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Development of in situ-forming hydrogels for hemorrhage control

Henry T. Peng · Pang N. Shek

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Abstract We report the preparation of in situ-forming hydrogels, composed of oxidized dextran (Odex) and amine-containing polymers, for their potential use as a wound dressing to promote blood clotting. Dextran was oxidized by sodium periodate to introduce aldehyde groups to form hydrogels, upon mixing in solution with different polymers containing primary amine groups, including polyallylamine (PAA), oligochitosan and glycol chitosan. A series of experiments were conducted to identify the optimum gelation condition for the Odex-PAA system. The polymer concentration appeared to have a major effect on gelation time and the polymer weight ratio affected the resulting gel content and swelling. Other influencing factors included pH of the buffer used to dissolve each polymer, PAA molecular weight, and the type of individual material. The latter also contributed significantly to gel content and swelling. Thromboelastography was used to examine the effects of the in situ gelation on blood coagulation in vitro, where the Odex-PAA combination was found to be most pro-hemostatic, as indicated by a decrease in clotting time and an increase in clot strength. The results of this study demonstrated that in situ-forming hydrogels could promote clotting in vitro; however, further studies are required to determine if the same hydrogel formulations are effective in controlling hemorrhage in vivo.

1 Introduction

Hemorrhage remains the leading cause of death on the battlefield. The latest statistics from Iraq wars shows that approximately 90% of combat deaths among potentially preventable casualties are contributable to uncontrolled hemorrhage, and a little more than half of those are caused by non-compressible (torso) hemorrhage [1].

A variety of hemostatic agents have been developed, including chitosan-based HemCon and zeolite-based QuikClot that are currently used in the field [2]. These materials are solids and normally need to be applied with manual pressure to control external bleeding, which may be less suitable for non-compressible internal bleeding.

In situ-forming hydrogels, often referred to as injectable hydrogels, are a class of hydrogel materials that can transition from a liquid state to a gel at the application site. These hydrogels have been exploited for broad applications in tissue engineering [3], drug delivery [4], and sealing the leakage of blood and other body fluids during surgical procedures [5, 6]. When associated with the latter use, these materials are often called tissue sealants/adhesives/glues, which can attach to a tissue surface to form a physical barrier or seal.

The application of hydrogels for wound and surgical management remains relatively limited. Nevertheless, hydrogel has been used as the constituent of a fibrin sealant, which is formed instantaneously from mixing liquid forms of fibrinogen and thrombin. Such sealant is widely used in different medical procedures, including hemorrhage control, wound healing, suture support, and tissue sealing [5]. The in situ-forming hydrogels can be formulated to incorporate other hemostatic agents prior to use and adhere to tissues immediately after application. They can be designed for topical applications to both external and internal bleeding sites of any irregular shape and size.

Different strategies have been used to obtain in situ-forming hydrogels. These strategies may be classified into three categories: (1) chemical methods, such as

H. T. Peng (✉) · P. N. Shek
Defence Research and Development Canada – Toronto,
1133 Sheppard Avenue West, Toronto, ON M3M 3B9, Canada
e-mail: henry.peng@drdc-rddc.gc.ca

photocrosslinking [7], Michael-type reactions [8], Schiff-base reactions [9], and oxidative polymerization [10]; (2) physical crosslinking, such as thermal gelation of temperature-responsive polymers [11], ionic gelation of polysaccharides [12], affinity interactions or self-assembly of biological synthetic copolymers [13]; and (3) biological crosslinking that involves enzymatic reactions, such as enzyme-mediated gelation of dextran-tyramine [14] and poly(ethylene glycol)-fibrinogen γ-peptide conjugates [15]. Compared to chemically formed hydrogels, physical gels may be more biocompatible, but they require a longer gelation time and have a lower gel strength, possibly accounting for differences in biological performance between the two hydrogel types [16]. A microsphere-based gelling system has also been obtained by mixing dispersions of oppositely charged dextran microspheres [17].

