Advanced Applications of Thiol-Ene Formulations

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ABSTRACT

Thiol-Ene polymerization has gained ever increasing interest during the last decade. Advantages such as low oxygen inhibition and shrinkage, uniform networks with significantly improved mechanical properties are accompanied by up to now unsolved disadvantages such as unpleasant odor and poor storage stability. We have addressed the latter issue with two-component stabilizer systems that provide thiol-(meth)acrylate compositions with nearly no increase in viscosity after one year. We have also explored the advantages of thiols to help increase the reactivity of vinyl esters and vinyl carbonates. These monomers are low toxic alternatives to acrylates that are not only suitable for biomedical applications but also for classical coatings. Copolymerization of vinyl carbonates and vinyl esters with di- and tetra-thiol monomers is shown to proceed at rates intermediate to (meth)acrylates. Material properties are strongly influenced by degree of crosslinking. Processing of these novel thiol-ene compounds by multiple modes of photo-based AMT will also be presented.

INTRODUCTION

Although the reaction of alkylthiols with vinyl groups has been known for more than 100 years,[1] the use of this reaction to build polymers remained for a long time unexplored. Interest in thiol-ene polymerization increased however as distinct advantages over acrylate polymerization were discovered. In the last twenty years researchers have shown that, unlike acrylates, thiol-ene reactions have reduced oxygen inhibition,[2] significant lower shrinkage,[3] and better mechanical properties. Despite these advantages, thiol-ene systems have some drawbacks, such as limited shelf-life stability and bad odor. This latter problem has been addressed with high molecular weight multifunctional low-odor thiols that are now commercially available. The main issue appears now to be the stability of the formulations with shelf lives varying greatly from a few seconds to months.

Thiol-Ene polymerization has been studied for a wide range of applications such as coatings, printing inks and 3D printed objects. In the last decades, development of cytocompatible hydrogels with defined microstructures has also gained interest with potential applications of soft tissue engineering and regenerative medicine. The use of light to trigger polymerization provides spatiotemporal control to the hydrogel crosslinking process that would not be possible by thermal or redox initiation. In fact, photopatterning of bioactive ligands(e.g., RGD motifs) within hydrogel matrices has enabled user-defined manipulation of cell functions in 3D. Acrylate-based monomers are commonly used, although the possibility for peptide amino-groups to undergo Michael Additions with the acrylates does raise toxicity concerns. In response we present vinylesters and vinylcarbonates[4] as suitable alternatives to acrylates, which when copolymerized with thiol monomers undergo rapid curing similar to acrylate-based monomers.[5,6] 3D-printing[7] of thiol-ene hydrogels is possible by both stereolithography and by two-photon polymerization (TPP). With highly efficient two-photon initiators it is possible to achieve micrometer-scale resolution.

