Injectable PEG-Genipin Hydrogels for Tissue Engineering

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Introduction

The rapid advances in the fields of tissue engineering and regenerative medicine require the development of novel, biocompatible scaffolds. Biodegradable polymer hydrogels that are capable of gelling in vivo are of immense clinical significance. Our lab has previously reported a biodegradable, non-toxic, polymer hydrogel, PEG-genipin.[1] We would like to propose an in situ forming PEG-genipin hydrogel. This *in situ*-forming hydrogel will have significant impact in the fields of tissue engineering, cell therapy, and regenerative medicine. Particularly, this hydrogel will lead to significant advances in musculoskeletal biomaterials.

The objective of this study was to decrease the gelation rate of PEG-genipin, moving towards an *in situ* forming hydrogel. It has been observed by us and others[2] that exposure to air enhanced the gelation rate of genipin-cross-linked gels. We hypothesized that exposure to oxygen would decrease the gelation time of PEG-genipin, thus increasing the gelation rate. To test our hypothesis, PEG-genipin and genipin alone have been exposed to both air or oxygen. The hydrogels were monitored for color change (yellow, orange, red, brown, blue) and gelation.

Materials and Methods

PEG-genipin preparation. An 88 mM genipin solution was prepared. The dissolved genipin reacted with a 10% PEG solution, forming a cross-linked PEG-genipin hydrogel.

Exposure to air. A solution yielding a final concentration of 35.2 mM genipin was prepared as previously mentioned. The solution was allowed to react in a closed system (sealed vial), an open system (vial open to the environment), or an open system exposed to air, by bubbling air through the solution. The PEG-genipin solutions were monitored for gelation. Gelation was initialized when the clear solutions turned blue.

Exposure to oxygen. The 88 mM genipin solution was exposed to oxygen following sonication. The length exposure of oxygen to the genipin solution varied. PEG solution was then added to these modified genipin solutions yielding a final genipin concentration of 44 mM. The PEG-genipin solutions were monitored for gelation.

Results and Discussion

The PEG-genipin hydrogels were prepared and examined using SEM. The hydrogels are porous, with a porosity of 43.67 ± 10.36 (Figure 1). Exposure to air of the PEG-genipin solution (35.2mM genipin), decreased the length of time to reach initialization of gelation as well as the gelation time (Figure 2). The gelation times were 2:15, 48:00, and 120:00 for the solution exposed to air, the solution in the open system and the solution in the closed system, respectively.

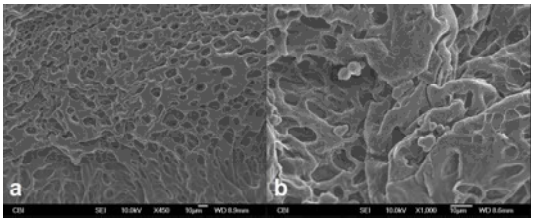

Figure 1. SEM of PEG-genipin scaffold (35.2mM genipin) (a) at higher magnification (b).

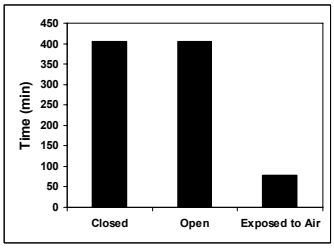

Figure 2. Time to attain initialization of gelation in a closed system, open system, and exposure to air.

Exposing the PEG-genipin solution to air decreased the gelation time. However, for a clinically applicable hydrogel we would like to combine two solutions to form a hydrogel *in situ.* We therefore examined the effects of exposing only the genipin solution to oxygen, then reacting this modified genipin solution with a PEG solution. Exposing the genipin solution to oxygen led to an enhanced rate of gelation when compared to genipin not exposed to oxygen. An increase in exposure time to oxygen resulted in an increase of gelation rate.

Table I. Length of time for solution to become blue ("initial gelation") after treating genipin with oxygen.

Oxygen exposure (min)	Initialization of gelation (min)
0	266
10	142
30	97

Conclusions

We have discovered a method to increase the gelation rate of the PEG-genipin hydrogel. By exposing the PEG-genipin solution to air we were able to reduce the gelation time. Exposure of the genipin solution to oxygen also led to an increase in the gelation rate, where an increase in exposure time enhanced the increased gelation rate. We are currently assessing the chemical structure of the modified genipin using FT-IR and degradation of the PEG-genipin hydrogels exposed to oxygen. The exposure of genipin to oxygen is a promising development towards our goal of an injectable PEG-genipin hydrogel.

References

1. Moffat KM; Marra, K.G. JBMR. 2004; 71B:181.
2. Mwale F, et al., J. Tissue Eng. 2005; 11:130.