Facile Synthesis of Rapidly Degrading Poly(ethylene glycol)-based Thiol-Norbornene Hydrogels

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Statement of Purpose: Norbornene-functionalized poly(ethylene glycol)s (PEGNBs) are widely used in tissue engineering and biofabrication for their amenable fabrication into hydrogels through photoactivated thiol-norbornene reaction. To functionalize PEG with norbornene moiety, esterification is commonly used. The resulting PEGNB contains hydrolytically-labile ester bonds, rending the PEGNB hydrogels susceptible to hydrolysis [1]. These PEG-based thiol-norbornene hydrogels have been widely used for in situ cell encapsulation and other tissue engineering applications [2]. PEGNB is commonly synthesized *via* Steglich esterification using 5-norbornene-2-carboxylic acid and hydroxyl-ended PEG (PEG-OH). However, arduous procedures were required to achieve products with high degree of substitution while minimizing the pungent odor of 5-norbornene-2-acrboxylic acid. We sought to circumvent the cumbersome steps in the conventional PEGNB synthesis and shorten the overall synthesis time using an alternative reaction involving carbic anhydride (CA), an odorless norbornene derivative containing diacid anhydride group. Moreover, the new $PEGNB_{CA}$ provides an additional carboxylic group suitable for secondary conjugation. We found that the hydrogels fabricated from these new PEGNBs exhibited accelerated hydrolytic degradation, which significantly differed from hydrogels prepared from conventional PEGNB.

Method: Norbornene was introduced to multi-arm PEG-OH through reacting with carbic anhydride assisted by 4-dimethylaminopyridine [3] in tetrahydrofuran. The additional carboxylic acid of $PEGNB_{CA}$ was subsequently conjugated with amine-bearing molecules including dopamine (D), tyramine (T), and isopropylamine (I) into PEGNB derivatives (PEGNB-X) through standard carbodiimide chemistry. The $PEGNB_{CA}$ and PEGNB-X (2.5-10 wt%) were photo-crosslinked into hydrogels by dithiothreitol (DTT) or protease-cleavable peptide linker KCGPQGIWGQCK (thiol-ene ratio=0.9-1) using lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP, 1-5 mM) as the photoinitiator. Hydrogels were characterized by dynamic shear rheology and swelling. The cytocompatibility of $PEGNB_{CA}$ hydrogel was examined by in situ photo-encapsulation of human induced pluripotent stem cells (hiPSCs), dental pulp stem cells (DPSCs), and human pancreatic cancer cells PANC-1 at 50k cell/gel.

Results: Thiol-norbornene hydrogels crosslinked by conventional PEGNB were stable for at least a month [1]. The new $PEGNB_{CA}$ gels were highly susceptible to hydrolysis and degraded completely within 14-16 days (Figure 1A). Interestingly, the ester linkages were further weakened by the second conjugation, as evidenced by the rapid degradation of hydrogels crosslinked by PEGNB-Xwith DTT. Specifically, PEGNB-D and PEGNB-I hydrogels degraded completely in ~2 hours, whereas PEGNB-I gels degraded entirely by 10 hours (Figure 1B). The delayed degradation of PEGNB-I hydrogels (compared with PEGNB-D and PEGNB-T) was likely a result of intermolecular hydrophobic interactions between the isopropyl groups. This could also explain the slight increase in the initial G'. Moreover, the newly synthesized $PEGNB_{CA}$ hydrogels also showed excellent cytocompatibility toward hiPSC, DPSC, and PANC-1 cells (Figure 1C). Live/Dead staining results showed that only minimal dead cells were visible for all cell types. After 7 days of in vitro culture, hiPSC and PANC-1 cells proliferated to form spheroids, while DPSC exhibited fibroblast-like morphology due to the degradation of peptide linker.

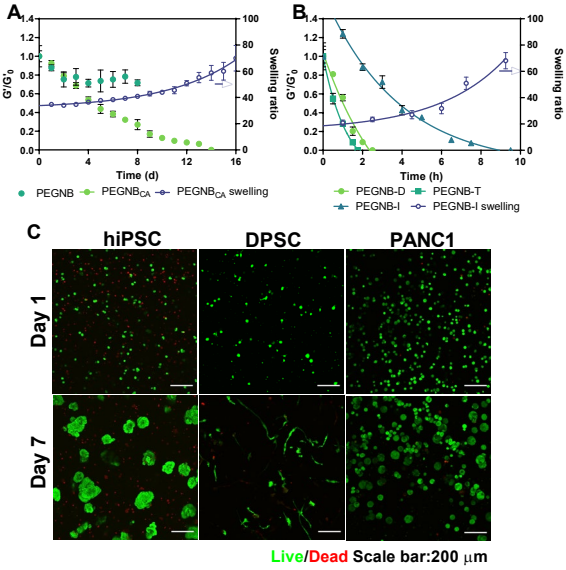

Figure 1. Degradation of (A) 2.5 wt% PEGNB, 4 wt% $PEGNB_{CA}$ and (B) 4 wt% PEGNB-X hydrogels at 37°C in PBS (n=3). G'_0: PEGNB=1.2 kPa, $PEGNB_{CA}$= 1.7 kPa, PEGNB-D= 1.5 kPa, PEGNB-T= 1.4 kPa, and PEGNB-I)= 1.4 kPa. (C) hiPSC, DPSC, and PANC-1 stained by live/dead calcein AM/ethidium homodimer-1 in 4 wt% $PEGNB_{CA}$ hydrogels crosslinked by CGPQGIWGQC with 1 mM CRGDS.

Conclusions: These results have established $PEGNB_{CA}$ hydrogels as a new class of readily degradable hydrogels suable for biomedical applications. The ultra-rapid hydrolytic degradation of PEGNB-X hydrogels also provide new opportunities for future biofabrication applications.

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References: [1] Shih H., Lin C.C., Biomacromolecules. 2012;13:2003–2012. [2] Lin C.C. J. Appl. Polym. Sci. 2015;132":1–11 [3]Sakakura A. J. Am. Chem. Soc. 2007;129:14775–14779.