RESULTS AND DISCUSSION

Storage Stable Thiol Ene Formulations

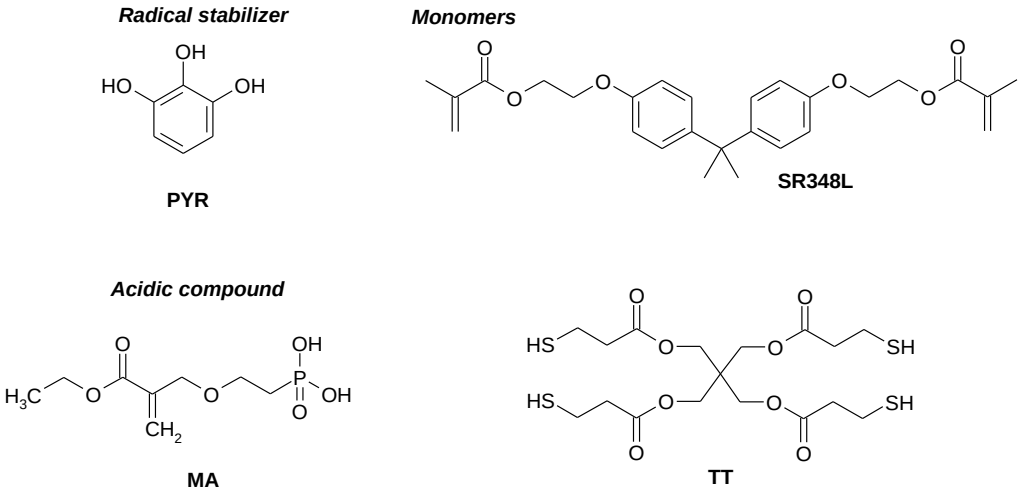


Figure 1. Stabilizer system (PYR and MA) and thiol-ene monomers

Due to low storage stability of thiol-ene resins, we have developed an efficient stabilizer system which exhibits no increase of viscosity at room temperature and significantly improved stability at elevated temperatures. The stabilizer system (Figure 1) prevents multiple modes of gelation and contains both a free radical scavenger, e.g pyrogallol (**PRA**) and a phosphonic acid buffer (**MA**). In formulations based on **SR348L** and **TT**, the addition of both **PRA** and **MA** displays a surprising synergistic stabilizing effect (Table 1). This stabilization system was also found effective in formulations with thiols and more reactive enes such as acrylates and vinyl ethers.[8] The synergetic effects of **PYR** in combination with phosphonic acid have also been successfully applied to reactive industrial formulations based on a combination of multifunctional dimethacrylate and diacrylate monomers.[8] After 110 days, no change in viscosity was noted for the formulation stored at room temperature, while at 65 °C gelation was avoided with viscosity increasing only by a factor of 3.

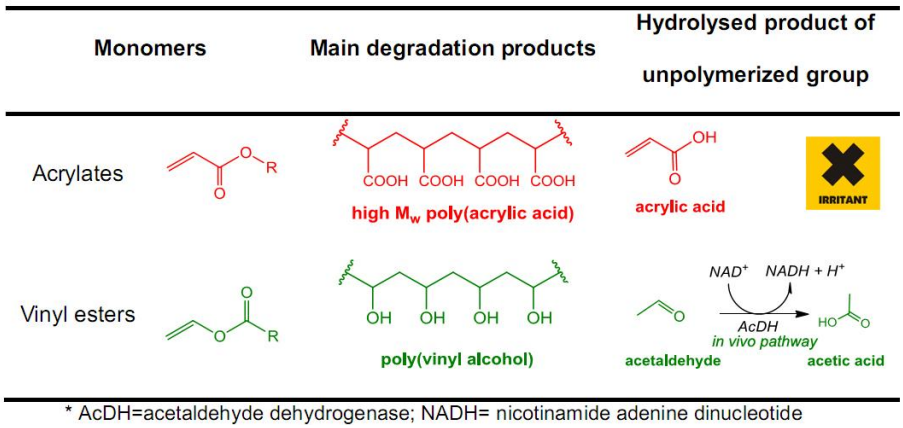
Table 1: Viscosity (Pa.s) of an equimolar mixture of SR348L and TT in the presence of different stabilizers after 110 days at room temperature and 65°C

Stabilizer [mM]		RT		65°C	
PRA	**MA**	Increase in viscosity [%]	Time until gelation	Increase in viscosity [%]	Time until gelation
-	-	-	2(d)	-	6(h)
-	90	-	22(d)	-	19(h)
90	-	16	-	3653	-
90	90	0	-	91	-
9	-	75	-	5275	-
9	90	0	-	90	-

