Hydrogel-based *in vitro* Glioblastoma Spheroid Models

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Statement of Purpose: Multicellular spheroids are well-accepted *in vitro* cancer models that are routinely used for cancer therapy development. Spheroids are typically grown on non-adhesive surfaces in the absence of cell-matrix interactions. Here, we developed a novel *in-vitro* hydrogel-based glioblastoma spheroid model by encapsulating glioblastoma cells in uniform polyethylene glycol (PEG) hydrogel microspheres. The hydrogel microspheres are encapsulated inside a bulk material, allowing the microspheres to degrade and multicellular aggregates to form in the hydrogel slabs. We are currently investigating the hypothesis that tumor spheroids grown in hydrogel-based spheroids will have different characteristics than liquid-grown spheroids. We also hypothesize that spheroid characteristics will be dependent on the stiffness of the hydrogel, namely normal versus cancerous tissue stiffness. The properties under consideration include spheroid growth in terms of cell viability, proliferation, and drug responsiveness.

Methods: Degradable cell-laden hydrogel microspheres were fabricated via manual pipetting or microfluidics. Microspheres were prepared from 4-arm PEG-acrylate (Ac) and DTBA or PEG-di-2-mercaptoethanoate (PEGDD-1) crosslinkers and then, encapsulated in slab gels made from PEG-diacrylate (PEGDA). Glioblastoma U87 and JX-12 cells were loaded in the microspheres by adding them directly to the precursor solution. The microsphere precursor solution was loaded into a volumetric pipette and droplets were collected in a collector of olive oil and allowed to gel for 10 minutes, then collected via centrifugation, and washed with buffer to remove oil. To fabricate microspheres via microfluidics, a custom T-junction droplet generator was developed and a 100 µl gas-tight syringe was loaded with microsphere precursor solution, placed on a micropump that was connected to the top port of a mixing tee via a luer lock and tubing. A 60 ml syringe loaded with olive oil was placed on a micropump and connected to the side port of mixing tee via luer lock and tubing. Lastly, tubing was connected to the remaining side port and placed in an olive oil bath for droplet collection. Microspheres were then placed in a well plate surrounded by a cloning ring. The slab gel precursor solution was added on top of the microspheres and allowed to gel for 30 minutes. The constructs were cultured at standard cell culture conditions for up to 2 weeks, during which time the microspheres degraded, leaving cells behind to form multicellular aggregates inside the bulk gel slab. For drug screening, cells were cultured in the PEG hydrogels and PEG hydrogels with 1% w/v 4-arm PEG-RGDS (i.e. 1% w/v 4-arm PEG-RGDS + 9% w/v PEGDA) to elicit cell attachment for 7 days and bolus drug doses of paclitaxel (PTX), doxorubicin (DOX), carmustine (BCNU), lomustine (CCNU) and temozolomide (TMZ), were added to the cell medium at concentrations ten times higher than the reported IC_{50} for monolayer cells seeded on TCP. Live/dead staining was performed to assess cell viability.

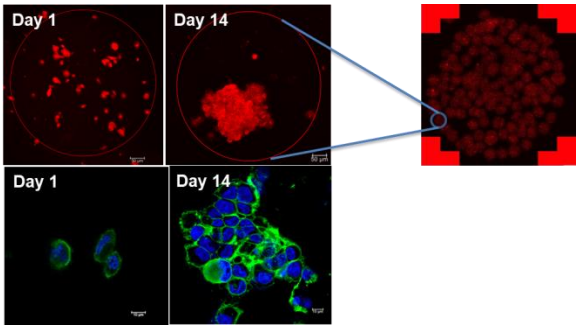

Figure 1: Culture of U87 cells encapsulated in degradable PEG microspheres formed spheroids within 14 days. Fluorescent images of microsphere with encapsulated U87 stained using AO, DAPI, and PI. Scale bar = 50 µm.

Results: The 4-arm PEG-Ac and PEGDD-1 microspheres degraded in 24 h and the microspheres made with DTBA in 6 days, leaving spherical pores behind into which the cells were deposited. For non-confined microspheres, the microspheres were completely degraded at 24 h in serum medium, which was quantified by the increase in microsphere size. In contrast, it was observed that confined microspheres had a slower degradation time of 7 days in serum medium and resulted in a decrease in microsphere size. For cell encapsulation, the cells were allowed to grow for up to 2 weeks to form multicellular spheroids (**Figure 1**) within the hydrogel slabs. The multicellular spheroid model supported spheroid growth and cell viability of >90%, demonstrating the suitability of the gel constructs for prolonged cell culture. In addition, a small drug screen with five FDA-approved anti-cancer drugs: PTX, DOX, BCNU, CCNU, and TMZ, was conducted to demonstrate how spheroid-matrix interactions affect drug responsiveness. PEG gels with and without RGDS were used. Our results show that all tested drugs led to reduced cell viability compared to no drug control and that RGDS presence (elicits integrin binding) reduced spheroid sensitivity to CCNU and TMZ.

Conclusions: In conclusion, we developed a hydrogel-based multicellular spheroid tumor model of uniform size and varying stiffness. Glioblastoma cells encapsulated in the spheroids were able to retain high cell viability over 14 day period and form cell spheroids. A small drug screen demonstrated that spheroid drug responsiveness is affected by matrix properties. Ongoing work is aimed at characterizing the effect of matrix mechanical and biochemical properties on tumor spheroid growth.