Supplemental Information

For

Engineering Neuronal Networks in Granular Microgels to Innervate Bioprinted Cancer Organoids On-a-Chip

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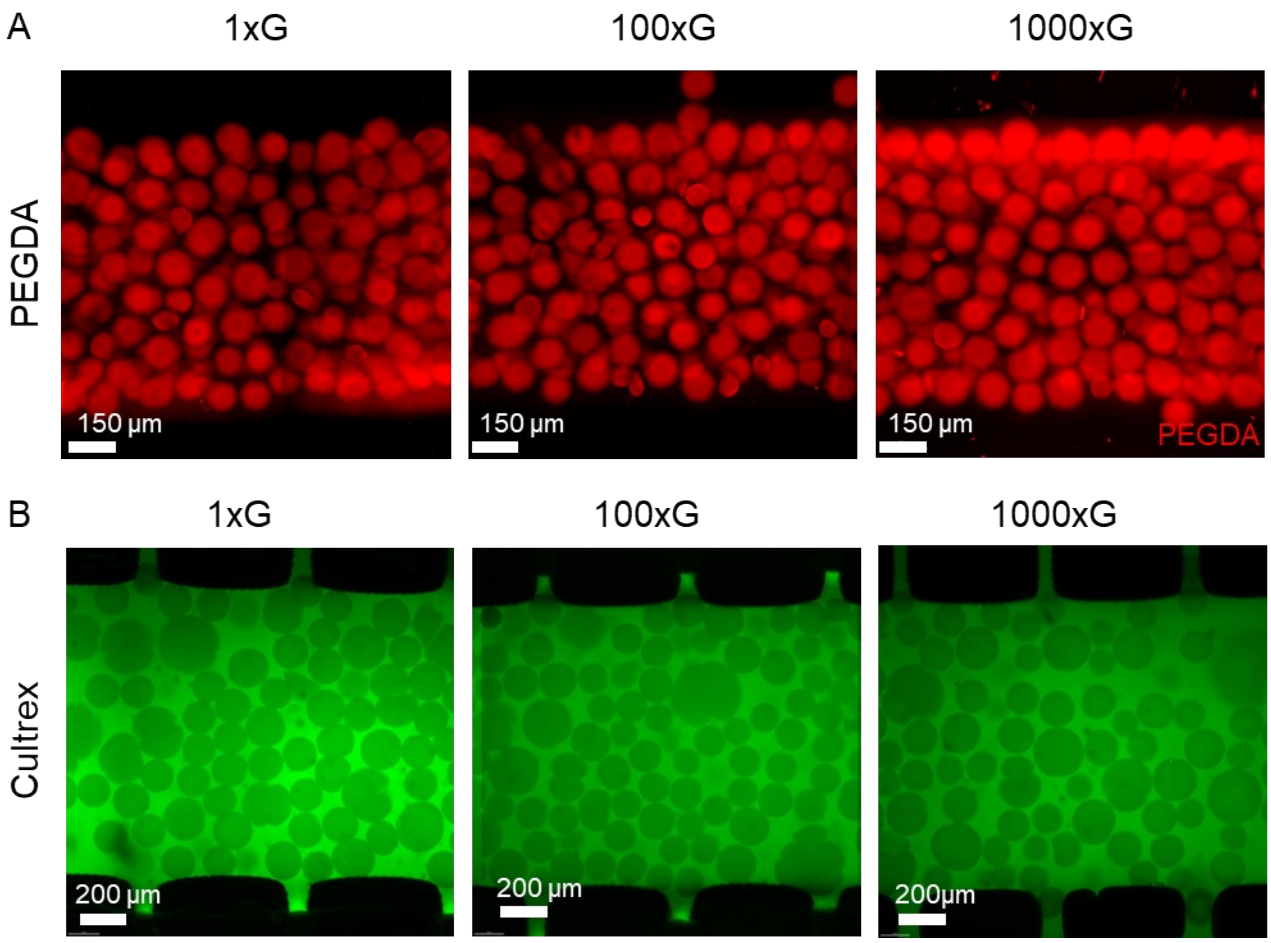
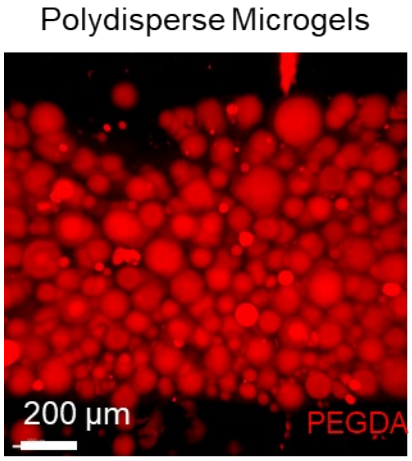
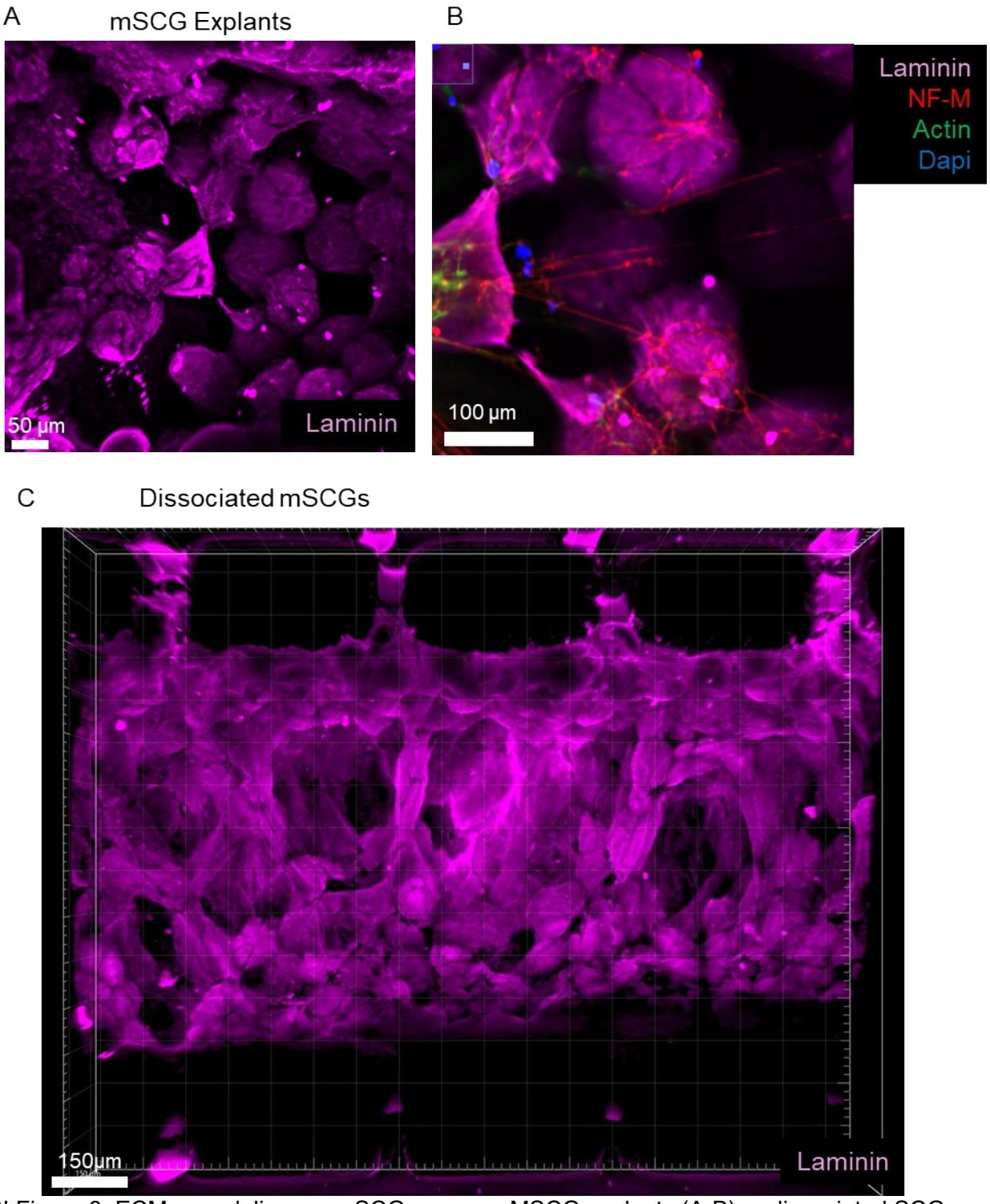


