Supporting Information

Efficient *in situ* gene delivery via PEG diacrylate matrices

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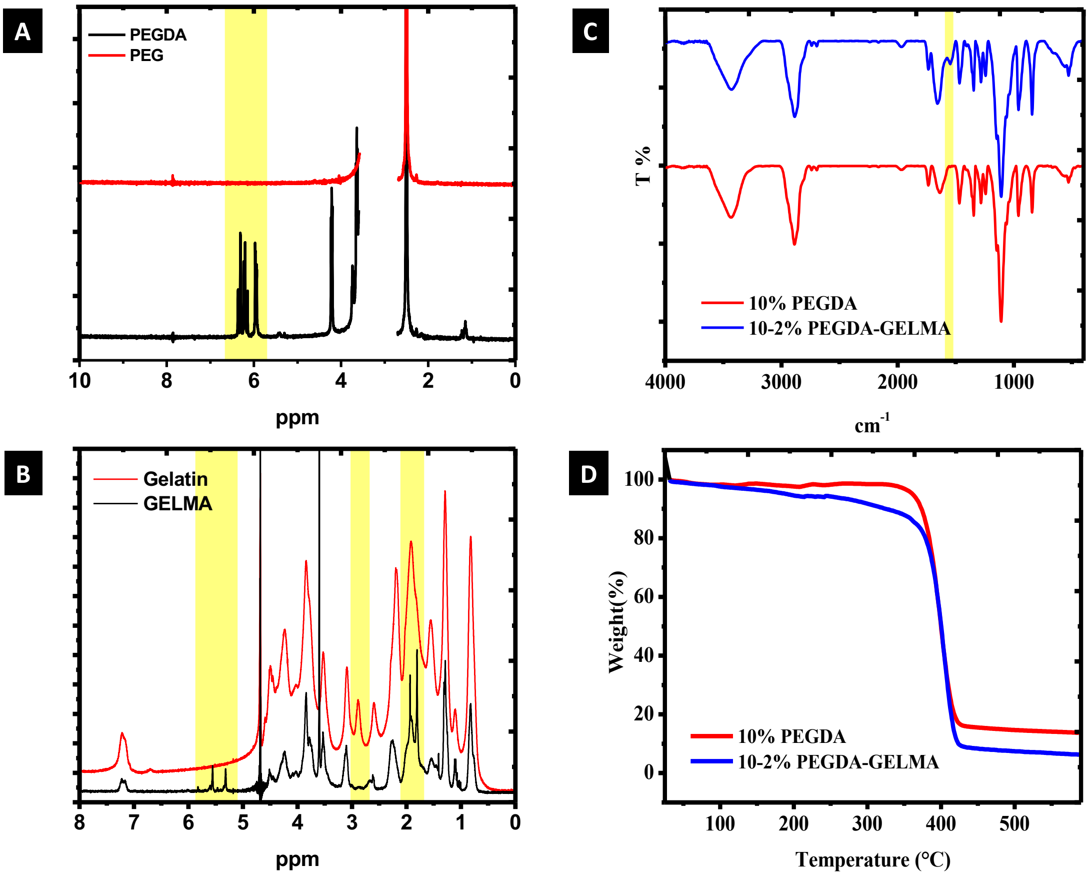

Figure S1: NMR spectra of synthesized polymers (**A**) PEGDA and (**B**) GELMA. Shaded regions indicate peaks corresponding to addition of acrylate groups.FTIR spectrum **(C)** comparing PEGDA cryogel with PEGDA-GELMA cryogel and thermal degradation profile **(D)** showing difference in thermal degradation profiles of PEGDA and PEGDA-GELMA cryogels.

