Supplementary Material

Penetration of Endothelial Cell Coated Multicellular Tumor Spheroids by Iron Oxide Nanoparticles

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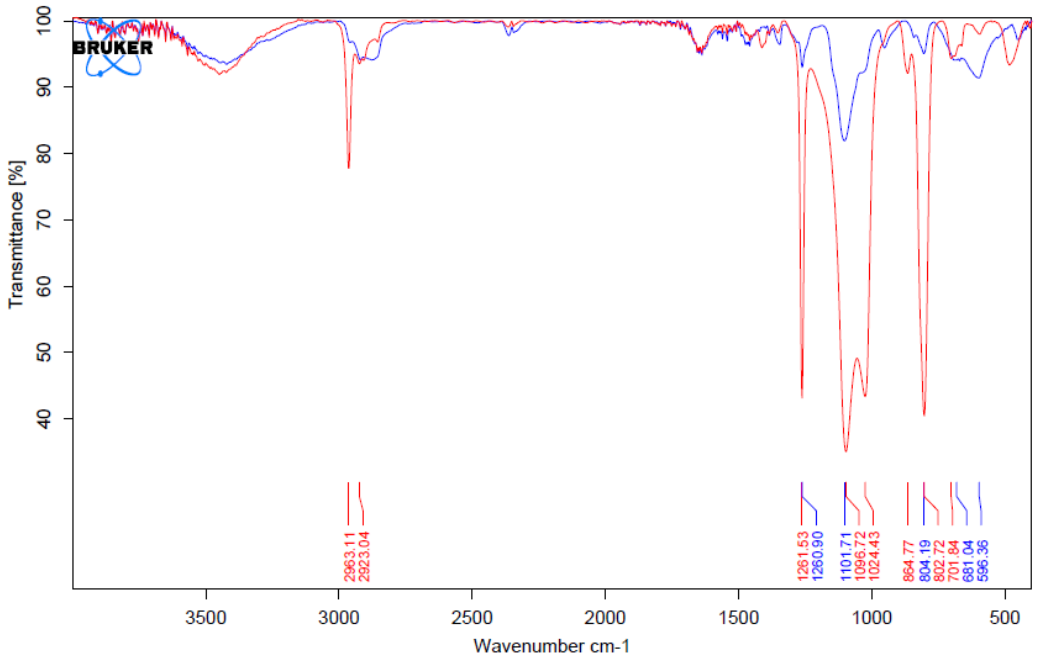

Figure S1: Fe_3O_4 NP in water after solubilization following ligand exchange with DPA-PEG(6000)-COOH show no strong presence of peptides before tumstatin conjugation (blue). After tumstatin conjugation and purification, tumstatin peptide peaks are observed with the nanoparticles (red).

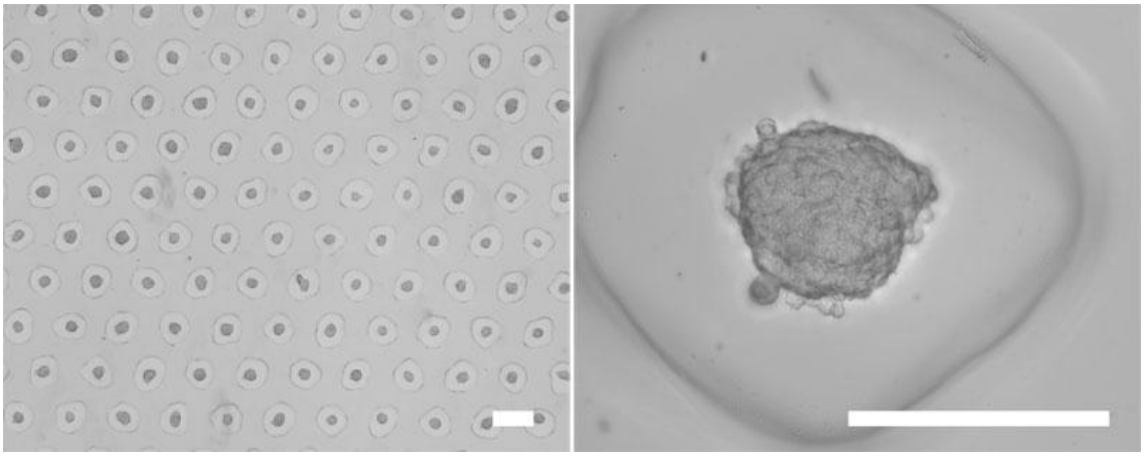

Figure S2: Assembled RG2 spheroids 24 hours after seeding in non-adhesive, micomolded hydrogels. Scale bars are 400 and 100 microns, respectively.

