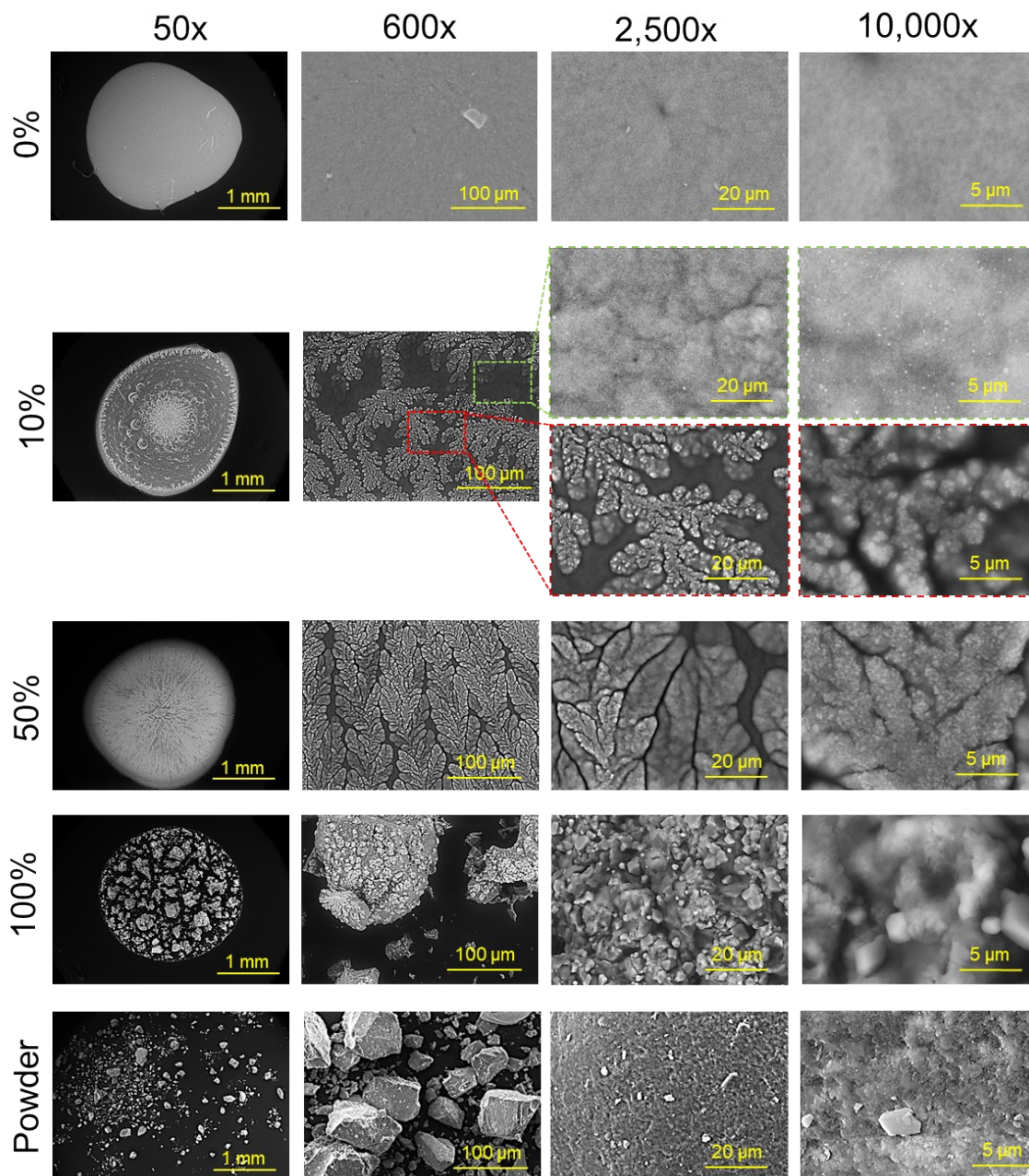


Figure S3. Scanning electron microscopy (SEM) images of Laponite® NC exfoliated in varying DPBS concentrations. The raw powder (bottom row) comes in large 1-100 μm chunks that exfoliate via the release of interstitial sodium ions in solution. At higher magnifications, the raw powder displays a surface of packed NC discs. At 100% DPBS, the NC is unable to exfoliate and remains as large clusters of tightly packed discs. As the salt concentration is reduced to 50% the size of the NC particles was reduced to micrometer features. At 10% salt solution, varying primary, secondary, and tertiary structures are observed through the exfoliation and interaction of NC discs with free ions in the solvent. The 10% solution formed two distinct phases: one that appeared fully exfoliated (green) and the other appeared to form micrometer clusters (red). The 0% (DI water) solution resulted in a smooth homogeneous surface with indistinguishable features.