In a previous study, we reported an in situ-forming hydrogel composed of a multi-functional poly(ethylene glycol) N-hydroxysuccinimide ester (PEG) and poly(allylamine hydrochloride) [18]. We demonstrated that such a biomaterial is promising in controlling hemorrhage. However, the relatively high cost of PEG renders it not attractive for most manufacturers. In this study, we investigated a more affordable replacement for PEG, which can be used for various gelling systems. Specifically, in situ-forming hydrogels were produced by mixing polymer solutions of oxidized dextran and different amine-containing polymers. Dextran is a bacterial polysaccharide, which has been extensively used as a biomaterial because of its excellent biocompatibility, low cost and easy functionalization [19]. Oxidation is a facile method to functionalize dextran with aldehyde groups, which can react with both small and large amine-containing molecules to produce hydrogels [20, 21]. Both synthetic and natural polymers with abundant amine groups were included in our study, such as poly(allylamine hydrochloride), glycol chitosan and oligochitosan. The former has been clinically used as a phosphate binder to treat renal disease [22], and the latter two are chitosan derivatives with potential applications for drug delivery [23] and drug detoxification [24]. Although oxidized dextran has been employed in a number of in situ gelation systems with N-carboxyethyl chitosan [25], gelatin [26], polylysine [27], hydrazide-modified carboxymethyldextran [21], and amine-terminated PEG [28], our study focused on using various amine-containing polymers as a sealant to stop bleeding. Such system may also be used to deliver pro-coagulation agents, while creating an instant seal over damaged tissues.

In this paper, Odex-PAA in situ-forming hydrogels were fabricated by titrating the following parameters: polymer solution concentrations; pH of buffers for dissolving different polymers; PAA molecular weights; and amine-containing polymer types. Our aim was to produce fast gelling materials with suitable physical and mechanical properties for applications in hemorrhage control. Fourier Transform Infrared (FTIR) spectroscopy and swelling experiments were used to reveal the composition, gelation mechanism and physical properties of the resulting hydrogels. A thromboelastography (TEG) method, a global measure of blood coagulation [29], was applied to evaluate the in vitro hemostatic effects of the hydrogels. This study generated sufficient data for further investigations of the biomaterials alone or in combination with other hemostatic compounds in animal bleeding models, which could lead to novel applications for hemorrhage control.

2 Materials and methods

Dextran from Leuconostoc mesenteroides, with a molecular weight range from 100,000 to 200,000, poly(allylamine hydrochloride) (PAA) with a weight-averaged molecular weight of approximately 15,000 and 70,000, medium-molecular-weight chitosan with 75–85% degree of deacetylation, glycol chitosan with a degree of polymerization above 400, sodium periodate with $\geq$99.0% purity, were purchased from Sigma-Aldrich (Mississauga, ON, Canada). The low-molecular-weight PAA and glycol chitosan were designated as LoMw PAA and Glycolchi, respectively. β-oligochitosan with an average molecular weight of 3,500 was kindly provided by Arabio Co., Ltd. (Guui-Dong, Gwangin-Gu, Korea) and designated as Oligochi. Other chemicals were ACS grade and used as received.

2.1 Oxidation of dextran

The reaction was carried out according to the process previously described [20]. Briefly, dextran and sodium periodate were dissolved in Milli-Q water, respectively and the two solutions were mixed in a glass beaker in the dark under continuous stirring. Different oxidation conditions (i.e., concentration and time) were used. Equal molar of ethylene glycol to sodium periodate was added to stop oxidation reaction, followed by dialysis against Milli-Q water in a membrane with a molecular weight cut-off of 12–14 kDa (Fisher Scientific Canada, Nepea, ON, Canada) for 3–4 days and vacuum freeze-drying to constant weight. The extent of oxidization was determined by a titration method previously described by Zhao et al. [30].

2.2 Hydrogel formation and gelation time

Hydrogels were prepared in glass vials (inner diameter: 17 mm and height: 61 mm) under different conditions.

Specifically, individual polymer solutions were prepared in aqueous pH buffers, respectively. Two solutions (150 µl each) were mixed at room temperature in the vial using a magnetic stirring bar (13 mm × 3 mm) at rpm of 60. The time required for the bar to stop stirring was defined as the gelation time [9].

2.3 Gel content and swelling ratio

Hydrogels formed within 3 min were soaked in Milli-Q water under shaking to remove any free polymers. Each hydrogel was weighed after removal of surface water, followed by freeze-drying. The gel content was calculated as the ratio between the mass of the dry hydrogel and the initial amount of polymers used. The swelling ratio of each hydrogel was determined as the mass ratio before and after the lyophilization.