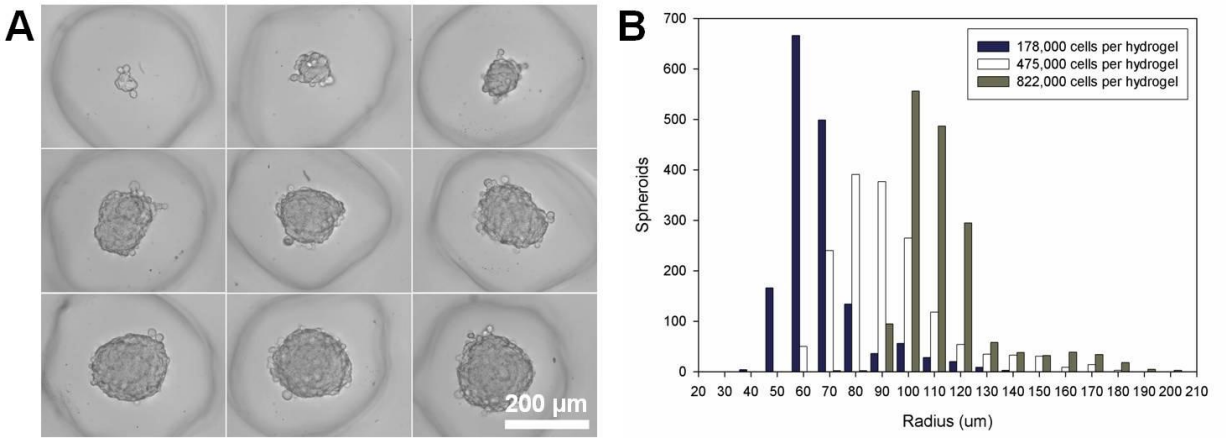

Figure S3: Size control of RG2 MTS through controlling the seeding densities. (A) Images of RG2 MTS formed at seeding densities that form spheroids with approximately 55, 108, 217, 433, 578, 770, 1027, 1521, and 1825 cells after 24 hours (top left to bottom right). (B) The size of assembled RG2 MTS fit with a Gaussian distribution with seeding densities of 178,000, 475,000, and 822,000 cells per gel after 24hours as measured with image analysis software.

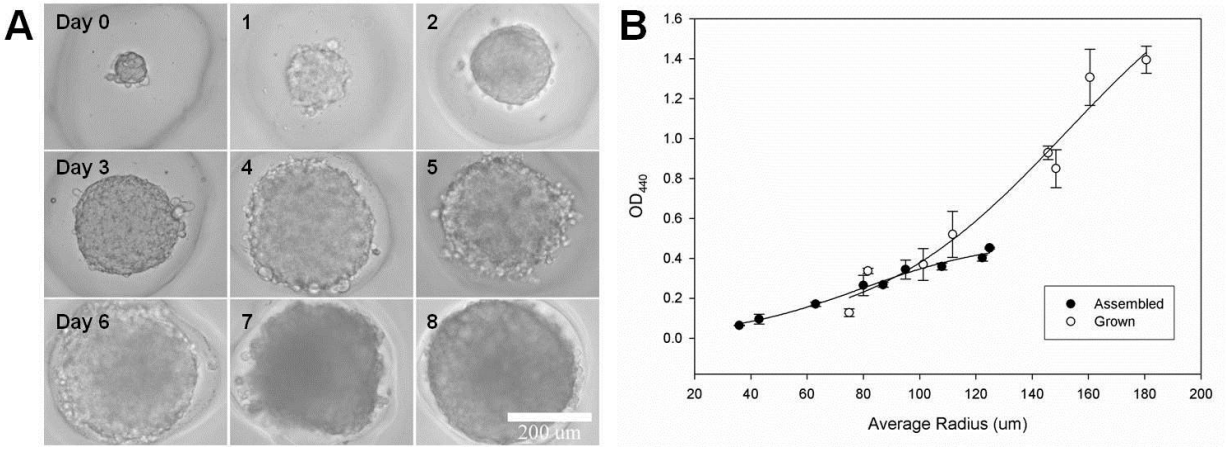

Figure S4: (A) Assembled MTS (day 0) and growth (day 1-8) of RG2 MTS following the cell seeding. (B) The assembly and growth process of MTS was accompanied with the increased metabolic activity, which indicated the proliferation of cells.