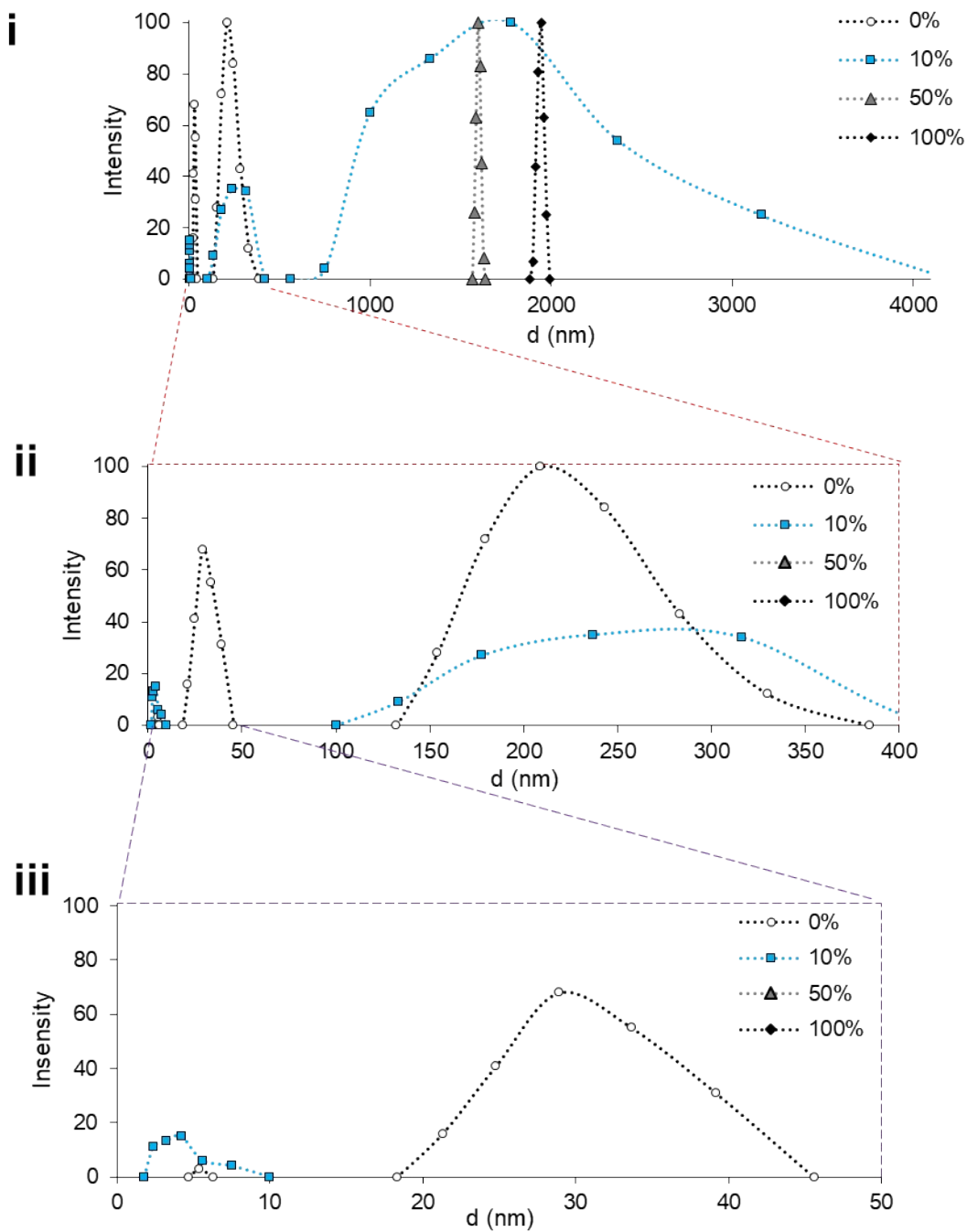


Figure S4. NC size analysis by varying DPBS concentrations. (i) Size analysis revealed that the NC was unable to exfoliate at high salt concentrations above 50%, resulting in single peaks on the μm scale. (ii) Reducing the salt concentration to 10% resulted in more exfoliation of the NC denoted by the reduction in the NC size. (iii) More individual NC discs (from 0-10 nm) were observed in the 10% DPBS solution than in the pure DI water (0% DPBS). Both 0% and 10% DPBS