2.4 Fourier Transform Infrared (FTIR) spectroscopy

Dextran, Odex, PAA and Odex-PAA gel were made into pellets, and then analyzed by FTIR. The pellet was made using Econo mounts and press (Fisher Scientific Canada, Nepea, ON, Canada). Pellet matrix contains approximate 100 mg of KBr, and 5–6 mg of sample material.

2.5 Thromboelastography (TEG)

TEG measures the in situ change in viscosity of blood as a function of time under a low shear [31]. The TEG machine consists of an inner cylinder (the pin) suspended on a torsion wire and an outer cylindrical cuvette (the cup). The suspended pin is immersed in whole blood or plasma in a cup. The cup oscillates back and forth constantly at a set speed through an arc of 4°45′. The torque of the cup is transmitted to the pin, via the fibrin strands in the blood clots as coagulation proceeds, and to the torsion wire for conversion by a mechanical–electrical transducer to emit an electrical signal, which is monitored by a computer [31].

TEG was used to determine the effects of individual polymers and their gelation on blood coagulation. Specifically, blood samples were taken from healthy volunteers via venipuncture and collected into separate Vacutainers (Fisher Scientific Canada, Nepean, ON, Canada) containing 0.109 M sodium citrate solution (citrate:blood volume = 1:9) and gently inverted 3 times. The citrated whole blood was stored at room temperature for at least 30 min prior to TEG analysis, since it has been consistently documented that a sample stored for less than 30 min is not stable [32, 33]. Thromboelastographic measurements were carried out using a computerized TEG® Hemostasis System 5000 (Haemoscope Corporation, Niles, IL). After the system has passed the electronics testing and quality control according to manufacturer's protocol, citrated whole blood (300 µl) was loaded into TEG cups pre-warmed to 37°C. Each polymer solution (30 µl) prepared in aqueous pH buffers and an aliquot of 30 µl of 0.2 M calcium chloride solution were then added into the TEG cups, respectively. The measurement was started immediately after uniformly mixing the whole solution by pipetting in and out once. TEG was also conducted after the addition of both polymer solutions at 30 µl each to 300 µl citrated human blood following the same procedure. Calcium chloride was included in the amino-containing polymer solution to initiate blood coagulation. Each polymer solution was fully loaded into a TEG cup to obtain values within detection ranges, but without significant gelation prior to any measurements. pH buffers or their mixtures were used as blanks. Analysis was run until all interested parameters were finalized. TEG was conducted as well for blood alone by adding 20 µl of 0.2 M calcium chloride solution to 340 µl of citrated human blood.

2.6 Statistic analysis

Data were presented as mean ± standard deviation and compared using a two-tailed t test with 95% confidence to identify significantly different groups.

3 Results

3.1 Oxidation of dextran

The reaction was optimized by altering reactant concentrations and reaction time. A band at around 1,730 cm^{-1}, corresponding to the stretching vibration of the carbonyl from aldehyde groups [20], appeared in the FTIR spectrum of Odex (Fig. 1), as dextran and sodium periodate concentrations increased 5 times at a constant ratio. The increase in reaction time from 4 to 24 h did not remarkably increase the intensity of the peak, implying lesser effects on the degree of oxidation. Table 1 summarizes the quantitative results obtained using the titration method, which is consistent with the FTIR analysis. Unless specified, all gelation was done by using oxidized dextran prepared under the 4-h condition.

3.2 In situ hydrogel formation and characterization

The hydrogels were formed rapidly through a Schiff-base reaction between the aldehyde and primary amine, by mixing Odex with all 3 amine-containing polymer solutions under appropriate conditions. When the two solutions of Odex and PAA were mixed by a stirring magnetic bar at room temperature, gelation took place immediately and the