The synthesis of PEGDA from PEG and GELMA from gelatin was confirmed using NMR spectroscopy as shown in Supplementary Figure S1. ^{1}H NMR spectrum of PEGDA shows peaks represented as δ in ppm as seen in Fig. S1A. Following are characteristic peaks for PEGDA: δ =6.3-6.37 (dd, 1H, J1=17.18 Hz, J2=1.83 Hz), 6.1-6.24 (dd, 1H, J1=17.54 Hz, J2=12 Hz), 5.93-5.97 (dd, 4H, J1=9.87Hz, J_2=1.83 Hz),4.21 (t, 2H, *J*=5.12Hz,), 3.74 (t, 2H, *J*=4.39Hz), 3.64(t, 2H, *J*=4.39Hz), 3.34-3.57 (m, 153H, PEG backbone). The presence of peaks at δ = 5.9-6.3 ppm in synthesized PEGDA confirms the substitution of terminal OH group of PEG with a vinyl group [1]. The degree of acrylation was calculated using NMR spectrum and was found to be >98%. ^{1}H NMR spectra (300 MHz, D_2O) of GELMA shows peaks at ~5.3-5.6 ppm from methylene proton and at 1.9 ppm from methyl proton of methacrylate group confirming the substitution of ξ-amino group of lysine and hydroxyl lysine on gelatin backbone [2] as seen in Fig. S2B. Degree of acrylation was determined from the loss of lysine peak at ~2.8-2.9 ppm and was found to be greater than 99% [2]. These polymers synthesized as above were then cross-linked by redox-mediated free radical polymerization using cryotropic gelation or cryogelation technique to form cryogel matrices. The incorporation of GELMA in PEGDA-GELMA cryogels was analyzed and confirmed using FTIR spectroscopy and TGA studies. FTIR spectrum of blended PEGDA-GELMA cryogel shows a small shoulder peak at 1550 cm^{-1} while that of PEGDA only cryogel does not show the same, as seen from the shaded region in Fig. S1C and also reported earlier [3]. This small shoulder peak in the spectrum confirms the presence of GELMA in the blended PEGDA-GELMA, which is non-existent in the PEGDA only cryogel. This peak is attributed to the amide-II (N-H) bending of both secondary and primary amides in GELMA. Since the amide bonds are present only in GELMA and not in PEGDA, we can conclude that GELMA is blended with PEGDA. Because the concentration of GelMA is very low in these blends, it is difficult to see the peaks that might occur due to interaction between PEGDA and GelMA. We also observed the following peaks: 1739-41 cm^{-1} and 1629-1648 cm^{-1}(C=O stretching, υ_s), 2885-2287 cm^{-1}(C-H stretching, υ_s), 1411 cm^{-1}(C-H bending, δ_s), 1467 cm^{-1}(C-H symmetric bending, δ_s), 1351-1352 cm^{-1} (C-O asymmetric bending, δ_{as}), 1243-1283 cm^{-1}(C-O asymmetric bending, δ_{as}), 1111-1112 cm^{-1} (C-O-C symmetric stretching, υ_s), 954-955 cm^{-1} (C-O-C stretching, υ_s), 840-841 cm^{-1} (C-O-C stretching, υ_s) and 523-524 cm^{-1} (C-H symmetric stretching, υ_s). FTIR studies confirm the incorporation of GELMA in PEGDA-GELMA cryogel. TGA was used as an indirect method to show stable covalent bond formation between GELMA and PEGDA and that it was not just an interpenetrating non-interacting network (IPN). It was done to study the differences in thermal degradation profile of

PEGDA-GELMA and only PEGDA cryogels. We observed a faster onset of thermal degradation in PEGDA-GELMA cryogels when compared to PEGDA cryogels between 150-300°C (Fig. S1D). This can be attributed to breaking of bonds between PEGDA and GELMA in PEGDA-GELMA cryogels. If GELMA and PEGDA were existing just as IPNs in the cryogels, then we would see independent peaks of GELMA and PEGDA in the PEGDA-GELMA system. FTIR and TGA studies confirm the successful incorporation of GELMA in PEGDA-GELMA cryogel system.

Mechanical properties of matrices are known to govern the success of gene delivery and were measured both as compressive and shear moduli to find stiffness **(Figure S2)**. To study viscoelastic properties of cryogels, rheological analysis was performed. An initial amplitude sweep was done followed by frequency sweep to determine storage and loss modulus from which complex modulus was calculated. We observed a similar trend for storage modulus, which was 610.10 ± 132 Pa for PEGDA only cryogel and 1019.52 ± 115 Pa for PEGDA-GELMA cryogel. We calculated elastic modulus of cryogels from the linear portion of the stress-strain graph. PEGDA-GELMA cryogels were found to have a higher elastic modulus (24.05 ± 7.4 kPa) compared to PEGDA only cryogels (13.21 ± 1.8 kPa) as seen in figure-S2C, possibly due to cross-linking between GELMA and PEGDA.