solutions resulted in the formation of secondary and tertiary structures from the complex hierarchical interactions of dislocated ions and NC edge and face charges.

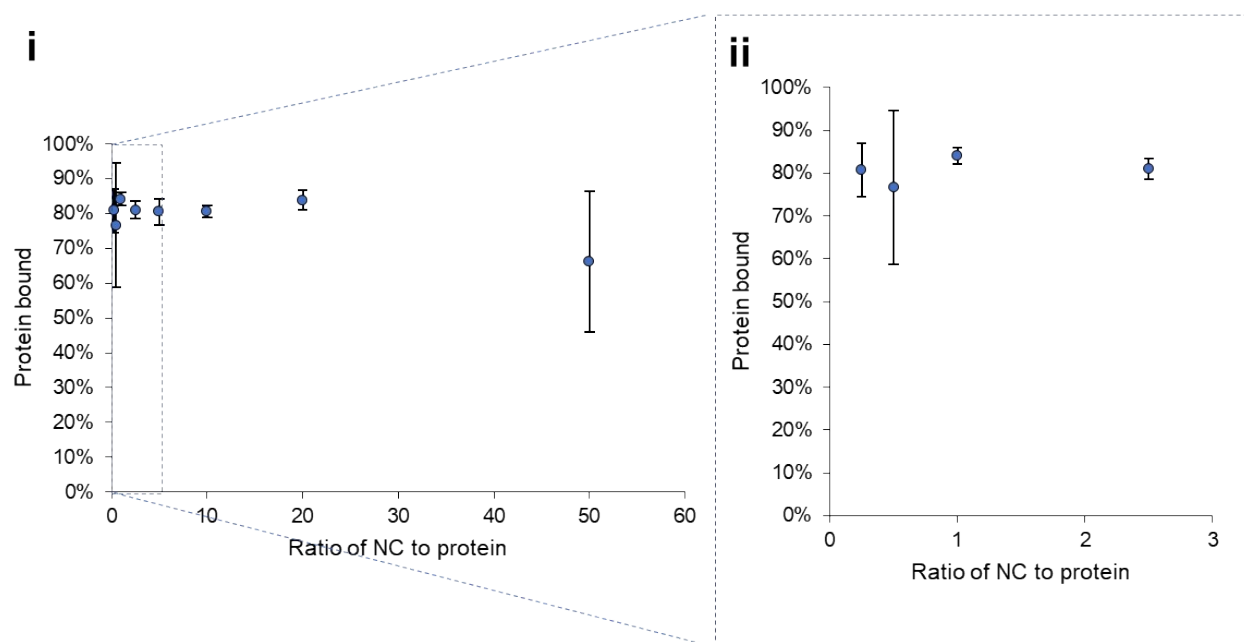


Figure S5. Expanded protein-binding assay by the ratio of NC to protein binding in a 10% DPBS solution. (i) A similar percentage of total protein-bound was observed for an NC to protein ratio ranging from 0.25-50. (ii) Even at extremely low NC to protein ratios almost all the protein was bound to the NC.