Biocompatible Monomers

Advanced applications for photopolymerization include dental materials, imprint lithography, and additive manufacturing technologies. Monomer toxicity has in some cases become a concern. While methacylate-based monomers are less reactive than acrylates, the irritancy and potential cytotoxicity of acrylates may preclude them from certain biological and medical uses. To address this issue, we have introduced biocompatible vinyl esters which are particularly promising for bone tissue engineering.[9] Comparative cytotoxicity studies on osteoblasts showed that vinyl esters are at least one order of magnitude less cytotoxic than (meth)acrylate analogues. This can be partially explained by the unique degradation mechanism of poly(vinylester) (Table 2). Upon hydrolysis, the polymer is converted to poly(vinylalcohol) (PVA) and small molecule acids. PVA is FDA-approved in numerous food and health products and should thus be preferable to the acrylate degradation product poly(acrylic acid). Low molecular weight acids released from vinyl ester hydrolysis will be readily metabolized or excreted from the human body. Since radical photopolymerizations never reach full conversion, the fate of unreacted vinyl monomer must be considered. While acrylate monomers and hydrolysis product acrylic acid are cytotoxic, vinyl ester monomers are hydrolyzed to acetaldehyde which is a common metabolite easily processed by natural enzymatic pathways.

Table 2. Schematic degradation mechanism of polyacrylates and polyvinylester



* AcDH=acetaldehyde dehydrogenase; NADH= nicotinamide adenine dinucleotide

Photoreactivity of vinyl esters is generally between that of acrylates and methacrylates. We recently demonstrated that addition of alkyl thiols can improve the photoreactivity of vinyl esters to a level comparable to acrylates.[6] The underlying mechanism is supposed to be chain-transfer from the propagating carbon-centered radical to a thiyl radical. To test the applicability of thiol-ene based photo-processing of natural materials we chose two different proteins and one polysaccharide which were then post modified.

Gelatin/ Albumin based Photopolymers

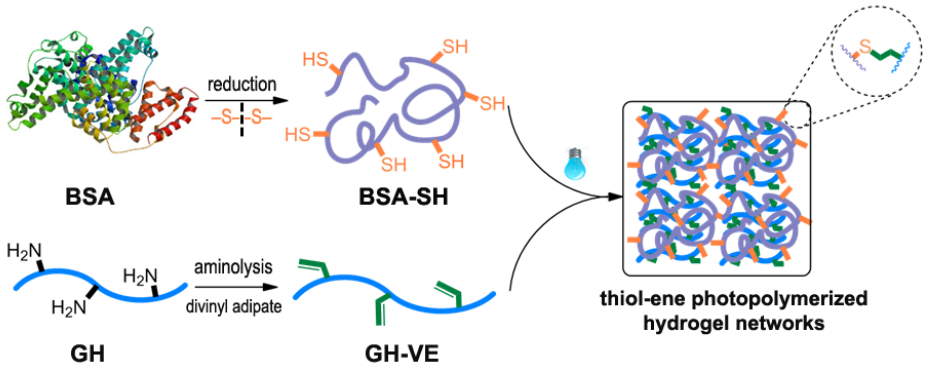


Figure 2. Thiol-ene photopolymerized hydrogels using GH-VE and BSA-SH as precursors

Bovine serum albumin (BSA), which is widely available and possesses a high degree of cysteine residues, was chosen as macrothiol. Since most of the cysteine residues in native BSA are trapped within disulfide bridges, a preliminary reduction process was required. Gelatin is interesting as a low molecular weight derivative of collagen and for its lysine residues. These pendant amine groups allow introduction of multiple vinylester groups along the gelatin chain (GH-VE).[10] The degree of substitution (confirmed by MALDI-TOF measurements) of GH-VE is intrinsically limited to the lysine residue concentration in the starting material-GH (Figure 2). Quantitative MTT assay on MG63 cells showed that GH-VE displayed negligible cytotoxicities after 24h culture as compared to culture medium alone.