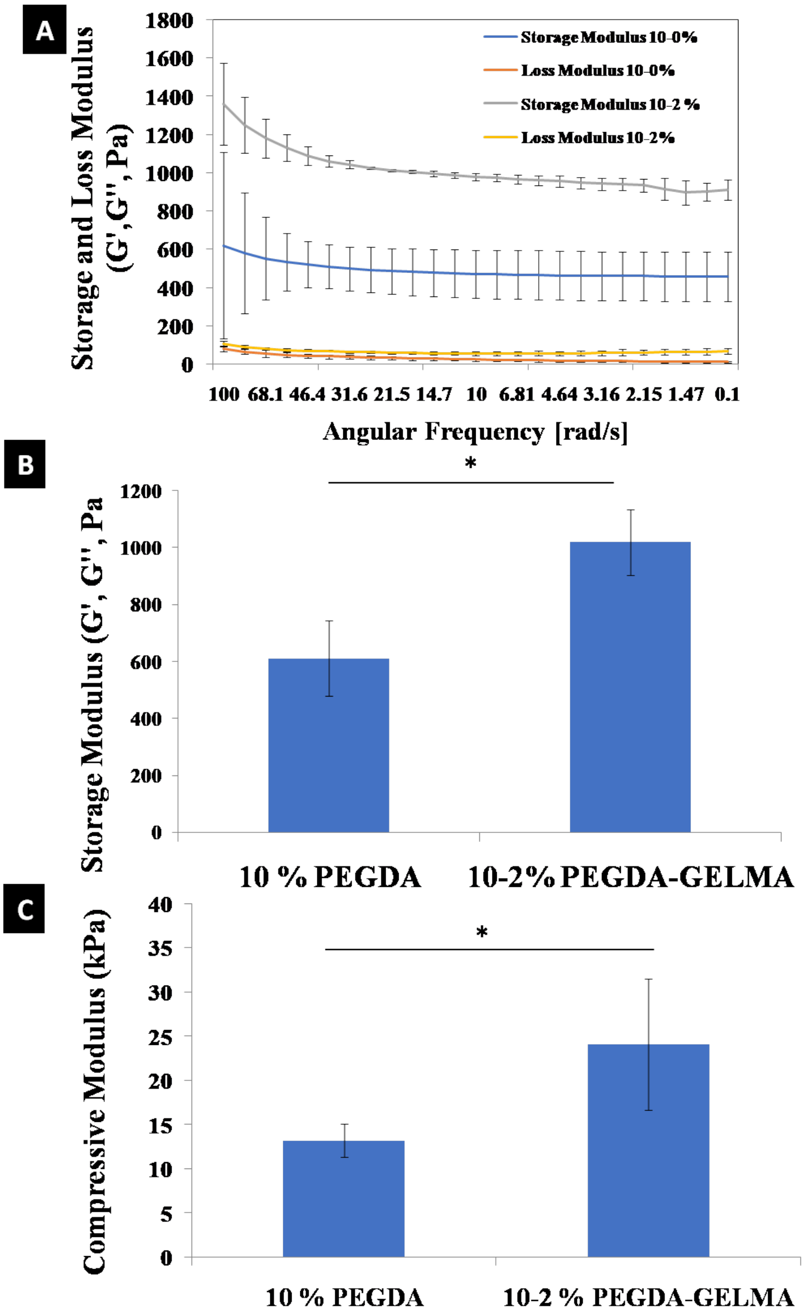

Figure S2: Physical properties of cryogel matrix. (A) Storage modulus and loss modulus for given angular frequencies (B) Storage modulus, (C) compressive modulus of PEGDA and PEGDA-GELMA . (* $p<0.05$, $n=3$)

Supplementary movies

Movie S1. Movie showing X-ray Microscopy 3D rendered image of PEGDA cryogel.

REFERENCES

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