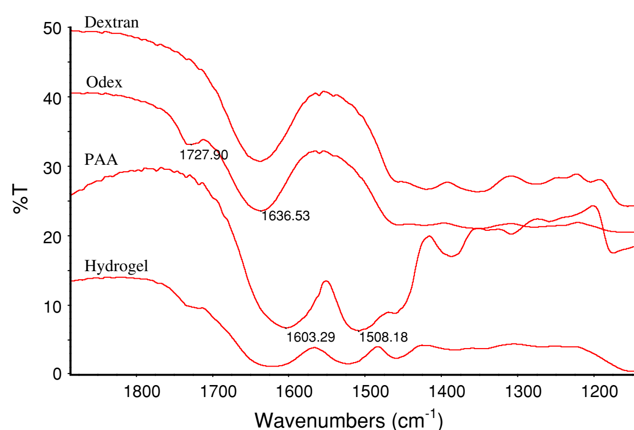

Fig. 1 FTIR spectra of dextran, Odex, PAA and their hydrogel. Odex was prepared at a dextran concentration of 0.625 w/v% and dextran to sodium periodate weight ratio of 0.935. The hydrogel was prepared by a 3-min gelation between 10 w/v% Odex in pH 8 buffer and 15 w/v% PAA in pH 10.4 buffer, washed in Milli-Q water and freeze-dried

stirring was stopped in less than 20 min under all investigated conditions, with the fastest gelation occurring in a few seconds. Figure 1 shows that although its intensity was reduced, the peak appeared at approximately 1,730 cm^{-1} due to the detectable presence of an aldehyde group in the hydrogel. The characteristic peak of C=N bond from the reaction may well overlap with the one at 1636.5 cm^{-1} from Odex [20]. The spectrum of the hydrogel is similar to that of PAA, implying the formation of a PAA matrix.

Figure 2 depicts the effects of Odex and PAA solution concentrations on their gelation time. In general, the gelation time initially decreased with increasing polymer concentrations; did not change within a concentration range; and then increased at a higher concentration. Specifically, the gelation time at a constant PAA concentration of 15 w/v% at pH 10.4 decreased as Odex concentrations increased from 2.5 to 5 w/v%; remained unchanged between 5 and 10 w/v%; and increased significantly at 15 w/v% (Fig. 2a). Similar effects were observed with PAA concentrations at a constant Odex concentration of 10 w/v%, i.e., an initial increase followed by no changes (at 10–20 w/v%) and an increase (at 20–30 w/v%) in gelation time (Fig. 2b).

Figure 3 indicates that gelation occurred in a pH-dependent manner. For an Odex solution buffer, lower pH seems to favour gelation, while an opposite effect was

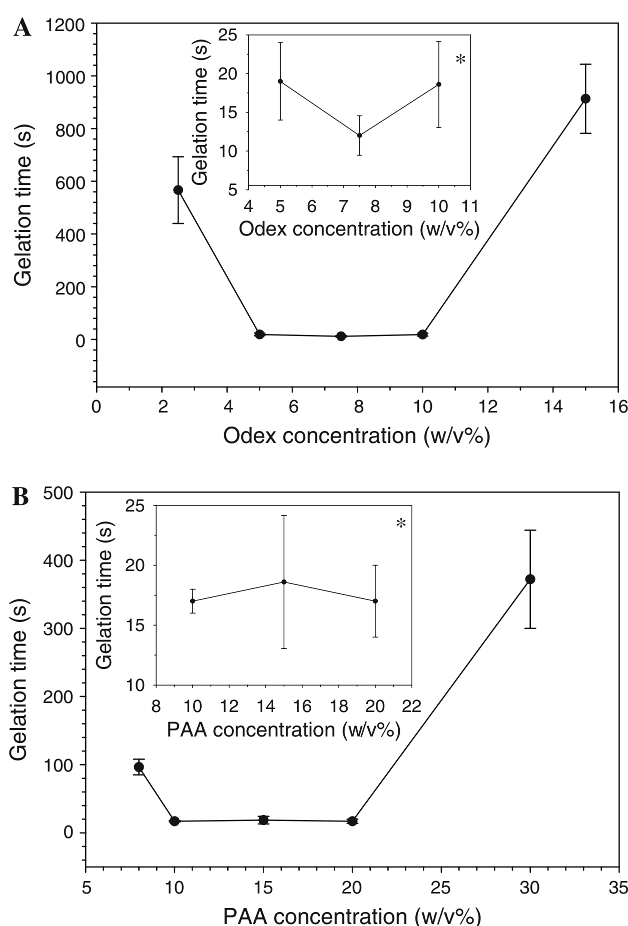

Fig. 2 Effects of the solution concentrations of oxidized dextran (Odex) and poly(allylamine hydrochloride) (PAA) on gelation time. **a** The PAA concentration was constant at 15 w/v% in pH 10.4 buffer. **b** The Odex concentration was kept constant at 10 w/v% in pH 8 buffer. * The inserts show the uneffecting concentration range. Data are expressed as mean ± standard deviation ($n = 3–6$)

seen for PAA. The gelation time was significantly reduced from 51.5 s to 18.6 s as PAA buffer pH increased from 8 to 10.4. In contrast, the effect of Odex buffer pH on gelation time is less profound.

