Electronic Supplementary Material (ESI) for Biomaterials Science.

Bicyclic RGD peptides enhance nerve growth in synthetic PEG-based Anisogels

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Additional supplementary data on 3D homogeneous distribution of fibroblasts and DRGs in PEG hydrogels, circularity and edge effect quantification, proliferation assay comparing Q-RGD and K-RGD and comparative study of fibroblast cell growth in 3D PEG hydrogels with Q-RGD and FN*.

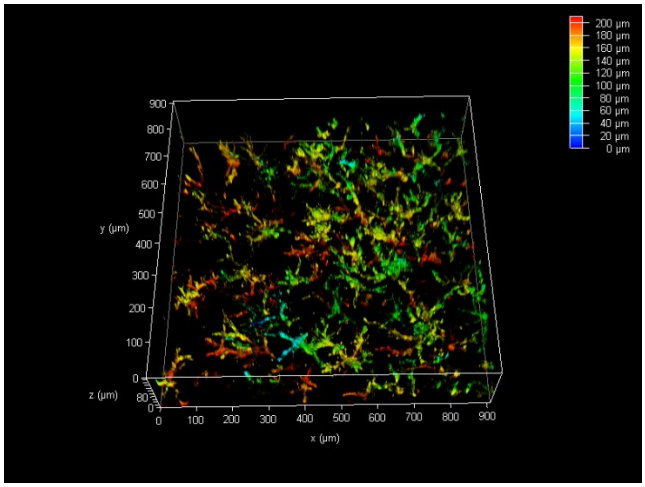

Figure S1. L929 fibroblasts were cultured in PEG hydrogels tethered with K-P2 RGD bicyclic peptide, fixed and immunostained for actin fluorescent markers, and imaged with a minimum z-stack size of 200 µm (color-coded), showing homogeneous cell distribution.

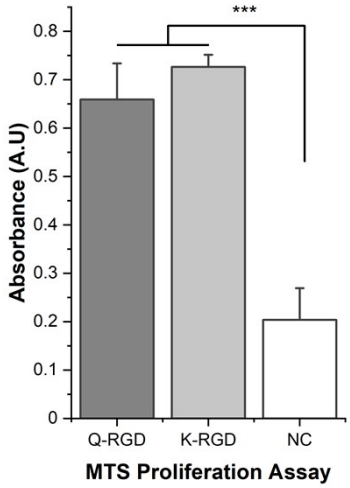

Figure S2. A similar RGD potency is observed between Q-RGD and K-RGD when a proliferation assay is performed with L929 fibroblasts cultured on thin PEG substrates coupled with Q-RGD (100 µM) or K-RGD (100 µM).

