

## Supplementary Information

### 3D bioprinting of dual-crosslinked nanocellulose hydrogels for tissue engineering applications

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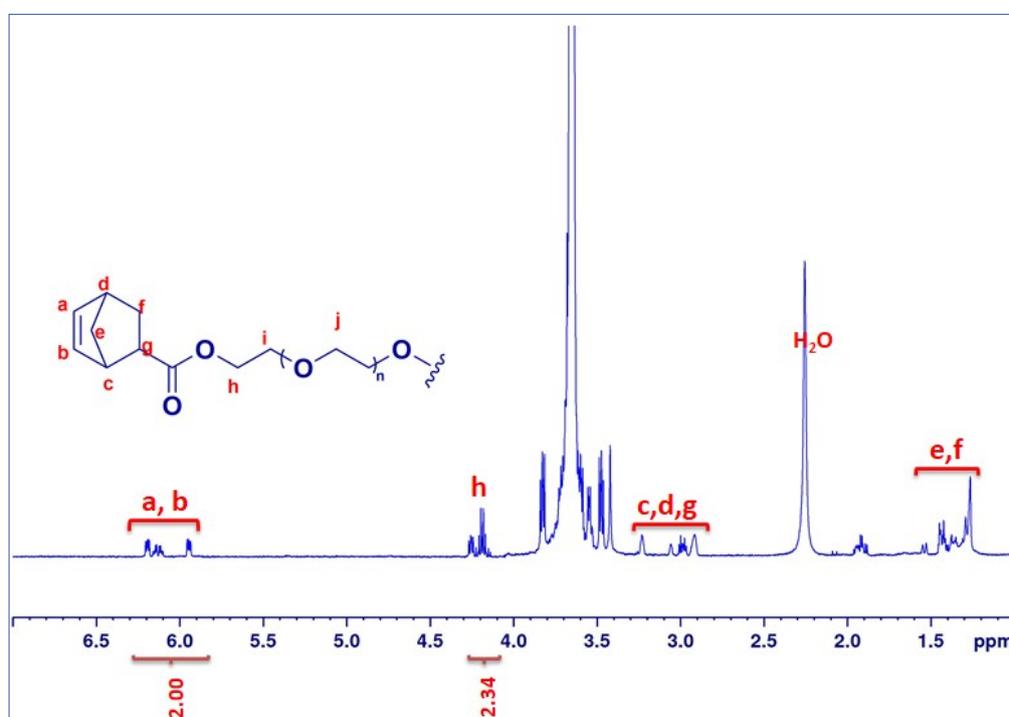


Figure S1. <sup>1</sup>H-NMR spectra of 4-arm PEG-NB

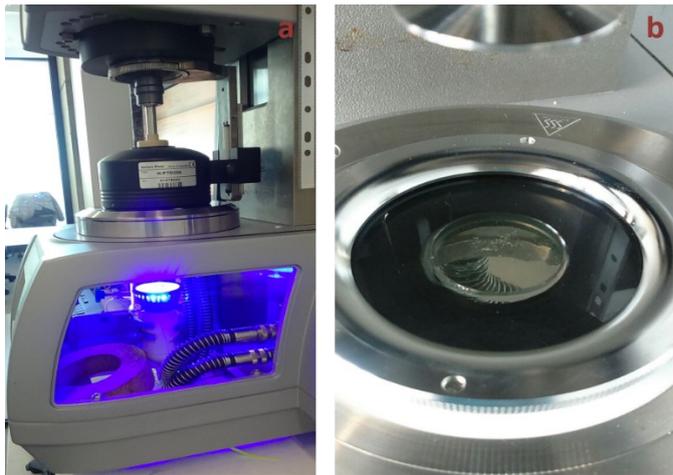


Figure S2. Photos of (a) a time sweep photorheometry of inks under blue light irradiation with 0.1% strain, 1 Hz frequency, and (b) a hydrogel after frequency sweep test.

