Supplementary Information

Enzymatically Crosslinked Gelatin-Laminin Hydrogels for Applications in Neuromuscular Tissue Engineering

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Supplemental Figure 1. GEL-LN hydrogels were prepared using 4% gelatin and 4% mTg.A) An amplitude sweep was performed to determine the LVD after which frequency sweeps were performed on 3 different samples to determine the B) storage modulus, C) loss modulus, D) complex modulus, and E) viscosity. These values were used to determine the F) Young's modulus of the sample.



Supplemental Figure 2. GEL-LN hydrogels were prepared using 6% gelatin and 4% mTg.A) An amplitude sweep was performed to determine the LVD after which frequency sweeps were performed on 3 different samples to determine the B) storage modulus, C) loss modulus, D) complex modulus, and E) viscosity. These values were used to determine the F) Young's modulus of the sample.



Supplemental Figure 3. GEL-LN hydrogels were prepared using 8% gelatin and 4% mTg.A) An amplitude sweep was performed to determine the LVD after which frequency sweeps were performed on 3 different samples to determine the B) storage modulus, C) loss modulus, D) complex modulus, and E) viscosity. These values were used to determine the F) Young's modulus of the sample.



Supplemental Figure 4. GEL-LN hydrogels were prepared using 10% gelatin and 4% mTg.A) An amplitude sweep was performed to determine the LVD after which frequency sweeps were performed on 3 different samples to determine the B) storage modulus, C) loss modulus, D) complex modulus, and E) viscosity. These values were used to determine the F) Young's modulus of the sample.



Supplemental Figure 5. GEL-LN hydrogels were prepared using 4%, 6%, 8%, and 10% gelatin and 4% mTg. A) The storage modulus, B) loss modulus, C) complex shear modulus, and D) viscosity were measured over the LVD region. Three samples were measured for each gelatin concentration and the average value was taken for each frequency.



Supplemental Figure 6. Schwann cells were cultured on gelatin (GEL) hydrogels and GEL-LN hydrogels for 2 days. Live/Dead was performed after 2 days on A) Gel and B) GEL-LN (n=2). Scale 100 μm. Edu assay was performed after 2 days to assess Schwann cell proliferation on C) gel and D) GEL-LN (n=8). Scale 100 μm. Schwann cell purity was assessed by performing flow cell cytometry for P75 on E) Gel and F) GEL-LN (n=2). G) Quantification of the percent of proliferating cells after 2 days in culture.



Supplemental Figure 7. The myogenic index was measured by determining the MI fraction of nuclei contained within myotubes. C2C12s were cultured for 28 days and fixed at weekly time points. A) Muscle was cultured on micromolded GEL and GEL-LN and myogenic index was determined over time. B) Muscle was cultured on GEL-LN with either 15 μ m x 15 μ m or 20 μ m x 10 μ m, grooves and ridges respectively. Myogenic index was determined at each time point. **p < 0.01.

Supplemental Movie 1: Representative video of SpS calcium signaling on LN coated surface after addition of 1 mM 4-aminopyridine.

Supplemental Movie 2: Representative video of SpS calcium signaling on GEL-LN condition after addition of 1 mM 4-aminopyridine.