To demonstrate the effectiveness of thiol and vinyl ester modification of the proteins, aqueous solutions containing BSA-SH and GH-VE were photo-cured to provide stable hydrogels. Addition of a reactive two-photon absorbing initiator to the thiol-ene formulation allows for two-photon polymerization (TPP) microfabrication.[11]

Hyaluronic acid vinyl ester

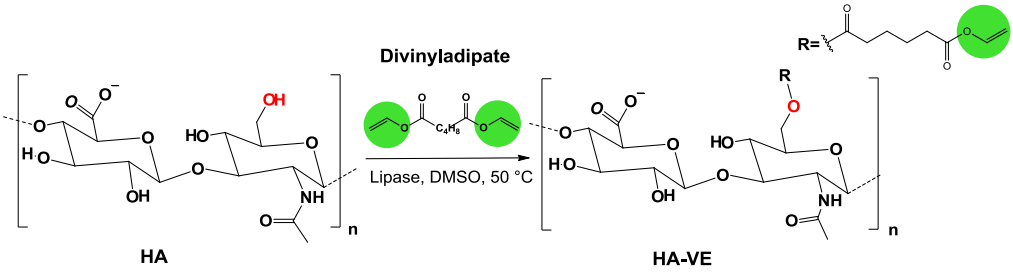


Figure 3. Synthesis of vinyl ester derivative of hyaluronic acid

Due to general therapeutic interest in the parent polymer, hyaluronic acid vinyl ester (HAVE) was synthesized via lipase-catalyzed transesterification reaction (Figure 3). Reaction time and stoichiometry were adjusted to provide degree of substitution across a wide range (from 0.13 to 1.25 vinyl groups per repeat unit).

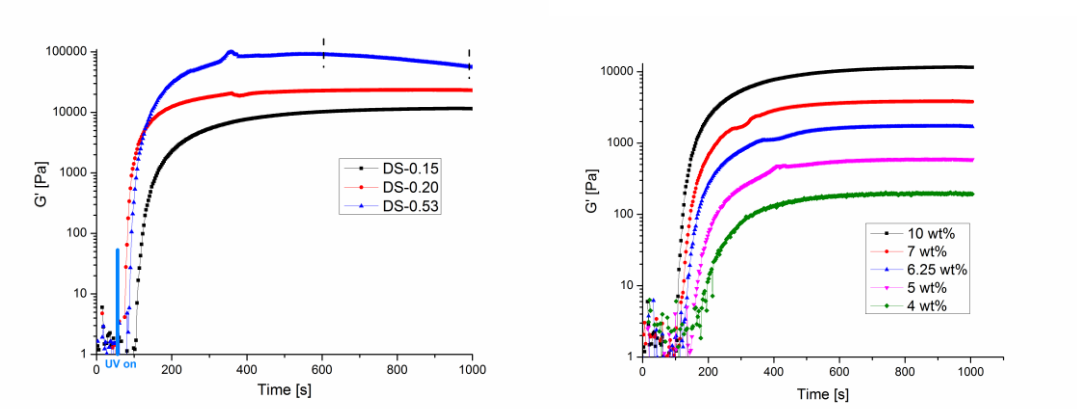


Figure 4. Influence of degree of substitution (DS) on photoreactivity (left) and influence of weight concentration on mechanical properties (right)

In-situ photorheology was used to monitor the curing and enzymatic degradation kinetics of HAVE hydrogels. It was found that the photoreactivity of HAVE towards homopolymerization increased with higher degree of substitution (Figure 4 left). More importantly, we demonstrated that the thiol-ene click chemistry was able to improve the photoreactivity of HAVE (data not shown). Additionally, the control over the concentration of HAVE as well as the crosslinking density provided hydrogels with a wide range of gel stiffness from 0.1 kPa to 10 kPa (Figure 4 right). MTT assay of both HAVE solutions and extracts of HAVE pellets proved that HAVE present negligible cytotoxicity and therefore shows great promise for potential clinical applications.