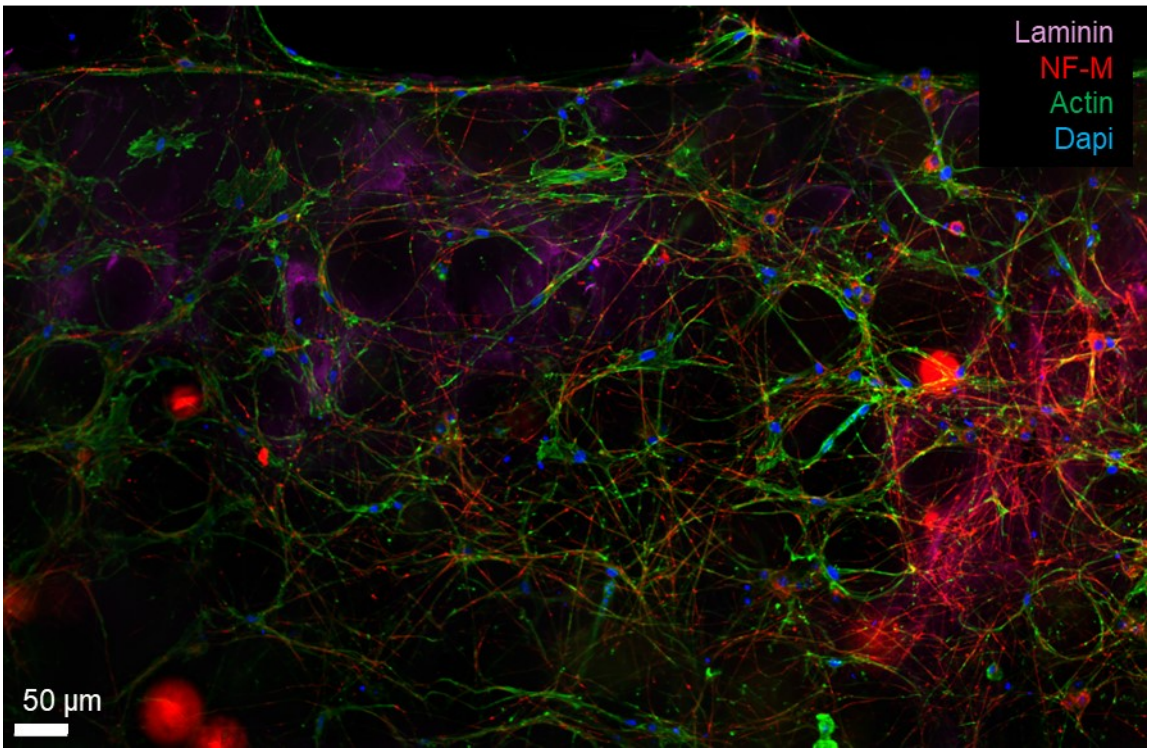
SI Figure 1: Microgel Packing Density. PEGDA (A) or Cultrex (B) microgels were loaded into the device and allowed to settle by gravity or were centrifuged at 100-1000 xG for 20 seconds



