Tuning stiffness of cell-laden hydrogel by photo-reversible host-guest interactions

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Statement of Purpose: In recent years, there is an increasing interest in designing supramolecular hydrogels based on host-guest recognition.[1] Hydrogels with tunable stiffness are particularly useful for studying the effect of biomechanical properties of 3D matrix on cell fate processes. In one example, hyaluronic acid (HA) was modified with cyclodextrin (CD) or with hydrophobic guest molecule adamantane (Ad). The gelation of these two modified HA was achieved via host-gust complexation.[2] The stiffness of these hydrogels can be adjusted by tuning the association and dissociation of physical bonds between host (CD) and guest (Ad) molecules. However, the changes in stiffness are slow (take between hours to days) or this strain-controlled stiffness reversible system is that. Alternatively, photoresponsive guest molecule azobenzene (azo) can switch its conformation rapidly from *cis* to *trans* upon visible light exposure and from *trans* to *cis* upon long-wave UV exposure.[3] The association between CD and azo establishes when azo is in the *trans* conformation. On the other hand, when azo is in the *cis* conformation, the assembly between azo and CD would be disrupted. Here, we present a cytocompatible cell-laden Michael-type thiol-vinyl sulfone (SH-VS) hydrogel with tunable stiffness regulated by photoresponsive host-guest relationship between CD and azo.

Methods: Azo-$(AEEA)_3$-cysteine (azo(A_3)cys) peptide was synthesized via solid phase peptide synthesis (Figure 1A). To characterize the photoresponsiveness of azo(A_3)cys, the spectrophotometric properties of the peptide were measured after exposing the peptide solution to visible light (400-700nm, 10mW/cm^2 at 555nm) or UV (302nm at 10mW/cm^2) at 30 seconds intervals. To form macromer poly(ethylene glycol)-tetra-thiol/teta-CD (PEG4SH4CD), α-CD was functionalized with allylether and photoconjugated with 8-arm PEGSH (PEG8SH, 20kDa) at a molar ratio of 4 to 1. A Michael-type based SH-VS hydrogel was formed after 16 hours of incubation (37ºC) with PEG8VS (20kDa), azo(A_3)Cys, and PEG4SH4CD at a unity molar ratio of thiol to ene. Swollen hydrogels were exposed to visible light or UV at 2 minutes intervals, and the elastic moduli of gels were measured 5 minutes after each light exposure. Human mesenchymal stem cells (hMSCs, 5×10^6 cells/mL) were encapsulated within this Michael-type hydrogel, stained with live/dead staining kit, and imaged with fluorescence microscope to evaluate the cytocompatibility of the system.

Results: In Figure 1B, the exposure of azo(A_3)cys peptide solution to UV light resulted in a decreased of absorbance at 316nm while the exposure of visible light resulted in an increased of absorbance at 350nm. These results indicate that the cys-modified azo maintained its reversible photoresponsiveness. Furthermore, step-growth Michael-type addition SH-VS hydrogel was formed using peptide azo(A_3)cys, PEG8VS, and PEG4SH4CD (Figure 1C). The gel appeared slightly yellow due to the presence of azo group. Live/dead staining result showed that the azo/CD containing hydrogel was cytocompatible to hMSCs (Figure 1D, >99% viability). In addition, hydrogels with the presence of both azo and αCD showed an increased in hydrogel stiffness upon the exposure of visible light. On the other hand, an exposure of UV light resulted in a decreased in hydrogel stiffness. Stiffness reversibility was not observed in the control groups (+azo/-CD and –azo/+CD). These results confirmed that the photo-reversible host-guest interactions only occurred in the presence of both azo and αCD. Further optimization of the system is in progress.

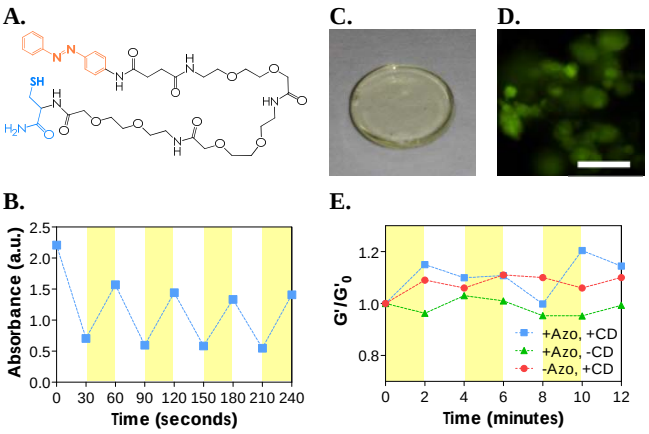

Figure 1. (A) Schematic of azo(A_3)cys peptide. (B) Spectrophotometric scan of azo(A_3)cys peptide after the exposure of UV and visible light. Absorbance was measured at 316nm and 350nm, after exposing to UV (white regions) and visible light (yellow regions), respectively. (C) Michael-type thiol-ene hydrogel formed by azo(A_3)cys, PEG8VS, and PEG4SH4CD. (scale: 5mm) (D) Fluorescence image of hMSCs cells-laden hydrogel stained with Live/Dead staining kit. (scale: 20μm) (E) Effect of light on stiffness reversibility of thiol-ene hydrogels. White and yellow regions indicate UV and visible light exposure, respectively. (3wt% PEG8VS, and $R_{[VS]/[azo]}$=1/3, N=3, error bars are omitted for clarity)

Conclusions: In summary, we have demonstrated that hydrogel stiffness could be reversibly tuned by photoreversible host-guest interactions between network conjugated CD and azo. This hydrogel system was cytocompatible to *in situ* encapsulation of hMSCs and should be of great interest in the study of biomechanical properties on cell fate processes. Current work is focusing on evaluating the effect of local changes of matrix stiffness on morphogenesis and differentiation of hMSCs.

References: [1] Jun L. Advances in Polymer Science 2009; 222:79-112. [2] Rodell CB *et al*. Biomacromolecules 2013;14:4125-34. [3] Tamesue S *et al*. Angewandte Chemie International 2010; 49:7461-64.