Characterisation of Redox Initiators for Producing Poly(Vinyl Alcohol) Hydrogels

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Summary: This work focuses on the characterisation of ascorbic acid/persulphate initiating system. Three different persulphates were used (ammonium, potassium and sodium), and a range of initiator concentrations were tested. Gel time, gel quality, initiator toxicity, and cell survival upon encapsulation were measured. No significant differences were observed between the three types of persulphates. Higher concentrations of the initiators resulted in faster gel times (5 min for 0.05 wt% initiator) and higher quality gels (less than 20% sol fraction), although the lower initiator concentrations were better in terms of cell growth inhibition and survival upon encapsulation. Overall, this system shows great promise for use in biomedical applications, however there is a need to minimise the initiator concentration to increase cell compatibility while maintaining a high enough concentration for adequate gel formation.

Keywords: biocompatibility; crosslinking; hydrogels; poly(vinyl alcohol); redox polymers

Introduction

Hydrogels are beneficial for many biomedical applications. In particular they have been used as embolic agents, drug delivery devices, or tissue engineered (TE) constructs. These applications have several key design criteria that scaffolds must meet including mimicking the native tissue, low toxicity and ability to be delivered and gelled *in situ*. It is also critical that cells survive the encapsulation process. Previous work has shown that poly (vinyl alcohol) (PVA) based hydrogels have minimal effects on cell growth and can be tailored to match the properties of soft tissues.[1,2] Ultimately, the application of this research is the formation of biosynthetic scaffolds that can encapsulate cells/drugs and cure in vivo. Previous research has focused on the polymer and scaffold properties,[3–6] whereas this study focuses on the initiating agents. UV initiation is a standard method of polymerising PVA macromers, however this method is limited to topical applications. A more flexible initiating system that allows a liquid macromer solution to be delivered to deep tissues and then gelled in place is desirable. Redox systems based on a metal catalysed Haber–Weiss reaction have been studied,[1] however these systems cured instantaneously and fine control of gel time was challenging. The ability to tailor the time it takes for gelation is an important design criteria for *in situ* curable hydrogels. The aim of this research was to adapt a persulphate/ascorbic acid redox curing system for the polymerisation of poly (vinyl alcohol) (PVA) macromers into hydrogels. Specifically, the effect of initiator type and concentration on gel time, gel quality, cell growth inhibition (toxicity of the polymers) and cell survival upon encapsulation was studied.

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Materials and Methods

PVA polymers (14KDa, 83% hydrolysis) that were modified with seven pendant acrylate groups per chain were used. Hydrogels were formed via redox initiation (reductant = ascorbic acid (AA); oxidant = ammonium, potassium or sodium persulphate (APS, PPS, SPS; 0.01% - 0.1 wt% initiator). Gel time was determined through physical observation, and recorded when a solid mass was formed. The gels were swollen in PBS at 37 °C and the sol fraction/mass loss was determined.

Cell growth inhibition (CGI) studies were conducted using L929 mouse fibroblast cells. Cells were incubated with the test solution for 48 hrs (37 °C, 5% CO_2), then counted using a Coulter counter and compared to controls. Test solutions were 0.01–0.05 wt% ascorbic acid, or ammonium, potassium or sodium sulphate. Viability of cells following encapsulation was performed using a live/dead cell assay immediately after polymerisation and at 24 hours post-encapsulation.

Results and Discussion

Hydrogels were formed by redox initiation, and the initiating systems were tested for gelation time, sol fraction, cell growth inhibition and cell survival upon encapsulation. The gel time for these hydrogels needs to be within a clinically feasible time scale so that it is slow enough to be able to deliver the hydrogels without premature polymerisation in the delivery device, while also curing quickly once it is placed in the body. Gel times for the three different oxidants (APS, PPS, SPS) were compared over a range of initiator concentrations (0.01–0.05 wt%) at 20 °C (see Figure 1). There were no significant differences between the three different persulphate oxidants and as expected, the higher the initiator concentration the faster gelation occurred. The highest concentration (0.05 wt%) resulted in gel formation in approximately 5 minutes, and the lowest concentration (0.02 wt%) gelled in approximately 30 minutes. Formulations made with 0.01 wt% initiators did not produce a gel. Additional experimentation was performed at 37 °C to further mimic physiological conditions. These experiments resulted in gel times between 5 and 10 minutes for all initiator concentrations from 0.02 wt% to 0.05 wt% (data not shown). These times to reach gelation were considered to be acceptable given that mixing and delivery should be easily achievable within 5 to 30 minutes.