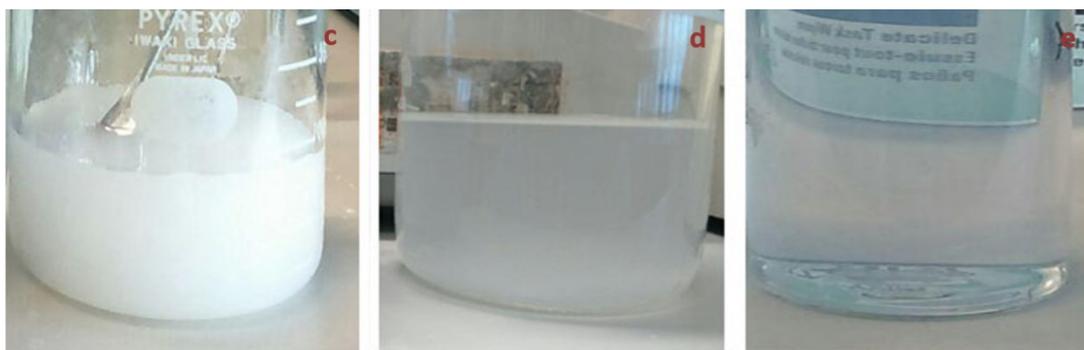
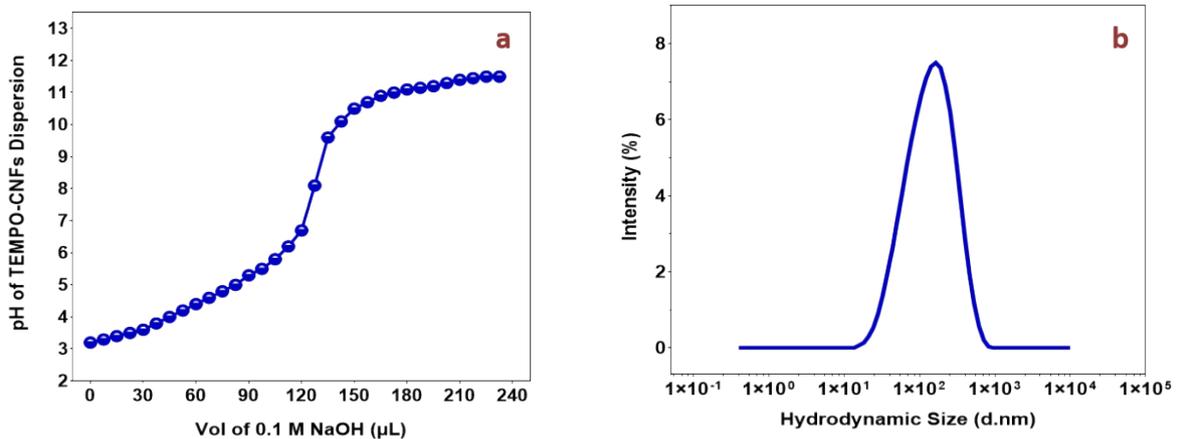


Figure S3. (a) pH measurements of TEMPO-CNFs solution by adding 0.1 M NaOH (mole of COOH = 1.27 mmol/g of TEMPO-CNFs) and (b) DLS measurements of TEMPO-CNFs at (0.1 w/v %) CNFs in milli-Q water and dispersion of cellulose nanofibers with high energy ultrasonication treatment. The CNFs aqueous solution after (c) just TEMPO mediated oxidation, (d) ultrasonication for 40 mins, (e) ultrasonication for 80 mins.

