Measurement of Tissue Adherence for *In Situ* Formed PEG Acrylate Hydrogels

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Statement of Purpose: Hydrogel coatings formed *in situ* have demonstrated commercial utility in applications such as sealants, hemostats, and adhesion barriers. In these biomedical applications tissue adherence is an important feature impacting the efficacy of the device. Adherence of the hydrogel to underlying tissue is influenced by many factors. This study describes a novel *in vitro* method to measure shear adherence for a set of modified PEG acrylate hydrogels formed *in situ* via free radical chemistry. The relevance of the *in vitro* data is validated by correlation with performance in an animal model.

Methods: Hydrogels were formed *in situ* by the free radical polymerization of acrylate terminated PEG-poly(lactate) macromers. An acrylate terminated 10kDa linear PEG with 5 lactate groups per chain end is designated as: 10K-PEG(L5A)2. Macromers were prepared and characterized according to previously described methods[1]. Two-part macromer formulations were prepared in DI water containing 0.6 wt % ferrous gluconate dihydrate in part-A and 0.035 wt% t-butyl hydroperoxide in part-B. Macromer was present at a constant wt% in part-A and part-B. A dual syringe mixing device was used to apply the formulation onto the target tissue. Shear adherence testing was performed using an Instron 5543 with a 5N load cell. Hydrated collagen membrane (Nippi Casing #320) was firmly attached to a steel substrate (Figure 1). A second piece of collagen membrane with a 4mm circular defect was layered on top. The test material was then applied to coat the entire defect extending at least 1mm past the defect borders. The sample was loaded onto the Instron and a constant uniaxial strain was applied. Shear adherence values were calculated from the maximum load on the sample during the test. A rat peritoneal sidewall defect model was used to determine the tissue adherence of hydrogels *in vivo*. Naive Sprague Dawley rats (~275g) were anesthetized, subjected to laparotomy, and a standardized 1cm^2 sidewall defect/circumferential suture procedure as described previously[2]. The defect was coated with 1.0mL of test material. Animals were evaluated postmortem at 1-, 3-, and 7-days for the presence/absence of test material.

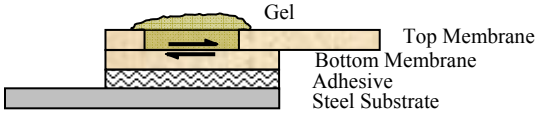

Figure 1. *In vitro* shear adherence test device

Results: 1. Effect of hydrogel crosslink density: Macromers of the general formula PEG(L5A)2 prepared from PEGs of various molecular weight (10, 20, and 29kDa) were formulated at several concentrations (4, 6, and 10 wt%). Shear adherence values were measured using our Instron method. All samples demonstrated adhesive rather than cohesive failure. These data are illustrated in Figure 2.

2. Effect of a low MW primer: A priming step was incorporated into the hydrogel forming procedure by first wetting the tissue with a priming solution containing 3.3K-PEG(L5A)2 macromer (10 wt%) and t-butyl hydroperoxide (0.035 wt%). As shown in Table 1, we found that without primer, the 29kDa macromer[3] showed low shear adherence with adhesive failure. When the primer protocol was used, an increase in adherence was observed, and cohesive failure was seen. The true adhesive-failure mode adherence is assumed to be greater than the experimental value reported in Table 1. Our rat sidewall adherence model confirmed that hydrogels with higher shear adherence values showed greater persistence on the sidewall defect *in vivo* (Table 1).

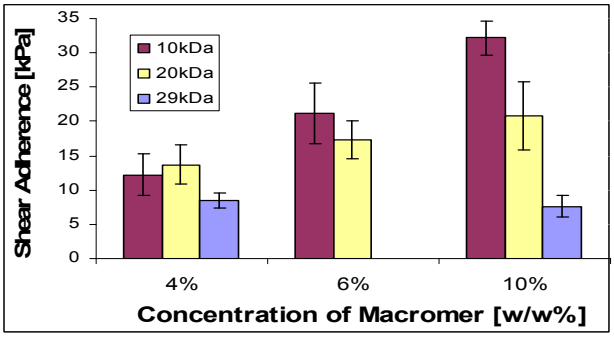

Figure 2. Effect of crosslink density on shear adherence

PEG MW (wt%)	(kPa) Shear Adherence	Failure mode	Animals with gel on defect		
			Day-1	Day-3	Day-7
29kDa (4%)	7.2 ± 1.9	Ad	0/3	0/3	0/3
primer + 29kDa (4%)	9.0 ± 2.0	Co	3/3	3/3	3/3
10kDa (10%)	29.7 ± 4.1	Ad	3/3	2/3	2/3

Table 1. Rat sidewall adherence model (n=3/time point)
Ad = adhesive failure, Co = cohesive failure

Conclusions: An *in vitro* method was developed to quantify the shear adherence of free radical polymerized PEG(L5A)2 hydrogels on a collagen membrane surface. Formulations with higher macromer content and lower macromer MW gave higher shear adherence values. Additionally, a primer step increased adherence and shifted the failure mode from adhesive to cohesive. An *in vivo* rat model verified that formulations with better *in vitro* shear adherence were well adhered to the sidewall defect over a 7-day period.

References: 1. Hubbell JA, Macromolecules 1993; 26: 581-587. 2. Skinner KC, Eur J Surg 1997;Suppl 577:40-48. 3. The 29kDa PEG macromer contains trimethylene carbonate (T) modification as well as lactate modification and is designated 29K-PEG(T10L3A)2.