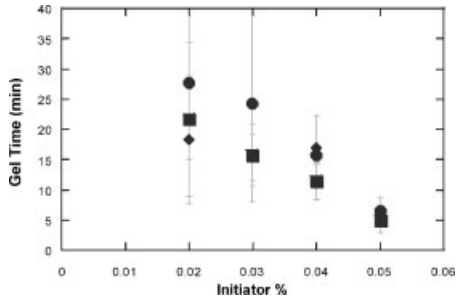

Figure 1.
Time to reach gelation as a function of initiator concentration. Ascorbic acid was the reductant in all formulations, and the oxidant was either ammonium (●), potassium (■) or sodium (◆) persulphate. Gelation was done at 20 °C with 20% PVA macromer.

The efficiency of gelation is another important parameter, and was related to the sol fraction of the gels, or the mass loss at 24 hours. All initiator concentrations (except 0.01% which did not produce a gel) and types resulted in less than 30% sol fraction, with higher initiator concentrations having lower sol fractions (see Figure 2). Again, no significant difference was noted between the three oxidants. PVA is known to have low toxicity, and the addition of different functional groups to the PVA did not significantly change the inhibitory effects of the polymer.[1] Therefore, the release of any polymer chains in the sol fraction is not expected to be detrimental to cells.

The relative toxicity of the initiators was tested using a cell growth inhibition assay. This assay was used to compare the effect of the different initiators on the cells. All three

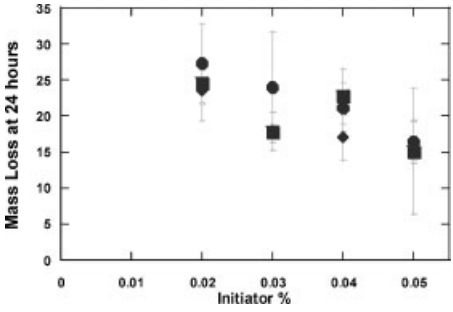

Figure 2.
Mass loss at 24 hours (sol fraction) as a function of initiator concentration. Ascorbic acid was the reductant in all formulations, and the oxidant was either ammonium (●), potassium (■) or sodium (◆) persulphate. Gelation was done at 20 °C with 20% PVA macromer.

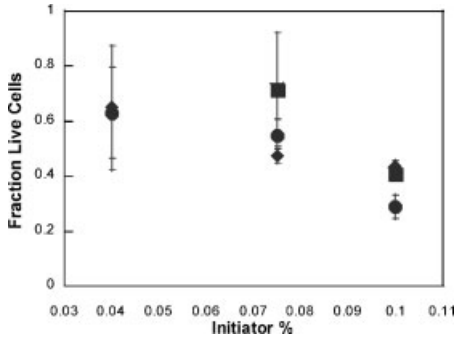

Figure 3.
Fraction of encapsulated cells that were viable immediately after polymerisation. Polymerisation was done 20% PVA macromer, and ascorbic acid as the reductant in all formulations. The oxidant was either ammonium (●), potassium (■) or sodium (◆) persulphate.

oxidants showed similar responses with between 60–75% inhibition of cell growth for all concentrations between 0.01–0.05 wt% initiator. While this is not an ideal result, it did demonstrate that there was a trend for lower cell growth inhibition as initiator concentration decreased, suggesting that initiator concentration should be minimized in all formulations. It is also important to keep in mind that this test is a relative measure and represents a worst case scenario, as the cells are directly exposed to the initiators for 48 hours. When applied *in vivo*, the reaction will occur much faster and the initiators will be rapidly consumed thus minimizing exposure of cells. A more realistic measure of response of the cells to the initiators is to encapsulate cells within polymer gels. The results of encapsulation studies for a range of initiator concentrations (0.04–0.1 wt%) are shown in Figure 3. The lowest concentration tested (0.04 wt%) showed over 60% viable cells immediate after polymerisation for all initiator types. The fraction of live cells decreased with increasing initiator concentration, as expected. At 24 hours post encapsulation, the fraction of live cells was similar at 50% for all concentrations and types (data not shown).

It was determined that when mixing cells and serum into the polymerization mixture the gel time decreased, and in some cases complete gels were not formed. As a result, higher initiator concentrations were used for the encapsulation study than were used in the previous studies, illustrating the need for optimising the polymerisation conditions.

Conclusions

Overall this work demonstrates that the ascorbic acid/persulphate redox system does have many beneficial features for use in biomaterial applications. The gelation time was able to be controlled sufficiently to allow for the appropriate mixing and delivery of the polymer solution, while also curing quickly enough to remain where it was placed within the body. The gels that were formed had good chain incorporation as was demonstrated by having less than 30% sol fraction.

All of the initiator types and concentrations had a similar effect on the cell growth of fibroblast cells. While this work showed a high level of cell growth inhibition, the more realistic measure of cell survival upon encapsulation revealed that for all cases studied at least 50% of the encapsulated cells survived the polymerisation process. Further work is underway to optimise the

concentration of initiator to maximise the number of viable cells while still maintaining a good quality hydrogel.

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