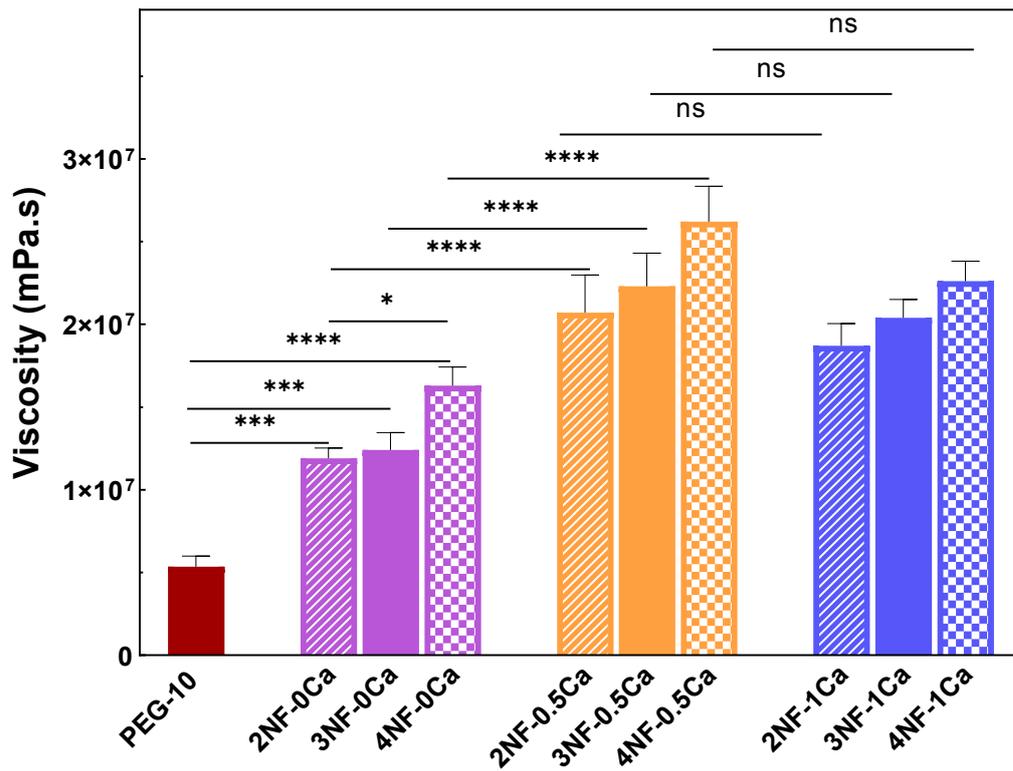


Figure S4. The viscosity of the formulated inks at near zero shear rate ( $0.001 \text{ s}^{-1}$ ). (one-way ANOVA, Tukey's post-test, ns = nonsignificant,  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ , and  $****p < 0.0001$ )

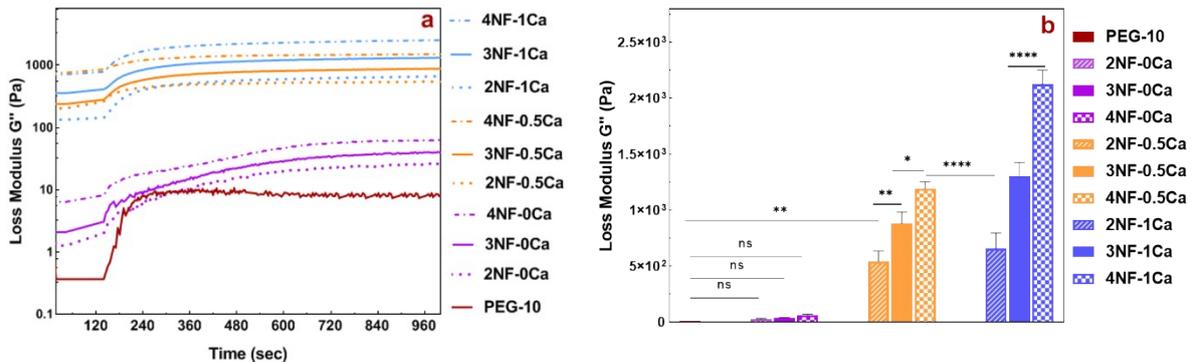


Figure S5. (a) Change of loss moduli of the PEG-CNFs hydrogels after blue light irradiation under oscillatory analysis; light was switched on at 120 s and continued until 1000 sec. (b) Loss moduli of the PEG-CNFs hydrogels at 12<sup>th</sup> min of blue light irradiation under oscillatory analysis. (one-way ANOVA, Tukey's post-test, ns = nonsignificant,  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ , and  $****p < 0.0001$ )



Figure S6. Cylinder samples for compression tests: (a) after photocrosslinking, (b) immersing in PBS, (c) swollen samples for compression test.