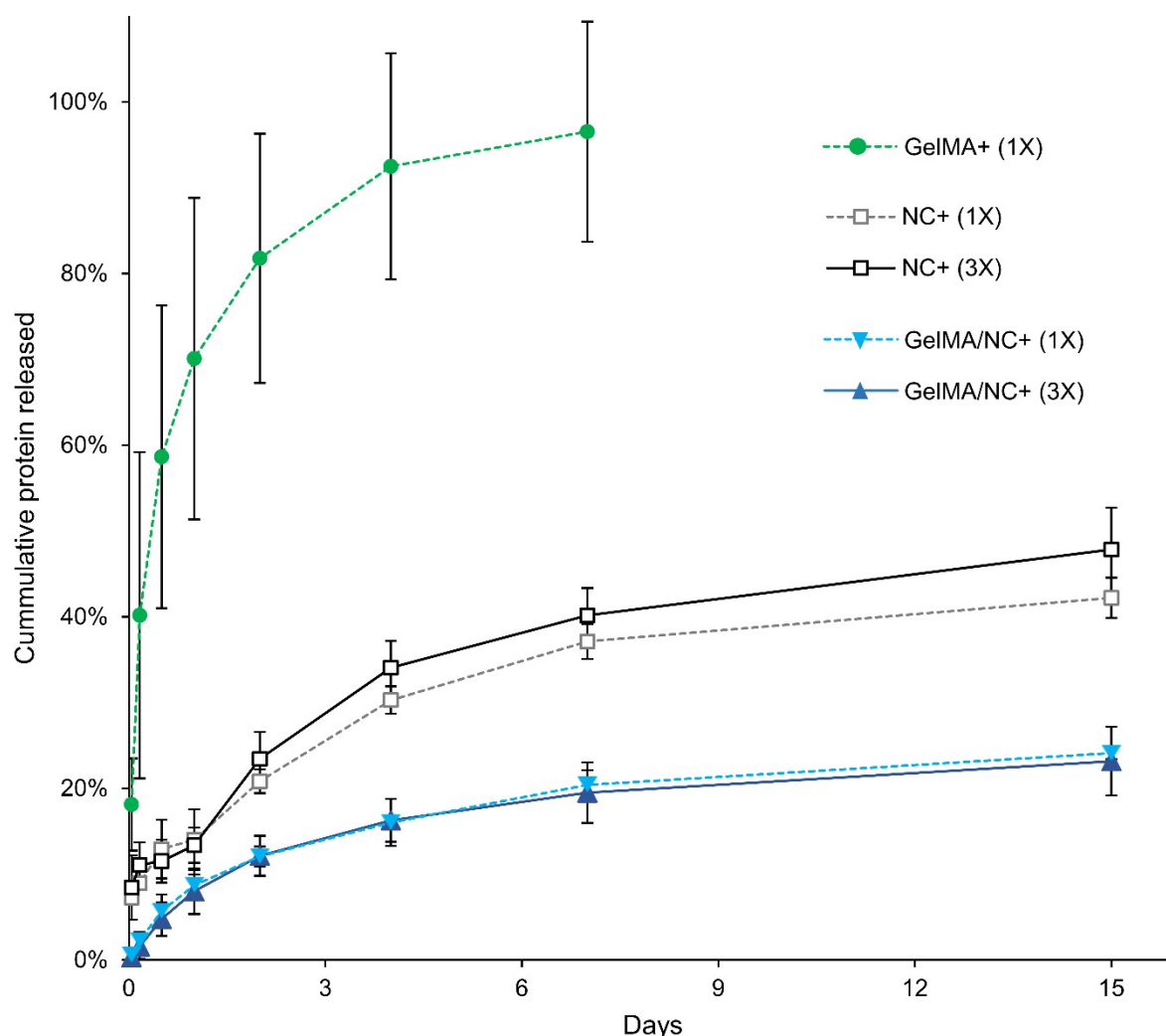


Figure S6. The cumulative percentage of protein mass that was released over 15 days. The GelMA+ (1X) group that did not have NC resulted in a burst release of the hydrogel that released almost all of the IGF-1 after 7 days of incubation. The NC+ (1X) and (3X) groups that were not encapsulated into hydrogel released more loosely bound protein within the first day but eventually resulted in controlled release. The NC+ groups that were encapsulated in hydrogel, GelMA/NC+ (1X) and GelMA/NC+ (3X), resulted in a slower release than unencapsulated NC or GelMA alone. The hydrogel encapsulated NC also retained a portion of NC bound IGF-1. Respective to whether encapsulated or not encapsulated, the 1X and 3X groups had very similar release curves demonstrating a consistent percentage of protein release. More protein mass is released from the 3X groups because of the higher initial protein mass.