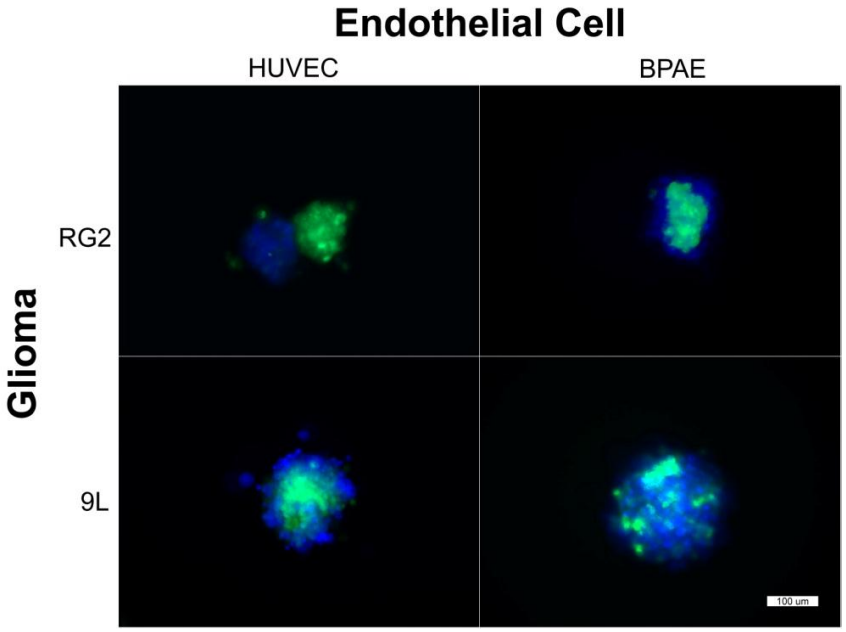

Figure S5: Assembly of RG2 glioma cell line (top, Green) for MTS formation with HUVEC (blue) or BPAE (blue) in separate spheroid assembly and endothelial-coated glioma assembly. In contrast, 9L glioma cell line (bottom, green) assemble with a patched coating with HUVEC (blue) and an indiscriminate mixture spheroid with BPAE (blue).

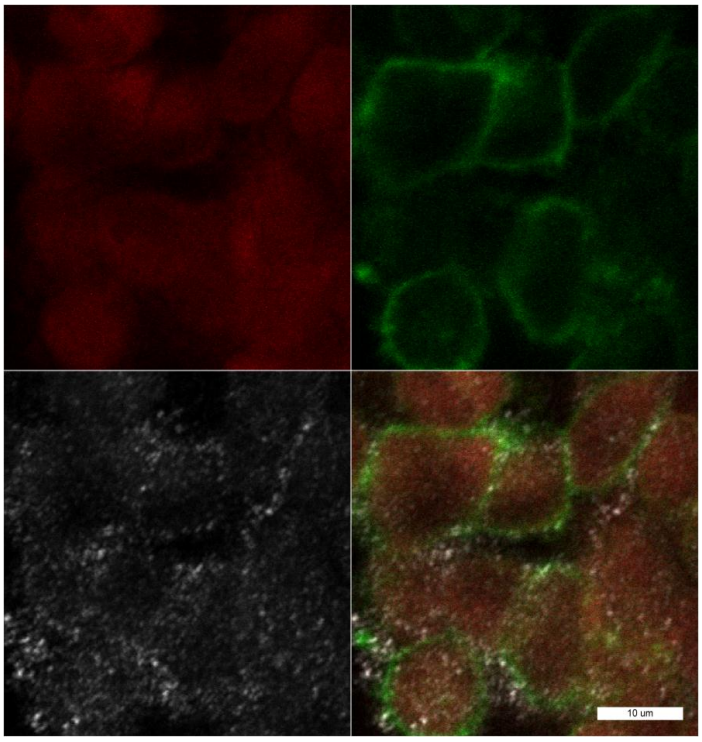

Figure S6: Tight junction formation is present in BPAE-coated RG2 heterogeneous spheroids. Tight junction markers ZO-1 (red) and Occludin (white) co-localize with actin cytoskeleton (green) at cell-cell contact regions.