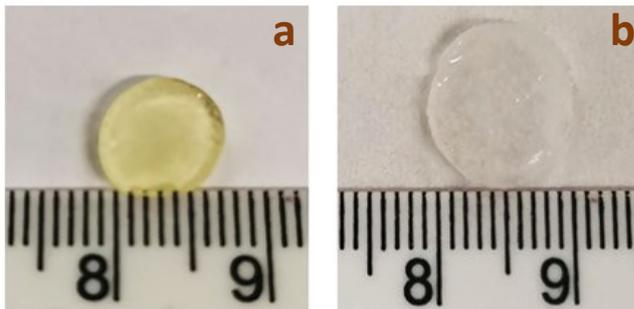


Figure S7. PEG-CNFs hydrogel disc: (a) before immersion in PBS and (b) after 24h in equilibrium swollen mode.

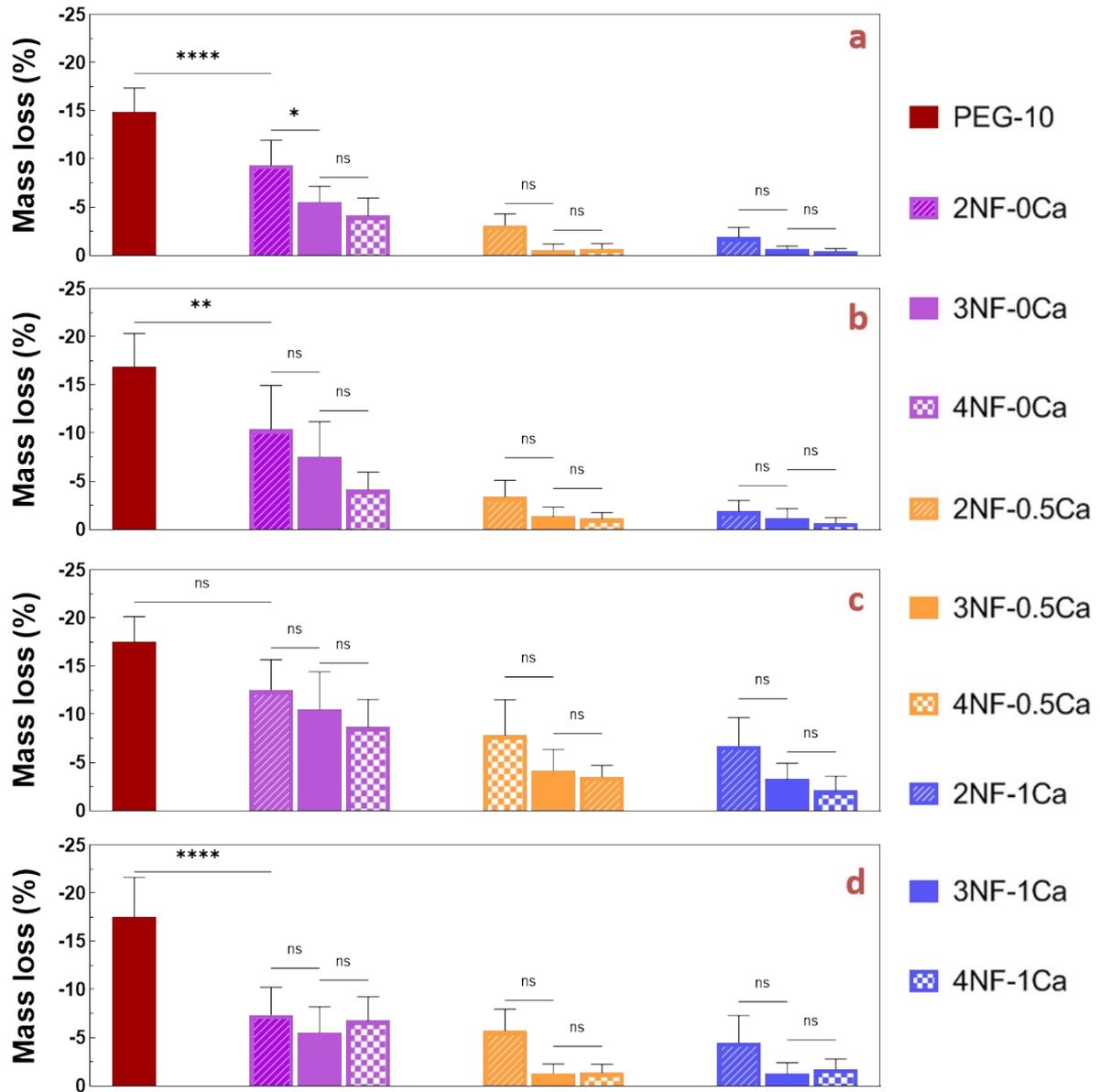


Figure S8. Mass loss of different PEG-CNFs hydrogels formed with various contents of CNFs or  $\text{Ca}^{2+}$  ( $n = 5$ , mean  $\pm$  SD) after (a) 1 week (b) 2 weeks (c) 4 weeks, and (d) 6 