Copper-free Click Chemistries for the Synthesis of Modular Poly(ethylene glycol) (PEG) Scaffolds

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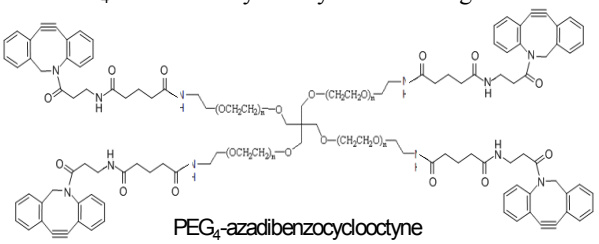
Statement of Purpose: A variety of chemistries have been introduced to produce hydrogels from synthetic and natural polymers. Click chemistries are a set of chemical reactions defined by their orthogonality (i.e. the specificity).[1] Thus, ideal click chemistries may be performed in the presence of proteins without risk of reacting with them. The classical click chemistry is the [3 + 2] cycloaddition between alkynes and azides. The cycloaddition occurs efficiently at 37°C in the presence of copper I ions. Unfortunately, copper ions are toxic to cells. The Bertozzi group has introduced a number of cyclooctynes that undergo cycloaddition with azides at 37°C without copper catalysts.[2] One such cyclooctyne, DIFO, was used by the Anseth group to produce PEG/peptide hydrogels.[3] A similar cyclooctyne (aza-dibenzocyclooctyne) is commercially available. We used this to produce PEG microspheres and scaffolds by adapting a modular approach that we previously developed.[4] The approach exploits phase separations between PEG and dextran or sodium sulfate to produce microspheres, which are then assembled into macroporous scaffolds in the presence of living cells. The pores in the scaffold may provide a mechanism for rapid migration and proliferation in the scaffolds, without requiring degradation of the scaffold.

Orthogonal chemistries may be useful for scaffold formation for a number of reasons. It is possible that the presence of reactive groups in a scaffold (vinyl sulfones, amines, acrylates, thiols, etc.) may lead to reaction of host proteins with the scaffold (e.g. proteins of the complement cascade, immunoglobulins, etc.), potentially enhancing the foreign body response. The use of chemistries that are practically unreactive towards amines and thiols may thus improve biocompatibility. They may also enhance drug delivery from hydrogel scaffolds, which may be complicated by reaction between chemical groups in the scaffolds and groups on protein growth factors, peptides, etc.

Methods: *Synthesis of PEG_4-azadibenzocyclooctyne:* PEG_4-amine was reacted with 1.5 eq. of azadibenzocyclooctyne (Click Chemistry Tools), 1.5 eq DIPCDI, 1.5 eq HOBT and 3 eq DIPEA in dichloromethane in an ice bath for 24 h.
Synthesis of PEG_4-azide: PEG_4-mesylate was reacted with 3 eq. of sodium azide in DMF at 70°C overnight.
Attachment of RGD peptide: Ac-GCGYGRGDSP-NH2 was added to PEG_4-azadibenzocyclooctyne in a 1:8 ratio in PBS. Eosin Y (40 μM) and triethanolamine (155 mM) were added and the solution was exposed to 480-520 nm light from a Xenon arc lamp for 4 min.
Scaffold formation: Microspheres were produced in 4% dextran or 500 mM sodium sulfate to cause the phase separation of 2% PEG-azadibenzocyclooctyne and 2% PEG-azide. The microspheres were buffer exchanged into 30% dextran and centrifuged at 500 g for 3 min to produce scaffolds.

Results: PEG_4-azadibenzocyclooctyne (i.e. four-arm PEG-azadibenzocyclooctynes) reacted rapidly with PEG_4-azide or PEG-diazide to form bulk gels in PBS without copper ions. However, PEG-dithiol was unable to react with PEG_4-azadibenzocyclooctyne to form a gel.



Microspheres were formed by causing the PEG-derivatives to undergo a thermally induced phase separation upon heating from room temperature to 37°C. Stable microspheres formed in 1-3 min (Fig. 1A). The microspheres were buffer exchanged into 30% dextran and centrifuged to compact them. This allowed crosslinking between the microspheres to occur, forming a macroporous scaffold (Fig. 1B).

Using visible light to generate free radicals, we exploited a thiol-yne reaction to add thiol-containing RGD peptide to PEG-azabenzocyclooctyne prior to microsphere/ scaffold formation. The peptide promoted spreading of endothelial cells on the scaffolds.

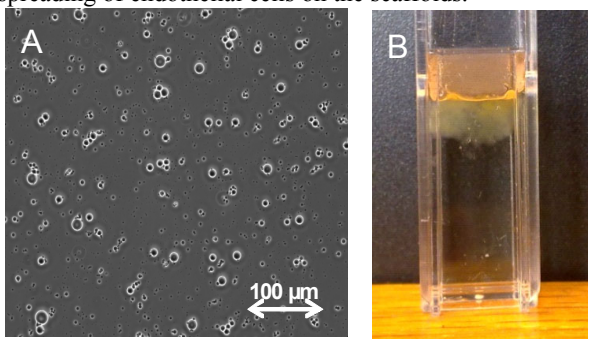


Figure 1: (A) Microspheres produced by reaction of 2% PEG_4-aza-dibenzocyclooctyne with 2% PEG_4-azide in the presence of 500 mM sodium sulfate. (B) Scaffolds produced by centrifuging microspheres in 30% dextran for 3 min at 500 g. The PEG microspheres rise to the top of the cuvette and crosslink.

Conclusions: The ability to use orthogonal chemistries to produce macroporous, modular scaffolds may enhance the development of advanced cell transplantation strategies.

References:

1. Kolb, H.C. et al., Ang. Chem., 40, 2004, 2009.
2. Jewett, J.C. et al, JACS, 132, 3688, 2010.
3. DeForest, C.A. et al., Nat Mater, 8, 659, 2009
4. Scott EA, et al., Acta Biomater 6, 29, 2009.