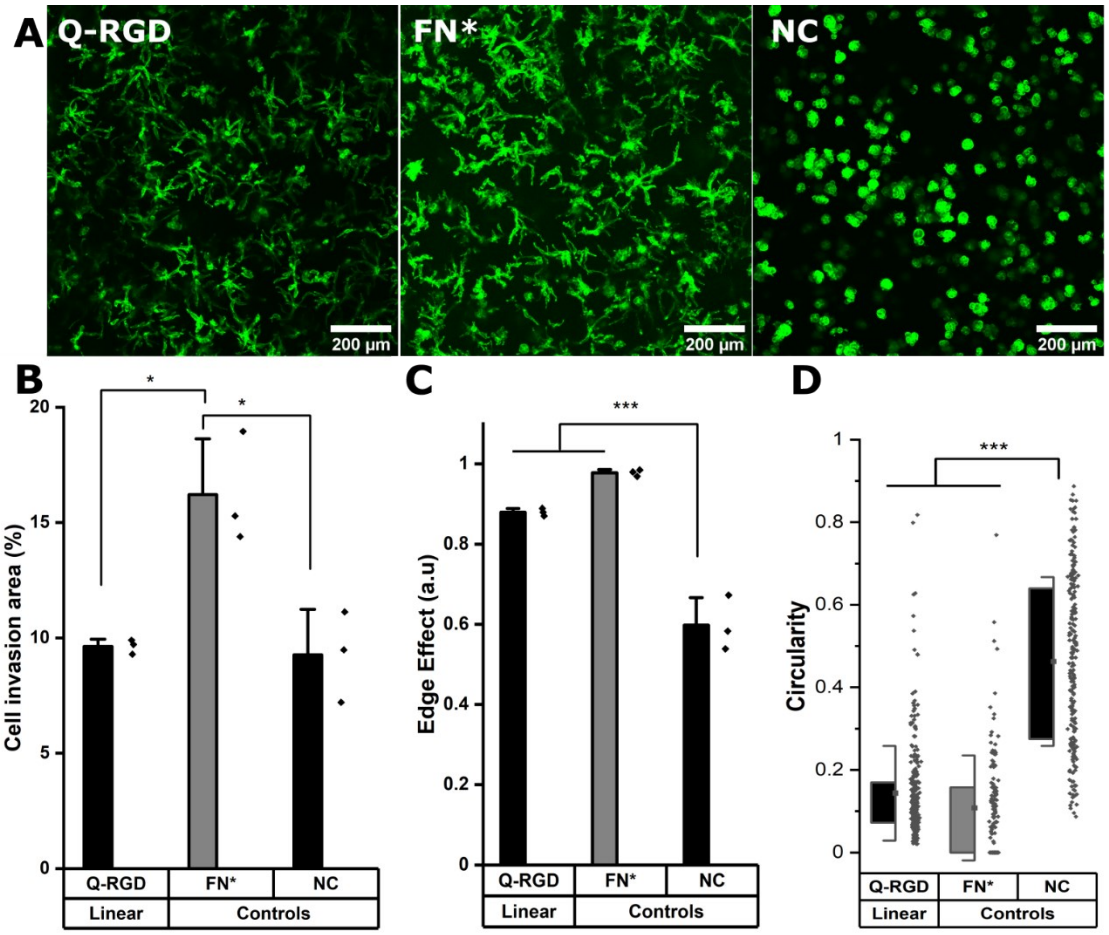

Figure S3. A) L929 cells encapsulated in PEG hydrogels tethered with Q-RGD and FN* showing actin filament (green) spreading across multiple planes in comparison to the negative control (NC) where the cells are predominantly rounded. B) Superior cell invasion is observed in FN* compared to Q-RGD and NC, however, the C) Edge effect and D) Circularity show that the cells are spreading well in Q-RGD and FN*.

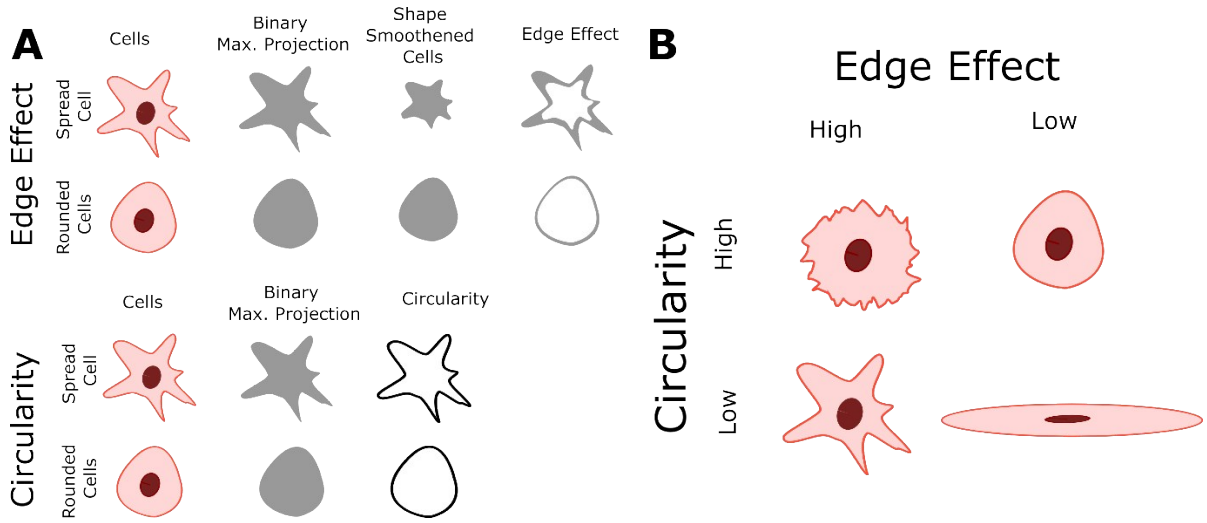

Figure S4. A) Schematic representation of stages in quantifying edge effect which is a combination of shape factor as well as the local protrusions, and circularity which measures the roundness of the cells. B) Interdependence of circularity and edge effect resulting in different cell morphologies relevant in 3D cell growth.

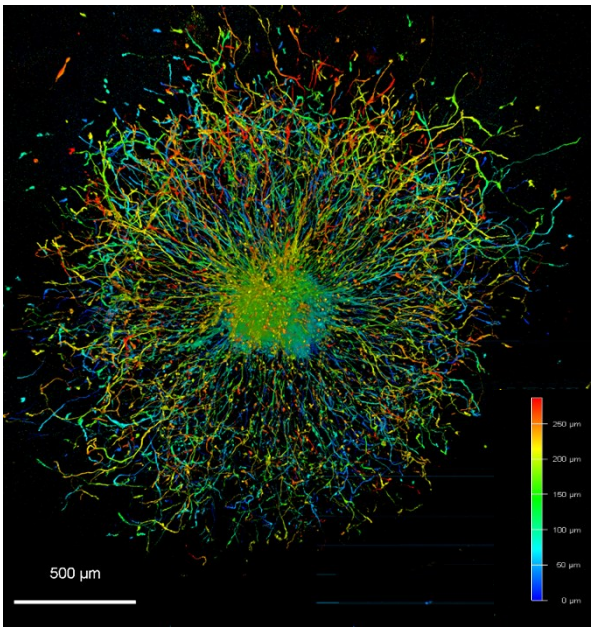

Figure S5. DRGs are cultured for 7 days in PEG hydrogels tethered with K-P2 RGD bicyclic peptide, fixed and immunostained for β-tubulin fluorescent markers, and imaged with a minimum z-stack size of 200 µm (color-coded).