Thiol-Maleimide Reaction Speed Effects on Hydrogel Homogeneity

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Statement of Purpose: The development of poly(ethylene glycol) (PEG)-based synthetic hydrogels for tissue culture and drug delivery in vivo and ex vivo has become favorable. Pro-cross-linking reactions such as the conjugate addition (Michael-type) of thiols onto unsaturated vinyl groups such as acrylates and maleimides have become a common coupling strategy.[1] PEG-maleimide (PEG-Mal) has been shown to be advantageous for cell culture while decreasing reaction time and increasing efficiency. The added benefit of the PEG-Mal reaction speed may also be leading to inconsistent gelation of the hydrogel throughout the bulk, leading to batch-to-batch variable cellular response to the heterogeneity of the cross-linked hydrogel. (Fig. 1a) We thereby aim to slow the reaction between maleimides and thiolates to allow for pre-gel mixing and more homogenously cross-linked hydrogels. The reaction can be slowed by reducing the temperature, or altering the concentrations of maleimide or thiolate groups present. The Henderson-Hasselbalch equation describes the relation between pH, pKa and concentration of thiolate groups and adding negative charges from aspartic acid and glutamic acid around the thiol will increase the pKa of that thiol which slows the reaction while maintaining physiological pH.[2] Through the rational design of peptides we altered the kinetics of the maleimide-thiol reaction while maintaining its high selectivity and efficiency.

Methods: Preparation of thiol peptides for pKa determination: Sodium chloride of strength 0.1M was added to 0.01M sodium acetate, potassium phosphate, tris base, or sodium tetraborate to create various pH buffers. Thiol-containing peptides (GCRDG, GDCDDG or GECEEG) (Genscript, Piscataway, NJ) were dissolved in previously mentioned pH buffers ranging from pH 4 to pH 12 at 0.1mM. The absorbance of the thiolates was measured at 240nm in a NanoDrop 2000c spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). Preparation of gel: Eight-armed PEG-Mal or PEG-Norbornene (fig.1b) 40,000Da (JenKem Technology USA, Plano, TX) was combine with one of the three MMP-degradable cross-linker peptides, GCRD-GPQGIWGQ-DRCG (CRD-MMP), GDDCD-MMP-DCDDG (DDCD-MMP), and GECEE-MMP-EECEG (ECEE-MMP) (Genscript, Piscataway, NJ), in 1xPBS at a final gel composition of 1.25mM PEG-Mal(PEG-Norb) and 4mM cross-linker. PEG-Norb gels were formed in the presents of 0.1mM Eosin-Y after 5min exposure to visible light. Gelation time: Plate-to-plate rheometer (Physica MCR 301, Anton Paar, Ashland, VA) was used to monitor the storage, G', and loss, G", modulus over time at constant frequency and strain, 1rad/s and 2.5%, respectively at 25°C. Statistics: Data is presented as a mean ± standard error. For pKa data analysis of variance (ANOVA) using the software GraphPad Prism 6 was performed and significance was established at $p < 0.05$.

Figure. 1

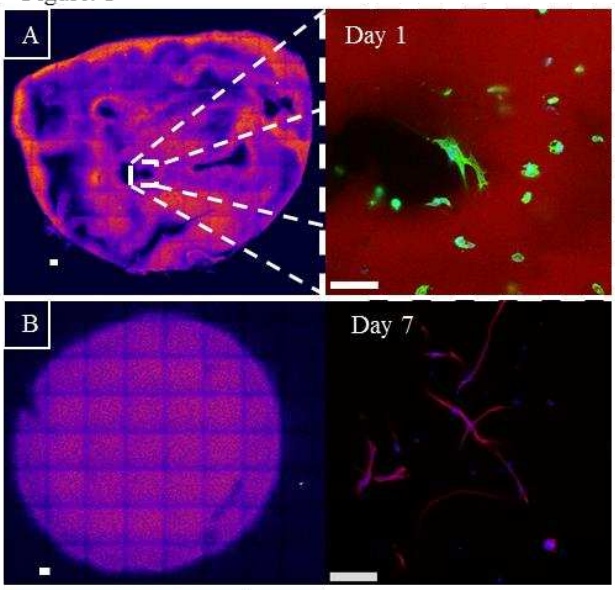


Table. 1	CRD	DCDD	ECEE
Charge	0	-3	-3
pKa	8.656	9.243	9.412
Gelation Time (sec)	100	220	660

Figure. 2

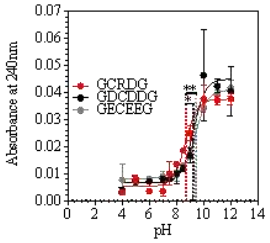
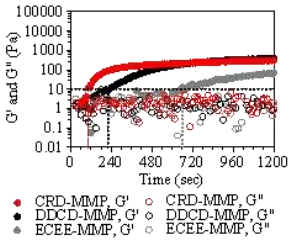


Figure. 3



Results: Effect of electrostatic environment on pKa of thiol groups: The absorbance data was fitted to the following form of the Henderson-Hasselbalch equation: $Absorbance_{thiolate}=Plateaus_{bottom}+(Plateaus_{top-bottom})/(1+10^{pka-pH})$. Fig. 2 demonstrates that increasing the negative charges surrounding the thiol group increases the pKa this reduces the concentration of the Michael-addition reactive thiolate at physiological pH and slows the reaction with maleimide. Effect of pKa on the gelation time determined through rheology: The gelation point of a material was determined to be the cross-over of G" and G' (G'~10Pa) as this point indicates the materials transition from the viscous regime to the elastic regime. Fig. 3 demonstrates that as the pKa increases the gelation time increases.

Conclusions: Through rational peptide design the thiolate maleimide gelation time was slowed, allowing for better mixing and ease of use.

References: (1) Phelps EA, Barker TH, Garcia AJ, et.al Adv. Mater. 2012;24:64-70 (2) Lutolf MP, Hubbell JA, et.al Bioconjugate Chem. 2001;12:1051-1056