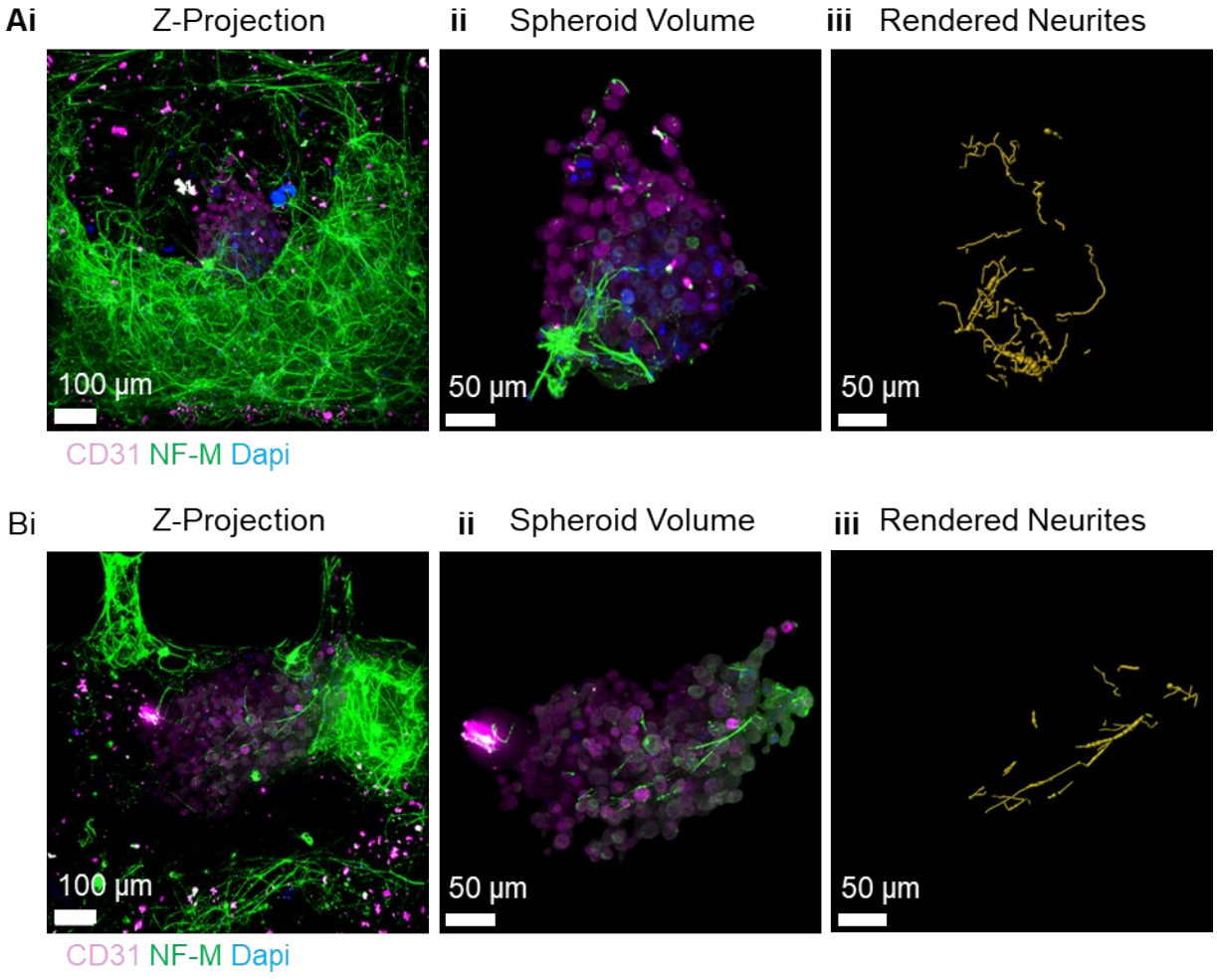
SI Figure 2: Polydisperse PEGDA microgels packed on the device

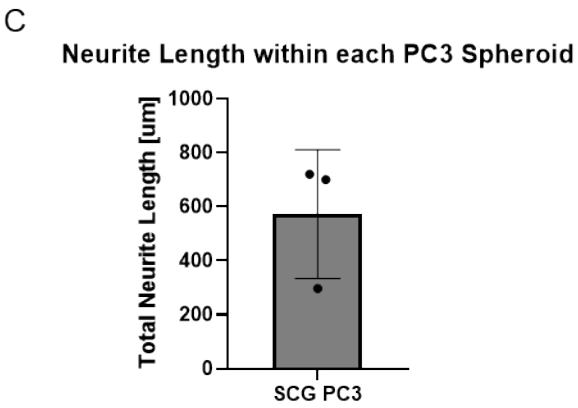


SI Figure 3: ECM remodeling my mSCG neurons. MSCG explants (A-B) or dissociated SCGs (C) were grown on chi and fixed. Antibodies for laminin were used to visualize the Cultrex microgels.



