3D In Vitro Vascularized Liver Cancer Model

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In order to devise an in vitro model which accurately recapitulates drug interactions with cancer, a vascularized 3D liver cancer model is devised using tissue engineering techniques. Anti-angiogenic cancer drugs aim to suppress cancer by halting the ability of cancer to use angiogenesis, the process in which new blood vessels are grown, in order to supply itself with gases and nutrients. So far anti-angiogenic drugs have been met with limited success prompting the development of better models to predict the outcome of using these types of drugs.

To this end, we propose an in vitro model which incorporates spheroids as a model of cancer and eventually combine it with a tissue engineering blood vessel construct to mimic cancer angiogenesis and drug reaction. Spheroids containing HepG2, fibroblasts and endothelial cells are incorporated in Matrigel to grow blood vessel sprouts. To confirm that the resulting structures are indeed blood vessel sprouts, immunohistochemistry is employed using CD31 antibodies and positive staining confirms their endothelial composition and thus are blood vessel sprouts. The length of these blood vessel sprouts are used as a measure of the effect of various dosages (0μM/control, 5μM, 10μM) of either anti-angiogenic drug sorafenib or chemotherapy drug doxorubicin. Exposure of anti-angiogenic drug sorafenib to spheroids composed with HUVEC shows that 5μM/10μM conditions are significantly lower than the 0μM/control condition over the course of 7 days. ($n=10$, $P<0.005$) Similarly exposure of sorafenib to spheroids composed with RAEC shows that 5μM/10μM conditions are significantly lower than the 0μM/control condition on days 4 and 7 day. ($n=10$, $P<0.005$) Exposure of chemotherapy drug doxorubicin shows that 5μM/10μM conditions are significantly lower than the 0μM/control condition over the course of 7 days, except for 5μM to 0μM on day 1**.** Overall, the presence of the drugs are able to arrest/decrease blood vessel sprouting compared to the control condition. Furthermore, exposure of spheroids to the drugs show a decrease in viability as revealed by LIVE/DEAD assay. These results match what is expected in vivo.

To assemble the blood vessel construct, chitosan is first wetspun by injecting a syringe of the substance into a NaOH:EtOH bath. This fiber is then crosslinked with heparin using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and n-hydroxysulfosuccinimide (NHS) crosslin**k**ing agents and coated with fibronectin to promote eventual endothelial cell adhesion. FTIR of chitosan, heparin, and crosslin**k**ed heparin to chitosan shows similar pea**k**s at 1230 nm^{-1} and 1040 nm^{-1} representing sulfate groups specific to heparin. Toluidine Blue staining, which stains sulfates as found on heparin, is also used and shows purple and thus positive staining of crosslin**k**ed heparin to chitosan fiber versus a plain chitosan fiber. Thus FTIR and Toluidine Blue staining shows successful heparin crosslin**king.** Phase imaging and subsequent LIVE/DEAD imaging following exposure of such a fiber to endothelial cells shows that endothelial cells are both able to adhere and survive on the fiber.

The spheroid model and blood vessel construct are then combined and their interactions observed under microscope resulting in three scenarios: no interaction, migration, and/or anastomosis. In the migration scenario, the spheroid migrates onto the blood vessel construct. In the anastomosis scenario, the blood vessel sprouts emitted from both the spheroid and the blood vessel construct interact with one another.

In conclusion, an in vitro model which incorporates both cancer and vasculature is exposed to various dosages of anti-angiogenic or chemotherapy drugs in an attempt to mimic the effect of these drugs on cancer driven angiogenesis. In the future, a blood vessel construct would be included alongside the spheroid model to better mimic the in vivo conditions of angiogenesis.