weeks incubation in PBS ( $p < 0.0001$ , one-way ANOVA, Tukey's post-test).

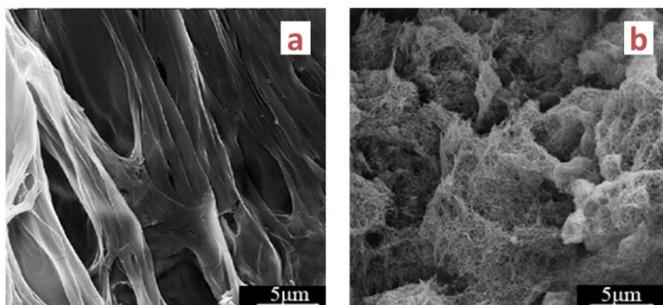


Figure S9. SEM images of hydrogels morphology (a) PEG-10 and (b) 3NF-0.5Ca PEG-CNFs.

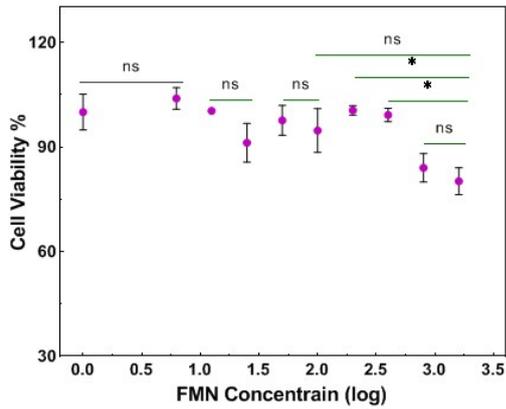


Figure S10. Comparison of cell viability at different concentrations of FMN photoinitiator ( $p < 0.0018$ , one-way ANOVA, Tukey's post-test).