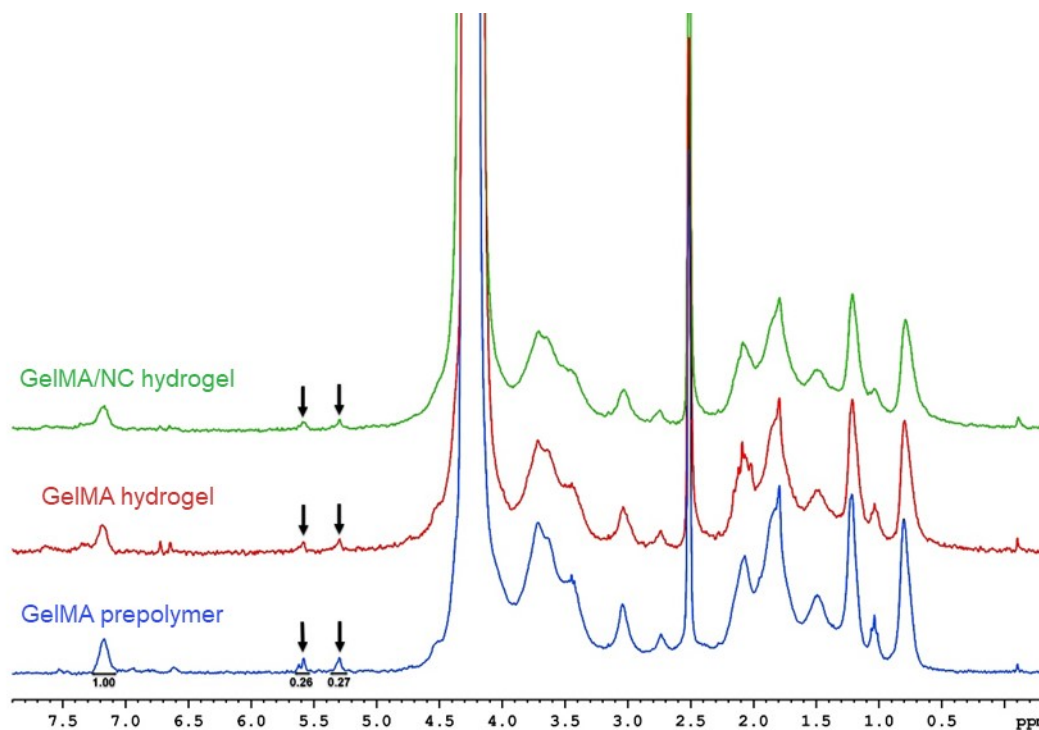


Figure S7. Representative ^1H NMR spectra of GelMA prepolymer, GelMA hydrogel, and GelMA-composite hydrogel. The prepolymer GelMA had a degree of functionalization of 53%. ^1H NMR analysis revealed the crosslinking degree of GelMA hydrogels and GelMA/NC hydrogel: 61% and 70%, respectively.

