Poly(ethylene glycol)-based Hydrogels *via* Metal-free Thiol-acrylate Click Chemistry: *In vivo* biocompatibility and 3-D organotypic culture studies

Yao Fu[1], Kedi Xu[2,3], Kyle Kleinbeck[1], Whitney Johnson[2], Cole Drifka[2], Evan Joyce[2], Scott Carpenter[2], W. John Kao[1,2,4*]

[1]School of Pharmacy, University of Wisconsin-Madison, Madison, WI 53705, USA
[2]Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, WI 53705, USA
[3]Department of Biomedical Engineering, Zhejiang University, Hangzhou 310027, China
[4]Department of Surgery, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI 53705, USA

Statement of Purpose: Poly(ethylene glycol) (PEG)-based hydrogels synthesized *via* metal-free click chemistry offer many attractive features for encapsulating cells during gel formation, such as ease of handling, not requiring the addition of initiator, metal catalyst, and the use of irradiation sources. The Michael-type addition between thiol-terminated PEG and PEG diacrylate under basic conditions will result in the step-growth of PEG chains and the formation of higher molecular weight PEG entanglements (**Fig. 1**). Complete gelation can be achieved from < 30 minutes to several hours. This newly developed PEG hydrogel matrix provides a potential platform for 3D cell encapsulation and organotypic cell culture for various clinical applications including artificial skin.

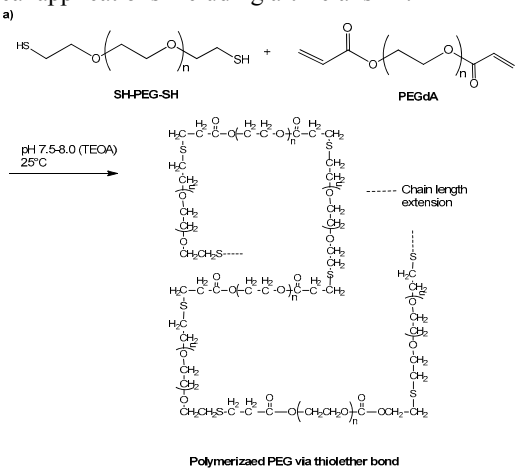

Fig. 1 PEG hydrogel synthesis via thiol-acrylate addition

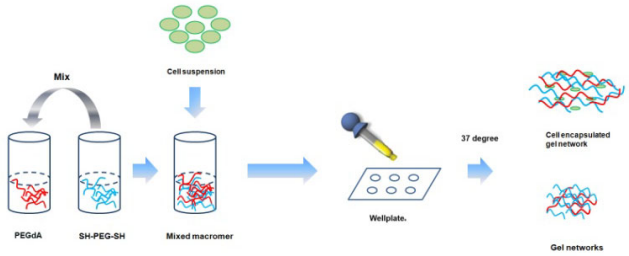

Fig. 2 Preparation scheme for cell encapsulation

Methods: Hydrogel preparation PEG diacrylate (PEGdA) and PEG dithiol (PEG-$(SH)_2$) were synthesized from PEG diol (Mw 2K and 3.4K) following previously established protocols [1]. Hydrogel constructs were fabricated from PEGdA and PEG-$(SH)_2$ in varying ratios by weight and molecular weights from ca 2-20 kDa. As shown in **Fig. 2**, PEGdA and PEG-$(SH)_2$ were dissolved in specific buffer solution, respectively. PEG-$(SH)_2$ solution was then transferred to PEGdA solution and mixed *via* vortexing. Curation of hydrogel was carried out at ambient room temperature or 37°C for cell encapsulation study.

***In Vivo* Biocompatibility** PEGdA and PEG-$(SH)_2$ macromers of Mw 3.4K (Mw 2K and 20K are to be included) were dissolved in PBS respectively. Sprague Dawley rats were anesthetized using isoflurane solution. After disinfection, 500 µL of premixed sterilized macromer solution was injected intramuscularly. At predetermined periods, rats were sacrificed and the hydrogel and its surrounding tissues were harvested. For histological examination, samples were fixed in 4% neutral buffered formalin and then stained with hemotoxylin-eosin (H&E) using standard techniques.

Cell encapsulation and cell viability Cell encapsulated PEG hydrogels were prepared as shown in **Fig. 2** and specific culture medium was used to dissolve PEGdA and PEG-$(SH)_2$. Keratinocyte and fibroblast were encapsulated into the PEG hydrogel matrices. At given time points (6, 12, 24, 48h), cells were stained with live/dead® assay and imaged under a computer-assisted video analysis system coupled to an inverted microscope (Nikon, Eclipse TE 300). Cell encapsulated hydrogel samples will be subject to cryosection and observation via transmission electron microscopy.

Results: PEG hydrogels were successfully formed *via* thiol-acrylate addition at room temperature or 37 °C. Hydrogels prepared from different macromer molecular weights displayed varying mechanical stabilities and gelation properties. *In vivo* studies showed PEG3.4K gels were able to form hydrogels *in situ* in rats. Cell encapsulation studies revealed that most of the keratinocytes and fibroblasts stayed alive within 48 h. To gain more insights, further studies are on-going.

Conclusions: *In situ* forming PEG hydrogel *via* thiol-acrylate addition serves as a promising scaffold for 3D cell encapsulation and a potential platform to incorporate biofunctional cues to develop organotypic cell culture system.

Acknowledgements
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References: Schmidt DR, Kao WJ. J Biomed Mater Res A. 2007;83:617-25.