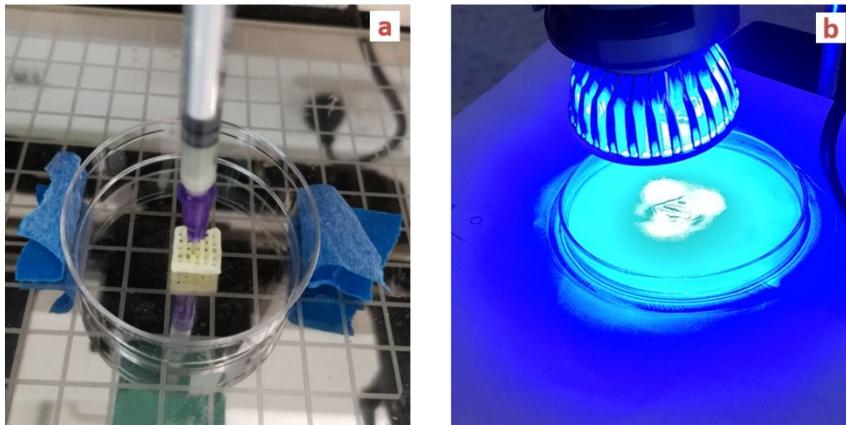


Figure S11. (a) Printing the whole layers of grid shape scaffolds of 3NF-0.5Ca ink contains 0.5wt% gelatin, (b) photocrosslinking the completed printed structure.

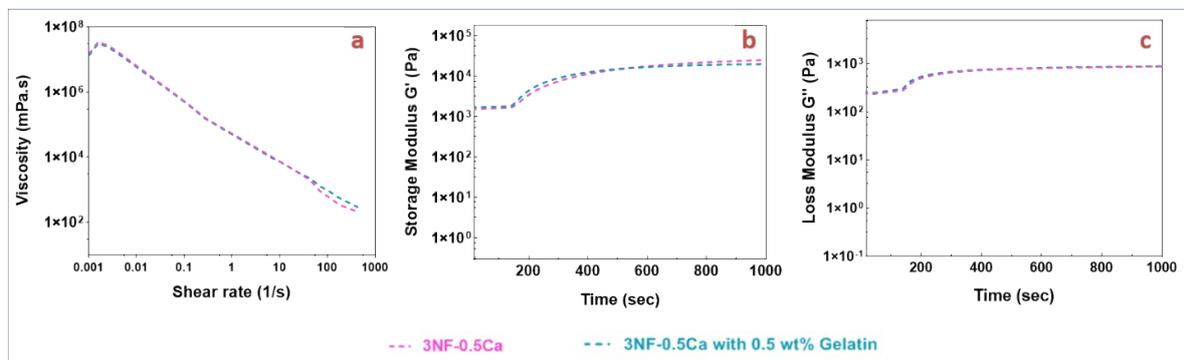


Figure S12. Effect of gelatin on the viscosity of PEG-CNFs ink and gelation kinetics. (a) variation of PEG-CNFs hydrogels viscosity over the shear rate for 3NF-0.5Ca with and without 0.5wt% gelatin. Change of (b) storage moduli and (c) loss moduli of the 3NF-0.5Ca hydrogels with and without 0.5 wt% gelatin in the mixture after blue light irradiation under oscillatory analysis; the light was switched on at 120 s and continued until 1000 sec.

