Engineered Tumor as an in Vitro Platform for the Assessment of Nanoparticle Drug Delivery System

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Statement of Purpose: There is a critical need to develop in vitro tumor models to study cancer biology with the purpose of interfering with cancer cell growth and development. When cultured in a 3D system in the presence of extracellular matrix molecules, cancer cells display different expression and activation profiles than they do when cultured on plastic. To better study cancer cell behavior, we have developed a hyaluronic acid (HA) based, in situ crosslinkable hydrogel system to encapsulate living cancer cells. We show that this system maintains cell growth and viability and that cancer cells within the HA hydrogel form a distinct aggregated structure reminiscent of native cancer tissue. The efficacy of the nanoparticle drug delivery systems was tested using the engineered tumor model. The 3D tumor model provides a more flexible, accurate and quantifiable way for screening the nanoparticulate cancer therapeutics.

Methods: HA derivatives carrying complementary functional groups, aldehyde (HAALD) and hydrazide (HAADH), were synthesized and characterized as previously described.[1] C4-2B cell pellet was dispersed in HAALD solution (1wt%) and were subsequently mixed with HAADH (1wt% in PBS). The mixture was pipette into a cell culture insert and was incubated for 10 min at 37°C before the medium was added. The cell/gel construct was incubated at 37 °C and 5.0% (v/v) CO_2. Cells cultured on 2D TCPS were used as the control. Cell viability was monitored by trypan blue exclusion. Immunostaining was performed to assess the cytoskeleton organization using AlexaFluor 488 phalloidin and Draq5.[2] Separately, amphiphilic block copolyesters carrying cyclic ketals in the hydrophobic segments were synthesized by ring opening polymerization of ε-caprolactone (CL) and 1,4,8-trioxaspiro-[4,6]-9-undecanone (TSU) using the free hydroxyls of methoxy polyethylene glycol (mPEG, 5000 g/mol) as the initiator and stannous octoate ($Sn(Oct)_2$) as the catalyst. Camptothecin (CPT)-loaded nanoparticles were prepared by nano-precipitation followed by extensive dialysis against DI H_2O. CPT-loaded nanoparticles were injected into a dialysis bag and CPT release was monitored using FluoroMax 4.[3] CPT-loaded nanoparticles were added into the media surrounding the cell/gel constructs and the apoptosis was detected using live/dead assay and a Cell Death Detection ELISA kit.

Results/Discussion: An in vitro tumor model was created by culturing poorly adherent bone metastatic prostate cancer cells (C4-2B) in a 3D HA matrix established by the direct mixing of HAALD and HAADH in the presence of the cells. Unlike 2D monolayer culture in which cells adopt an atypical spread morphology, cells residing in the HA matrix formed distinct clustered structures which grew and merged, reminiscent of real tumors (Figure 1).

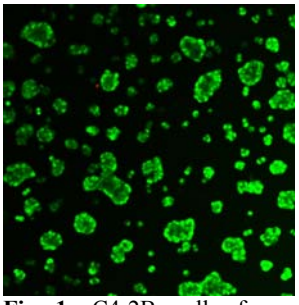

Fig 1. C4-2B cells form tumor-like aggregates in the HA matrix as revealed by live/dead assay.

Separately, we have developed a novel polymeric nanocarrier system that allows for controlled release of CPT. Amphiphilic block copolymers consisting of hydrophilic segments based on PEG and hydrophobic polyester bearing various amounts of pendent cyclic ketal groups were synthesized and characterized (Figure 2). Amphiphilic block copolymers with TSU content in the hydrophobic segments varying from 0, 14, 39 to 100 mol% were successfully synthesized. Compositional analyses indicate that TSU is randomly distributed in the hydrophobic blocks. When TSU content in the copolymers increased, the polymer crystallinity decreased progressively. Drug encapsulation was achieved during the particle assembly using a THF/DMSO/H_2O mixed solvent system. The particle size, drug encapsulation and drug release behavior are strongly dependent on the copolymer composition and crystallinity. At a TSU content of 14 mol%, CPT was released in a continuous and controlled fashion with a reduced initial burst and a 73% cumulative release by day 7.

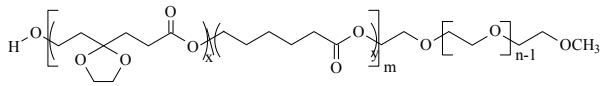

Fig 2. Chemical structure of amphiphilic block copolymers investigated.

The engineered tumor was used to assess the anticancer efficacy of CPT-loaded nanoparticles. Our results show that CPT-loaded nanoparticles added to the media surrounding the cell/gel construct were capable of killing the embedded cells. Modulating the physical characteristics of the amphiphilic copolymers via copolymerization offers a facile method for controlling the bioavailability of anticancer drugs. We suggest that the data obtained from 3D HA systems is superior to that from conventional 2D monolayers as the 3D system better reflects the bone metastatic microenvironment of the cancer cells.

References: (1) Jia, X. et al. *Biomaterials*, **2004**, *25*, 4797. (2) Gurski, L. A. *Biomaterials*, **2009**, *30*, 6067. (3) Wang, X. *Biomacromolecules*, submitted.