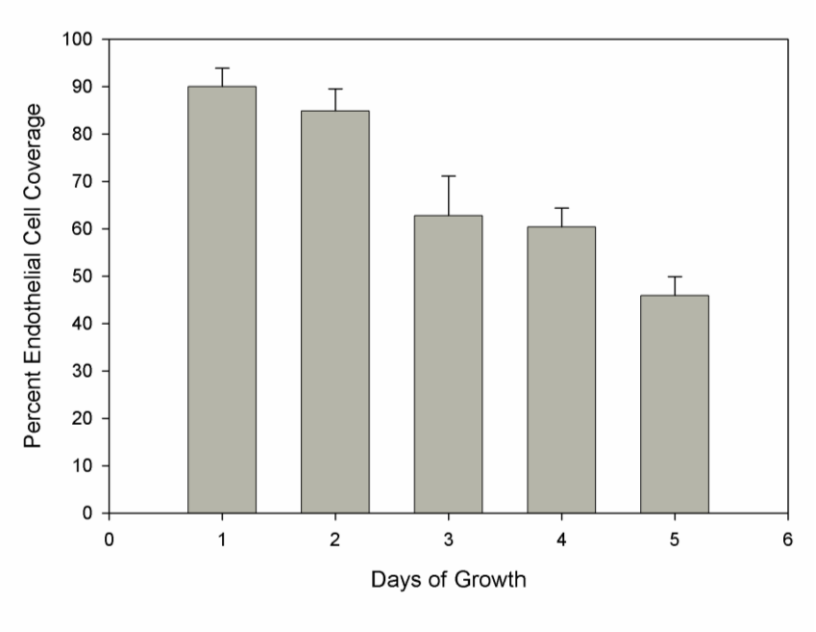

Figure S7. BPAE-Coated RG2 maintain high endothelial cell coverage for 2 days at 90% and 85% while coverage of the MTS decreased to 60% and below from day 3.

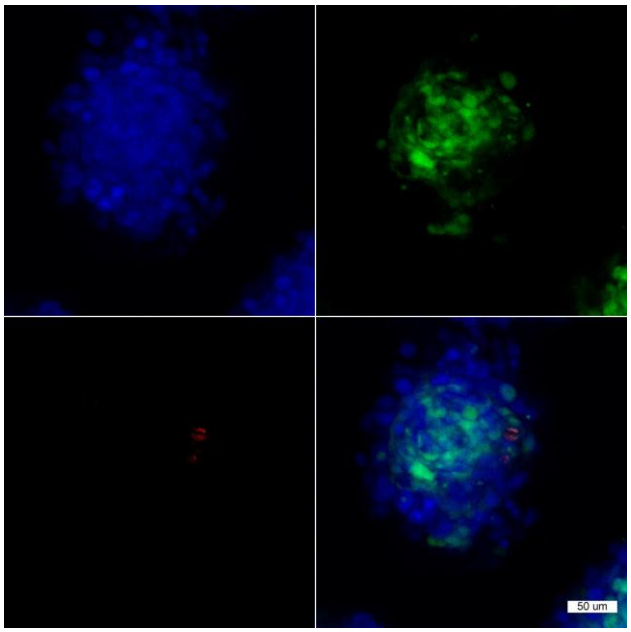

Figure S8. BPAE-Coated RG2 treated with Fe_3O_4-Rhod control. BPAE (top left), RG2 (top right), Iron Oxide-Rhod (bottom left), and Overlay (bottom right).

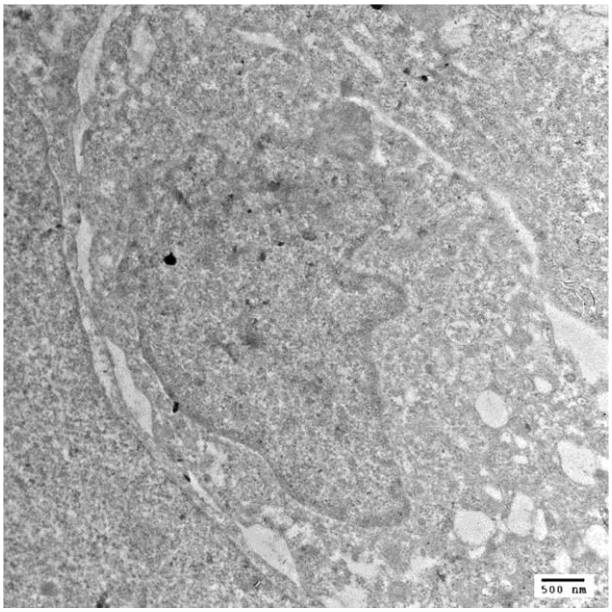

Figure S9. RG2 cells in the core of BPAE-Coated RG2 treated with tumstatin-Fe_3O_4 show little to no uptake under TEM.