Enzyme-Mediated Stiffening Hydrogels for Studying Pancreatic Cell Malignancy

Hung-Yi Liu[1], Chien-Chi Lin[1,2]

[1] Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN 47907, USA

[2] Department of Biomedical Engineering, Indiana University-Purdue University Indianapolis, Indianapolis, IN 46202, USA

Statement of Purpose: Pancreatic cancer has extremely poor prognosis and current therapeutic strategies have not yielded significant improvement in patient survival rate. The intense stromal reactions in pancreatic cancer have been suggested to play a key role in tumor progression.[1] It is also believed that the behaviors of the cancer cells are partially affected by matrix stiffness,[2] potentially through an epithelial-mesenchymal transition (EMT) process.[3] In order to mimic a stiffening tumor, we have previously developed a PEG-peptide hydrogel system capable of undergoing on demand matrix stiffening.[4] This was achieved through adding additional tyrosine residues on the peptide linkers. The additional tyrosine motifs were susceptible to tyrosinase-mediated di-tyrosine crosslinking. In this work, we present another biomimetic hydrogel system by using norbornene (NB) and hydroxyphenylacetic acid (HPA) dually functionalized gelatin, which was crosslinked by thiol-modified hyaluronic acid (THA) through orthogonal thiol-norbornene photopolymerization. The additional HPA moieties permit tyrosinase-mediated di-tyrosine crisslinking, which led to *in situ* gel stiffening. The stiffening HA/Gelatin hybrid gels were used to study pancreatic cancer cell malignancy in three-dimension (3D).

Methods: Gelatin-norbornene (GtNB) was synthesized as described previously.[5] GtNB was further conjugated with HPA through carbodiimide chemistry[6] to yield dually functionalized GtNB(HPA) (Fig. 1A). Desired amounts of GtNB or GtNB(HPA) (7 wt%), as well as thiolated hyaluronic acid (THA) (2 wt%) and photoinitiator LAP were mixed and irradiated under 365nm light (5 mW/cm^2) for 2 minutes. Hydrogels were stored in PBS at 37°C for two hours, followed by incubating in tyrosinase solution for 6 hours to achieve in situ stiffening. Gel elastic moduli (G' & G") were measured by oscillatory rheometry in strain-sweep mode. COLO-357 cells were used as a model to evaluate the influence of matrix stiffening on cancer cell fate. Cells were cultured in high glucose Dulbecco's modified eagle medium (DMEM, HyClone) containing 10% of fetal bovine serum (FBS, Gibco) and penicillin/streptomycin. Cells (2 million cells/mL) were encapsulated in gels composed of GtNB or GtNB(HPA) with THA. In situ stiffening of cell-laden hydrogels was performed one day post-encapsulation. Live/dead staining and confocal imaging were performed to evaluate cell viability and morphology.

Results: ^{1}H NMR analyses results (Fig.1B) demonstrated successful modification of gelatin into GtNB and GtNB(HPA). The crosslinking of GtNB(HPA) and THA yielded biomimetic hydrogels susceptible to tyrosinase-mediated *in situ* stiffening. To demonstrate this, we prepared GtNB-THA and GtNB(HPA)-THA hydrogels with initial moduli of around 1,000 Pa (Fig. 1C). Gels were incubated in tyrosinase (1 kU/mL) for 6 hours, transferred to and stored in PBS solution, and characterized with rheometry. After tyrosinase treatment, gel moduli increased 3-fold for GtNB(HPA)-THA hydrogels and the high stiffness maintained for several days. However, the modulus of control gels made by GtNB-THA did not increase after tyrosinase treatment (Fig. 1C). These gels were highly cytocompatible and the encapsulated COLO-357 cells were able to proliferate over a two-week culture period (Fig. 1D). More importantly, we observed significant cell spreading in stiffened GtNB(HPA)-THA gels, but not in GtNB-THA gels (Fig. 1D). Specifically, cells formed condensed clusters when encapsulated in hydrogels that were not susceptible to stiffening (i.e., GtNB-THA). On the other hand, cells were spread out significantly in the stiffened GtNB(HPA)-THA hydrogels, potentially due to a stiffness-induced EMT process.

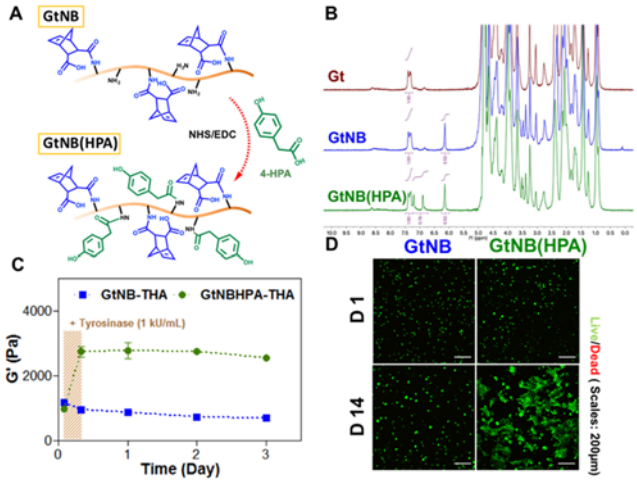

Figure 1. (A) Schematics of GtNB(HPA) conjugation. (B) ^{1}H NMR analyses of gelatin (Gt), GtNB, and GtNB(HPA). (C) *In situ* stiffening of GtNB-THA and GtNB(HPA)-THA hydrogels. (D) Confocal z-stack images of live/dead stained COLO-357 cells encapsulated in GtNB-THA or GtNB(HPA)-THA hydrogels on day 1 and day 14 (stiffening performed on day 1).

Conclusions: In summary, we have prepared a biomimetic and dynamic hydrogel system using orthogonal thiol-norbornene photochemistry and tyrosinase-mediated matrix stiffening. The processes of enzyme-mediated stiffening were cytocompatible for encapsulation of pancreatic cancer cells. Moreover, matrix stiffening increased cancer cell spreading, potentially driven by EMT. Current work is focused on evaluating how the dynamic matrix stiffness changes induce EMT in the encapsulated pancreatic cancer cells.

References: [1] Apte MV, *et al*. Gastroenterology 2013;144:1210–9. [2] Discher DE, *et al*. Science 2005;310:1139–43. [3] Wei SC, *et al*. Nat Cell Biol 2015;17:678–88. [4] Liu H-Y, *et al*. Acta Biomaterialia DOI: 10.1016/j.actbio.2016.10.027. [5] Mũnoz Z, *et al*. Biomaterial Sci. 2014;2:1063–11. [6] Wang L-S, *et al*. Biomaterials 2010;31:1148–57.