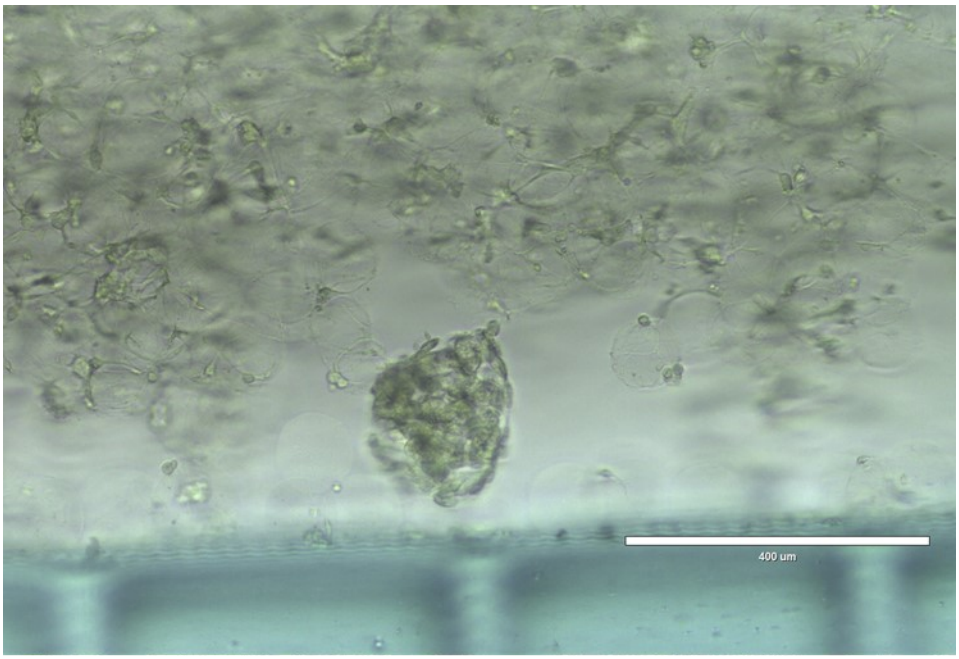
SI Figure 4: The bottom of the device cultured with dissociated mSCG neurons.



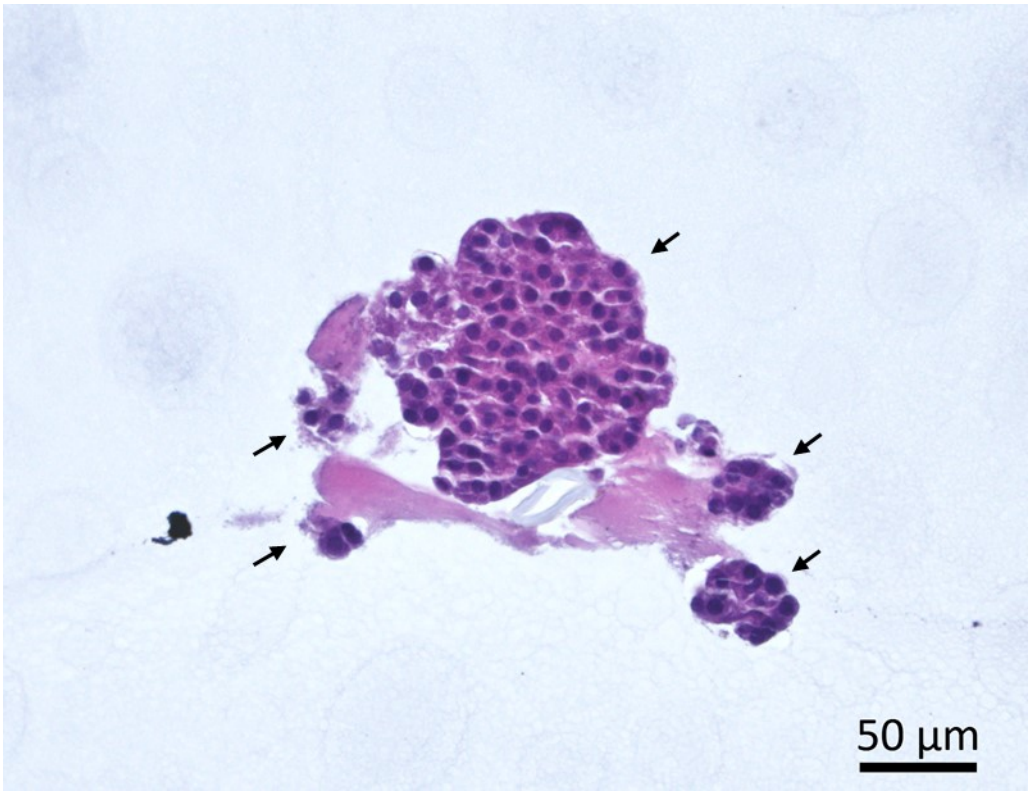


SI Figure 5: Innervation of prostate cancer spheroids. Dissociated mSCG neurons were cultured in a granular Cultrex hydrogels for 1 day. Subsequently, PC3 spheroids were placed into the granular hydrogel using aspiration assisted bioprinting an cultured for 2 days prior to fixation. Samples were stained for nuclei (Hoechst), CD31 (to distinguish PC3 cells), and NF-HM(Axons). (Ai, Bi) A z-projections of an innervated PC3 spheroids. (Aii, Bii) Surface