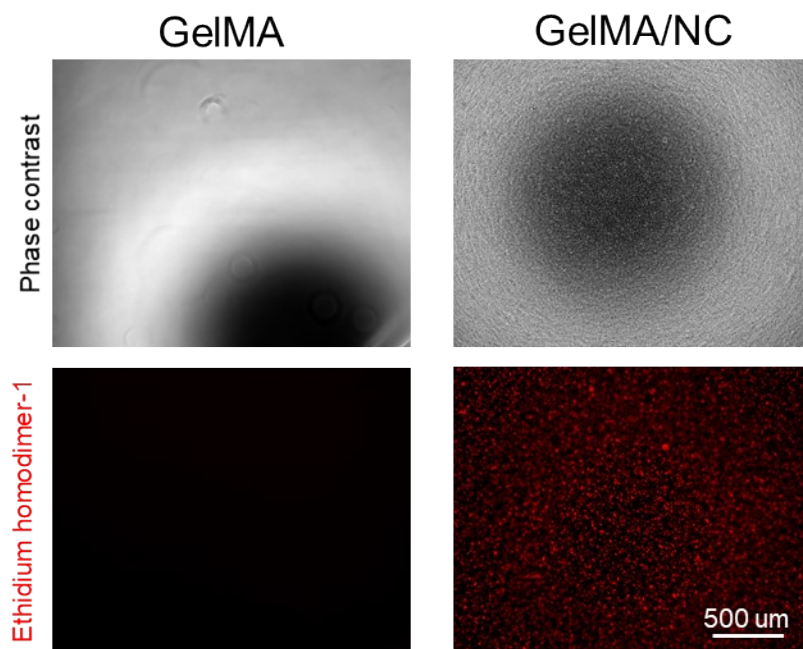


Figure S8. Distribution of encapsulated NCs in 4% GelMA hydrogel in Live/Dead material control samples visualized by the adsorption of ethidium homodimer-1.

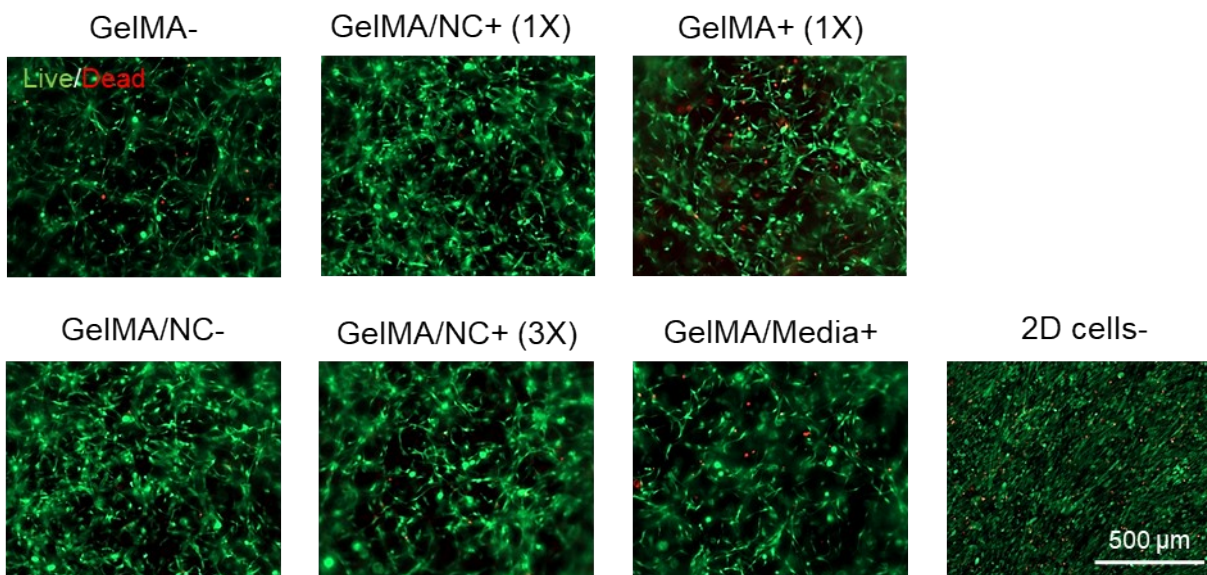


Figure S9. Biocompatibility of nanoengineered GelMA hydrogels with encapsulated nanoclay (NC) biocompatibility. Live/Dead assay performed on day 3 demonstrated viability of all of the NC groups and IGF-1 groups (+) relative to the GelMA- and 2D cells- controls. Live cells are shown in green and dead cells are shown in red.