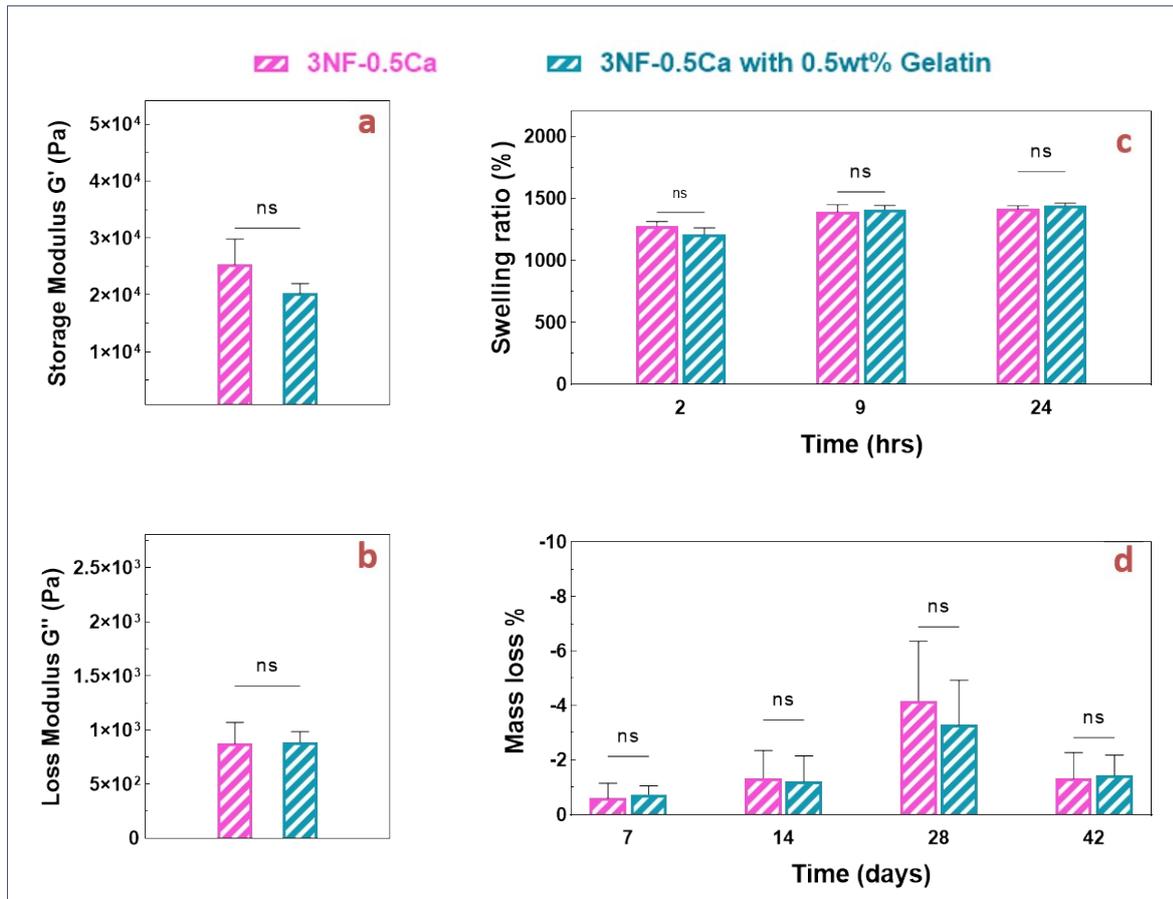


Figure S13. Effect of gelatin on strength of PEG-CNFs hydrogels, water uptake, and degradation. Final (a) storage moduli and (b) loss moduli of the 3NF-0.5Ca hydrogels with and without 0.5 wt% gelatin in the mixture (unpaired t-test). (c), (d) Equilibrium mass swelling ratios and mass loss of 3NF-0.5Ca group with and without 0.5 wt% gelatin over time, respectively ( $p < 0.0001$ , Two-way ANOVA, Sidaks's post-test,).

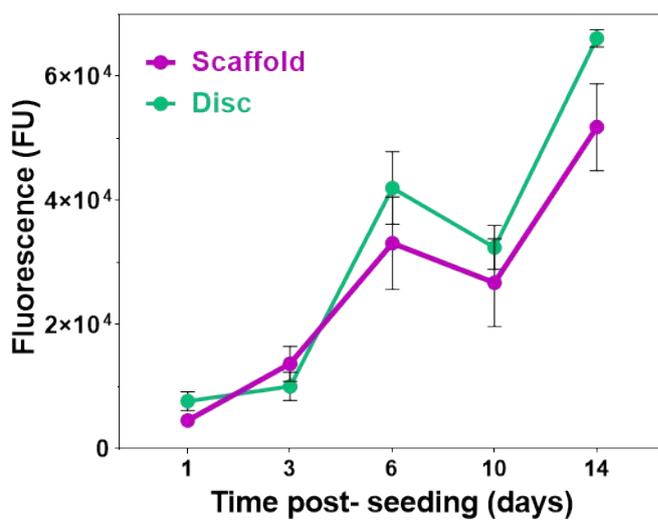


Figure S14. Fibroblast cell proliferation on hydrogel over 14 days quantified using Alamar blue cell metabolic assay with absolute fluorescence intensity (Two-way ANOVA, Sidaks's post-test,  $p < 0.0001$ ).