tools in Imaris were used to render the spheroid volume and exclude staining outside of the spheroid. (Aiii, Biii) Filaments tools in Imaris were used to trace neurites within the spheroid. Images are a Z-projections from the top and side views. (C) Graph of the total neurite length within each spheroid.

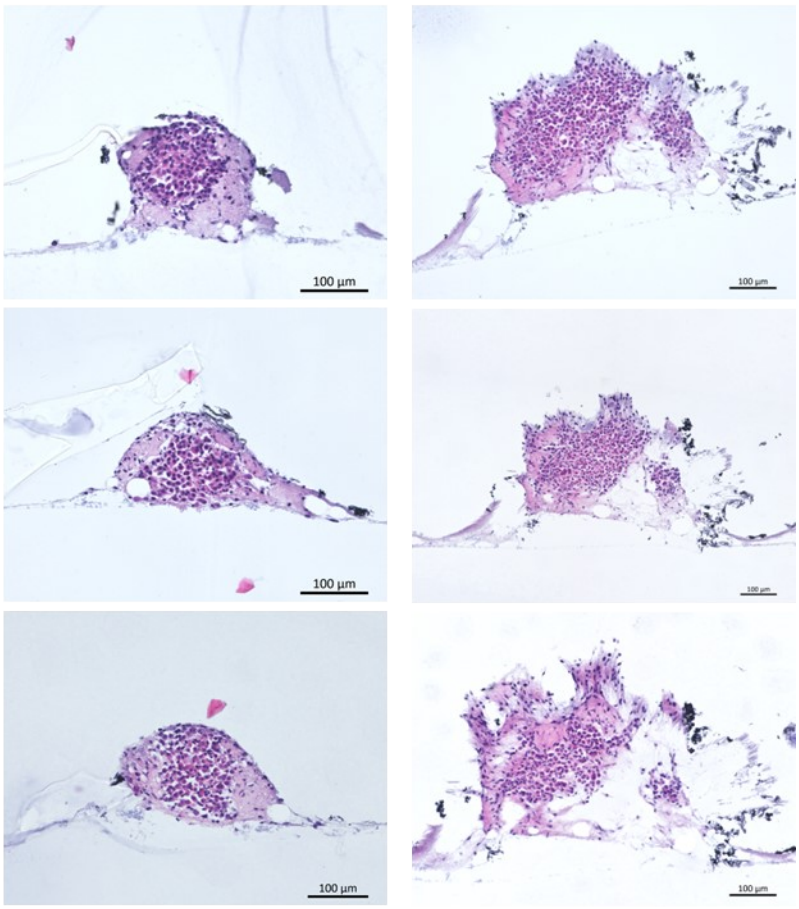


SI Figure 6: Representative brightfield image of a PC3 spheroid in the innervated granular hydrogel after 1 day of coculture.

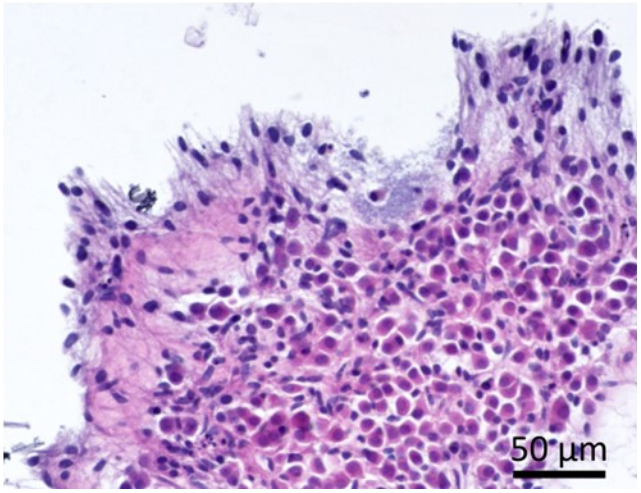


SI Figure 7: Hematoxylin and eosin stained FFPE section of extracted organoids from chip, sectioned at 5µm in the Z plane. Arrows in left panel indicate multiple sizes of organoids in view.