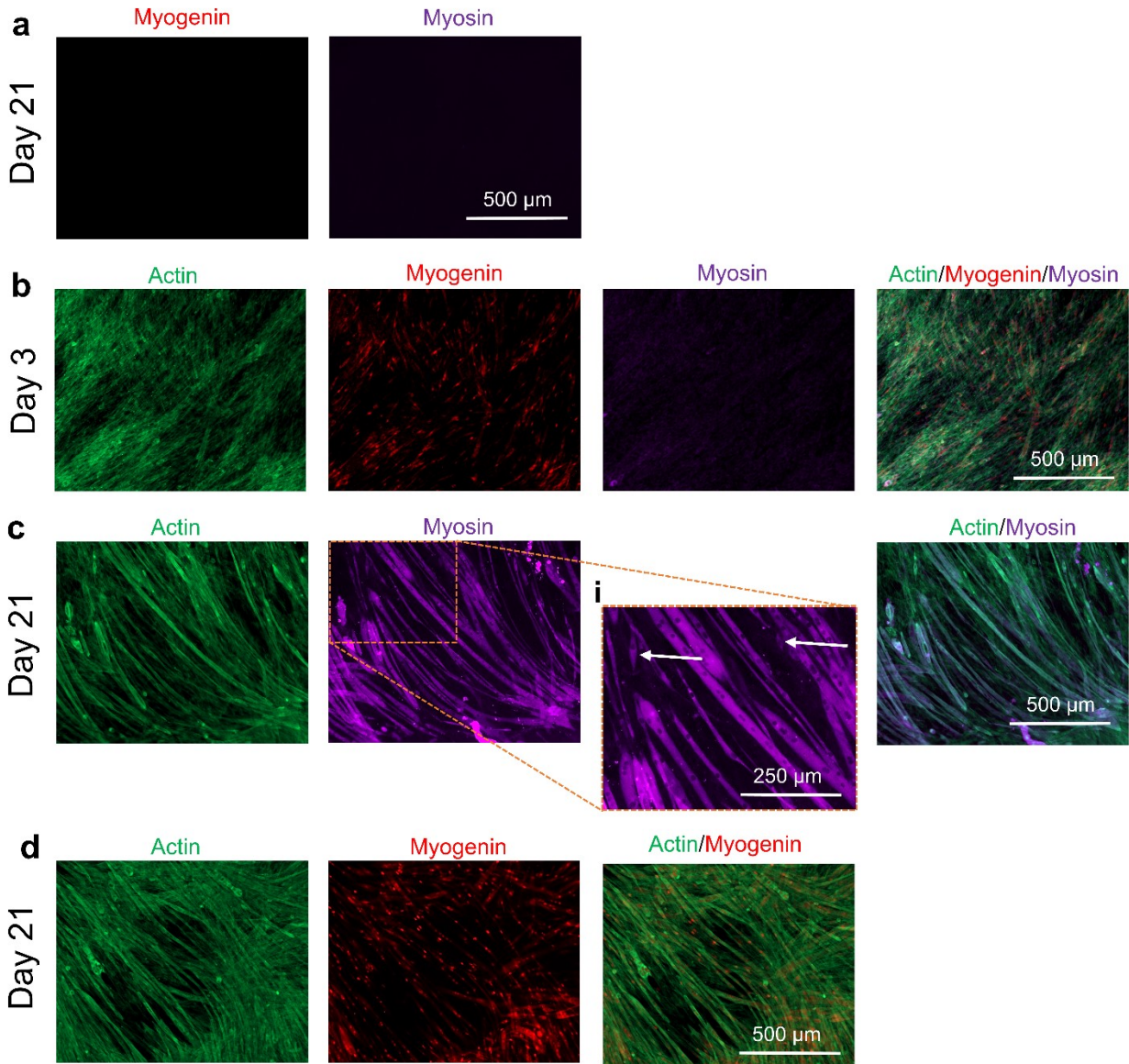


Figure S10. Immunofluorescent optimization on 2D controls. a) Day 21 cultured (14 day differentiated) controls that are only treated with myogenin (red) and myosin heavy chain (MHC, purple) secondary fluorescently-labeled antibodies. b) Fully stained day 3 cultured (undifferentiated) controls that demonstrate myogenin is expressed before serum-starved differentiation, but myosin heavy chain is not expressed. Actin (green) panels show that not all cells are expressing myogenin. c) Fully stained day 21 cultured (14 day differentiated) controls stained for MHC. (i) A callout demonstrating single-nucleated cells expressing MHC (white arrows); scale bars are 250 μm . Actin panels demonstrate not all cells are expressing MHC by day 21. d) Fully stained day 21 cultured (14 day differentiated) controls stained for myogenin. All scale bars except c(i) are 500 μm .