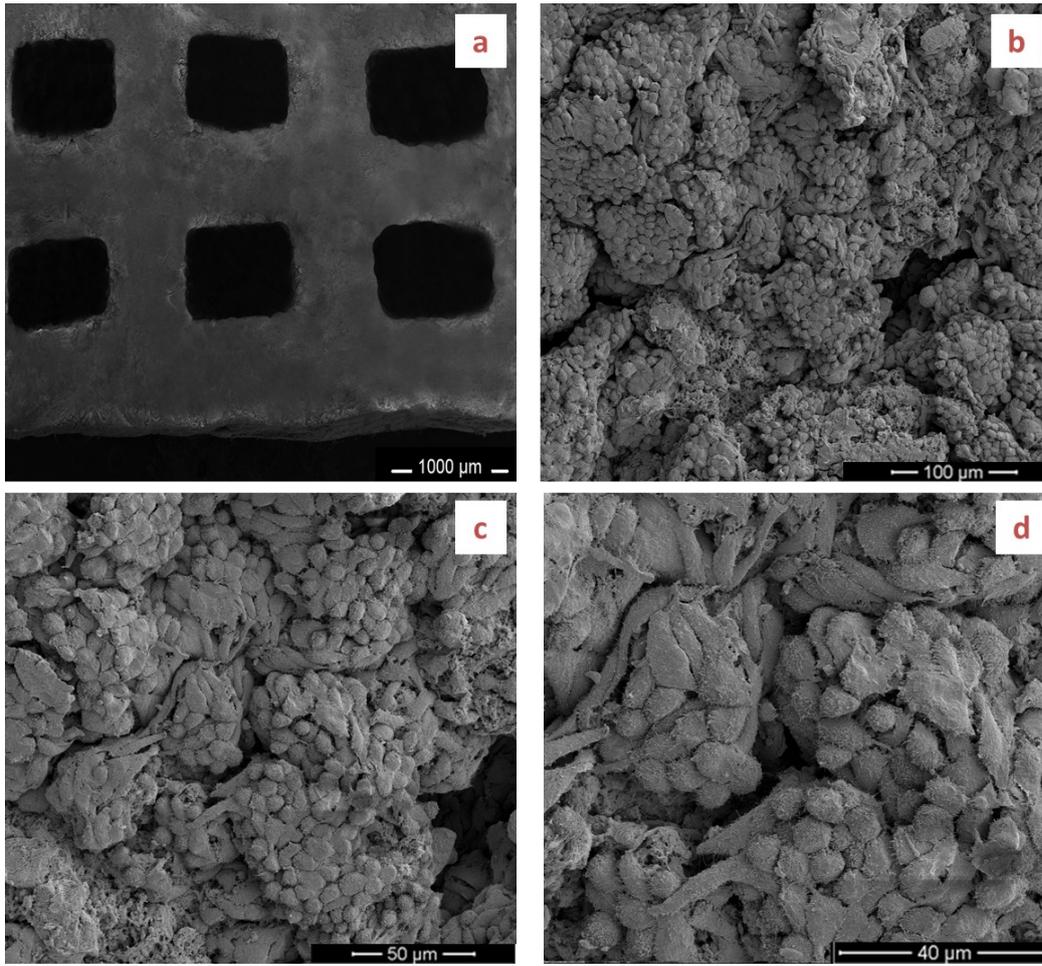


Figure S15. SEM images of the (a) printed scaffolds from cross-section view, (b-d) scaffold post seeded with L929 cells on day 14.