# Modular gelatin hydrogels formed by orthogonal thiol-ene photochemistry for 3D hepatocyte culture

Tanja Greene and Chien-Chi Lin
Department of Biomedical Engineering, Purdue School of Engineering and Technology,
Indiana-University Purdue-University Indianapolis, Indianapolis, IN46202, USA

**Statement of Purpose:** Challenges in liver regeneration include the rapid de-differentiation and the loss of liver specific functions of hepatocytes in conventional 2D culture platforms, which do not recapitulate native liver microenvironment.[1] Previously, it has been shown that hydrogels photopolymerized by using thiol-ene photochemistry provide a biomimetic microenvironment for 3D cell culture.[2, 3] However, these systems have used synthetic macromers and several biomimetic peptides are required to construct a relevant environment. Based on thiol-ene photo-click chemistry, our group has recently developed a covalently crosslinked biomimetic poly(ethylene glycol) (PEG)-gelatin hydrogel without the addition of synthetic peptides.[4] Here, we report the use of norbornene-modified gelatin (i.e., GelNB) to construct highly tunable bio-hybrid hydrogels with integrin binding and protease sensitive sites for 3D culture of hepatocytes.

**Methods:** Gelatin-norbornene (GelNB) and poly(ethylene glycol)-tetra-norbornene (PEG4NB, 20kDa) were synthesized from previously established protocols [4] [5]. Poly(ethylene glycol)-tetra-thiol (PEG4SH, 10kDa) was obtained from JenKem Technology. Hydrogels were formed by exposing pre-polymer solutions under long-wave low intensity ultra violet light (365 nm, 5mw/cm$^2$) for 5 min with lithium arylphosphinate (LAP, 1.0mM) as the photo-initiator. Gel points and elastic moduli at various macromer concentrations and thiol to norbornene ratio ($R_{SH/ene}$) were examined using a digital rheometer (Bohlin CVO100). To investigate the viability of hepatocytes within hydrogels, Huh7 cells were photo-encapsulated, stained with live/dead staining kit, and imaged with confocal microscopy.

**Results:** Figure 1A shows the effects of GelNB concentration and $R_{SH/ene}$ on the evolution of hydrogel elastic moduli as a function of time. As expected, an increase in $R_{SH/ene}$ and GelNB concentration led to increased shear modulus of the hydrogel. However, these parameters had little effect on the gel point (6 ± 1 sec for both 3wt% and 2wt% GelNB at $R_{SH/ene}$=1 and 8 ± 1 sec for 2wt% GelNB at $R_{SH/ene}$=0.5). Uniquely, the stiffness of these gelatin-PEG hybrid hydrogels can be systemically increased with increasing concentrations of PEG macromers (Figure 1B) at a fixed GelNB (type A or B) content (2wt%). This result indicates that a constant amount of bioactive motifs can be incorporated while adjusting the stiffness of the material. Meanwhile, Figure 1C demonstrates stiffness can be held constant while the concentration of the bioactive motif (i.e., GelNB) is easily tuned. Moreover, the cross-linking of GelNB hydrogels using thiol-ene photochemistry was cytocompatible with in situ encapsulation of Huh7 cells, as demonstrated by the live/dead staining result day 1 post-encapsulation (Figure 1D). However, at day-20 post-encapsulation, Huh7 cells formed larger multi-cell clusters in hydrogels containing 2wt% GelNB but not in gels containing 0wt% GelNB, suggesting the importance of bioactive motifs on gelatin in supporting cell survival in 3D (Figure 1D).

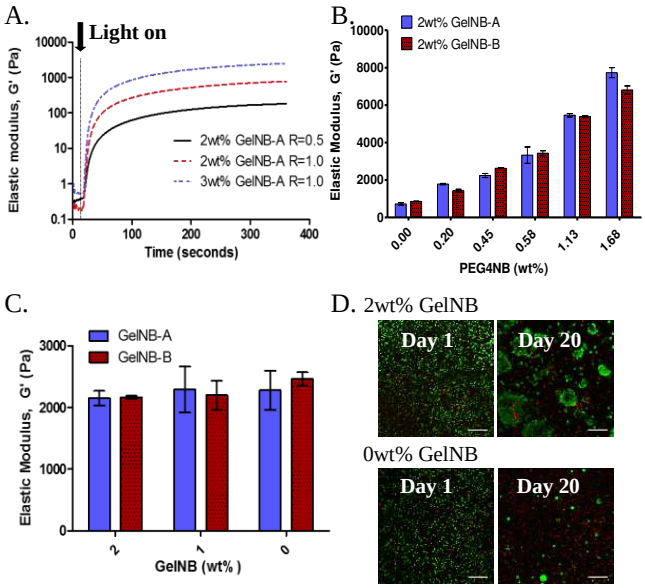


**Figure 1.** (A) In situ photo-rheometry (Type A GelNB; 2wt% or 3wt%; $R_{SH/ene}$=0.5 or 1. (Light was turned on at 30sec) (B) Tunable hydrogel modulus at constant gelatin content (2wt% GelNB-A or GelNB-B). (C) Constant gel modulus while adjusting the concentrations of bioactive components. (D) Viability of Huh7 cells in GelNB/PEG4NB/PEG4SH (top) or PEG4NB/PEG4SH (bottom) hydrogels at day-1 or day-20 post-encapsulation. Cells were stained with Live/Dead staining kit and imaged by confocal microscopy (scales: 200 μm).

**Conclusions:** We have prepared hepatocyte-compatible gelatin hydrogels based on orthogonal thiol-ene photochemistry. One major difference between the current step-growth GelNB hydrogel and the previously reported gelatin-methacrylamide (GelMA) hydrogel is that the later was formed via a chain growth photopolymerization that creates high concentration of radical species and may damage the encapsulated cells or proteins [4]. The gelatin/PEG bio-hybrid hydrogels prepared here can be used for 3D culture of human hepatocytes for the purpose of liver regeneration or studying liver hepatitis. This hydrogel system will also be used for delivering growth factors important in liver regeneration. In summary, this hydrogel system represents an alternate culture platform that may provide a relevant culture environment for hepatic tissue engineering applications.

**References**: [1] Lau TT, Lee LQ, Leong W, Wang DA. Biomedical materials 2012;7:065003. [2] Shih H, Lin CC. Biomacromolecules 2012;13:2003-12. [3] Raza A, Ki CS, Lin CC. Biomaterials 2013;34:5117-27. [4] Munoz Z, Shih H, Lin C-C. Biomaterials Science 2014;2:1063-72. [5] Fairbanks BD, Schwartz MP, Halevi AE, Nuttelman CR, Bowman CN, Anseth KS. Advanced Materials 2009;21:5005-10.