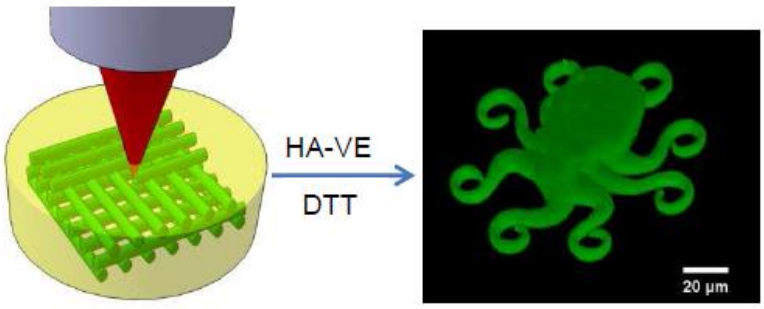


Figure 5. TPP microfabrication of a hydrogel based on HAVE with dithiol DTT

Although HAVE did undergo homopolymerization, reaction rate was greatly increased by addition of thiol. In this case, dithiothreitol (DTT) was found to more than double polymerization rate and allow for photo-fabrication via TPP. Figure 5 displays a hydrogel micro-octopus constructed from HAVE and DTT. Feature size of the individual arms is less than four micrometers. The ability to apply this technology in other biomedical applications is currently being explored.

CONCLUSIONS

Thiol-Ene polymerization has gained increasing interest due to reduced oxygen inhibition and tailorable mechanical properties of the final material. Disadvantages such as bad odor and poor storage stability have limited the wide-spread industrial application. Within this work we have demonstrated that a combination of radical stabilizer with an acid buffer gives significantly improved storage stability. In addition, we have applied thiol-ene step growth polymerization for the formation of biocompatible and biodegradable hydrogels for high-resolution two-photon-induced polymerization. To do this gelatin and hyaluronic acid were modified with vinylester groups. MTT assays showed low cytotoxicity of both monomer and polymer and confocal laser scanning microscopy proved the successful 3D writing of thiol-ene hydrogels with micrometer-scale resolution.

REFERENCES

1. T. Posner, *Chem. Ber*. **1905**, *38*, 646-57.
2. S.C. Ligon, B. Husar, H. Wutzel, R. Holman, R. Liska *Chem. Rev.* **2014**, *114*, 557.
3. H. Lu, JA. Carioscia, JW. Stansbury, CN. Bowman, *Dent. Mater.* **2005**, *21*, 1129-36.
4. B. Husar, R. Liska, *Chem. Soc. Rev.*, **2012**, *41*, 2395-2405.
5. A. Mautner, X.H. Qin, H. Wutzel, B. Kapeller, D. Moser, G. Russmüller, J. Stampfl, R. Liska, *J. Polym. Sci. Part A: Polym. Chem*.**2012**, *51*, 203-212.
6. A. Mautner, X.H. Qin, B. Kapeller, G. Russmüller, T. Koch, J. Stampfl, R. Liska, *Macrom. Rapid Commun.* **2012** *33*, 2046-2052.
7. A. Ovsianikov, V. Mironov, J. Stampfl, R. Liska, *Exp. Rev. Med. Dev.* **2012**, *9*, 613-633.
8. R. Liska, P. Esfandiari: "Stable Curable Thiol-Ene Formulation"; WO2012126695 A1
9. C. Heller, M. Schwentenwein, G. Russmüller, F. Varga, J. Stampfl, R. Liska *J. Polym. Sci. Part A: Polym. Chem.* **2009**, *47*, 6941 - 6954.
10. X.H. Qin, J. Torgersen, R. Saf, S. Mühleder, N. Pucher, S. C. Ligon, W. Holnthoner, H. Redl, A. Ovsianikov, J. Stampfl, R. Liska, *J. Polym. Sci. Part A: Polym. Chem.***2013**, *51*, 4799.
11. J. Torgersen, A. Ovsianikov, V. Mironov, N.U Pucher, X.H. Qin, Z. Li, K. Cicha, T. Machacek, V. Jantsch-Plunger, R. Liska, J. Stampfl, *J. Biomed. Opt.* 17 **2012**, *10*, 1-10.