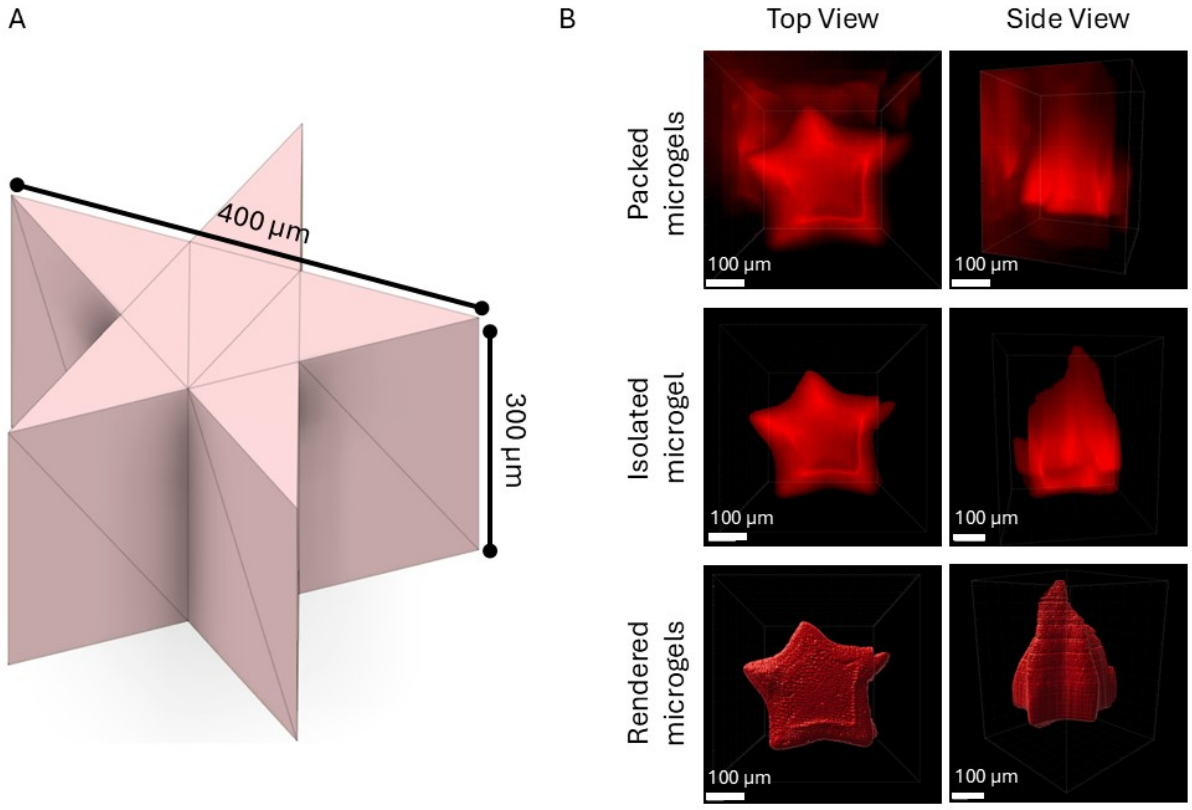
A



B



SI Figure 8: (A) Histological section through Z plane of extracted mSCG explant grown on open-top chip, stained with hematoxylin and eosin. Sections were cut from FFPE blocks at 5µm thickness. (B) Crop highlighting projections from explant core to periphery.



SI Figure 9: (A) CAD Drawing used for 3-D printed microgels. (B) Renderings of a single 3-D printed microgel from the device packed with geometrically controlled microgels.

SI Movie 1: SI Movie 1: Z-stack of mSCG explants. Blue = Nuclei, Green = Actin, Red = NF-M, Pink = Laminin (Cultrex microgels).

SI Movie 2: Z-stack of dissociated mSCG neurons. Blue = Nuclei, Green = Actin, Red = NF-M, Pink = Laminin (Cultrex microgels).

SI Movie 3: Z-stack of innervated PC3 spheroid volume. Blue = Nuclei, Green = NF-M, Pink = CD31.

SI Movie 4: Z-stack of innervated RCC organoids. Blue = Nuclei, Green = Actin, Red = NF-M, Pink = KI67.