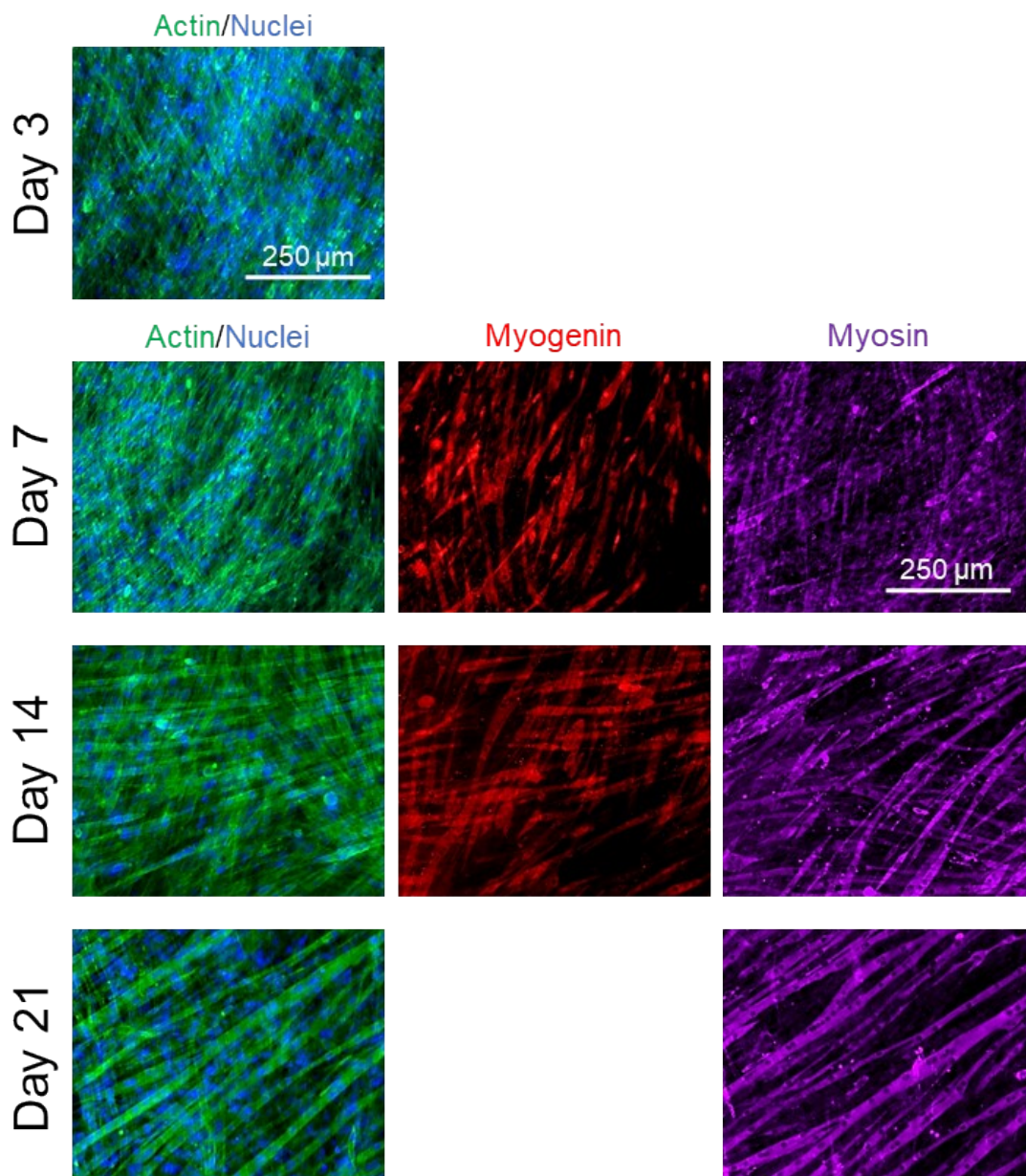


Figure S11. 2D control cell proliferation and differentiation immunofluorescent imaging. The left-most column displays actin (green) and nuclear (blue) staining demonstrating the normal morphological development and differentiation of C2C12 myoblasts without exposure to IGF-1 on a concentrated 2D surface. The middle column displays 2D control cells stained for myogenin (red) before (Day 7, top) and 7 days after differentiation (Day 14, bottom). C2C12s expressed myogenin before and after serum-deprived differentiation demonstrating their ability to cell differentiate at high cell concentrations. The right column displays 2D control cell myogenic differentiation and staining for myosin heavy chain (MHC, purple) positive myotubes. Scale bars are 250 μm for all images.