The hydrogels, obtained at a 3-min reaction time, retained approximately 60 to 90% of initial materials and swelled 25 to 50 times in water. The gel content decreased with the Odex:PAA weight ratio, corresponding to an increase in gel swelling (Fig. 4). A plot of gel content versus either the gelation time or swelling ratio shows that

Table 1 Degree of oxidization of dextan under different conditions quantified by the titration method

Sample	Dextran concentration (w/v%)	Dextran to sodium periodate weight ratio	Reaction time (h)	Degree of oxidization (%)
Odex 1	0.125	0.935	24	36.5
Odex 2	0.625	0.935	4	82.59 ± 0.02*
Odex 3	0.625	0.935	18	85.6
Odex 4	0.625	0.935	24	87.5

* Data are expressed as mean ± standard deviation ($n = 3$)

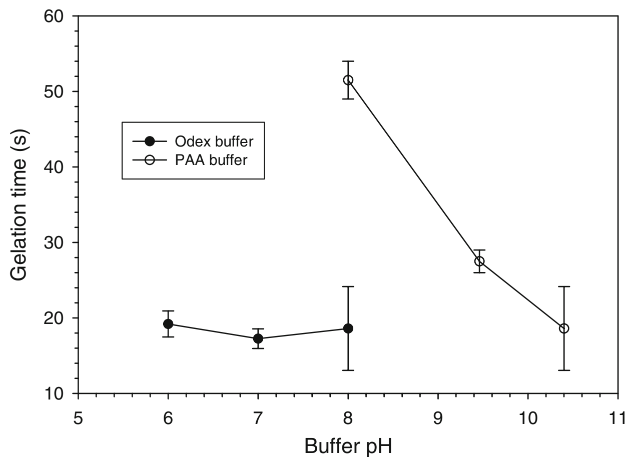

Fig. 3 Effects of buffer pHs on gelation time, with constant Odex or PAA concentrations of 10 and 15 w/v%, respectively and a constant buffer pH either at 10.4 for PAA or at pH 8 for Odex. Data are expressed as mean ± standard deviation ($n = 3{-}6$)

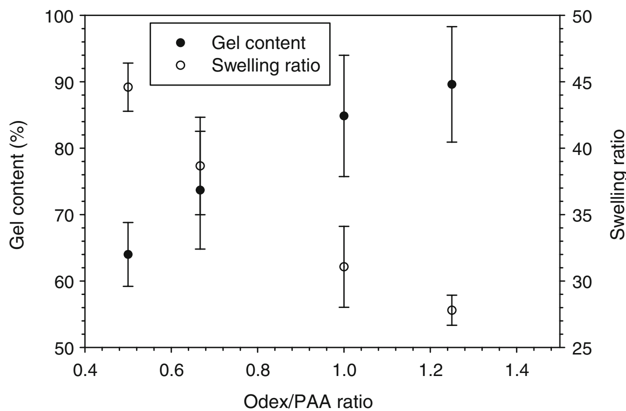

Fig. 4 Effects of Odex: PAA ratio on gel content and swelling ratio, with either a constant PAA concentration of 15 w/v% in pH 10.4 buffer or a constant Odex concentration of 10 w/v% in pH 8 buffer. Data are expressed as mean ± standard deviation ($n = 3{-}6$)

the swelling ratio was more correlated with the gel content in an inverse relationship (Fig. 5).

Table 2 compares the gelation time, gel content and swelling ratio of various systems comprising Odex and amine-containing polymers: PAA, LoMw PAA, Oligochi, Glycolchi, where all mixtures gelated within 3 min. The Glycolchi solution became too viscous at 6 w/v% and could not mix well with the Odex solution. The other amine-containing polymers were prepared at optimum concentrations. The results indicated that the polymer molecular weight and the type of individual polymers affected the gelation time. The Odex-PAA and Odex-Glycolchi system exhibited faster gelation time than the Odex-LoMw PAA and Odex-Oligochi system.

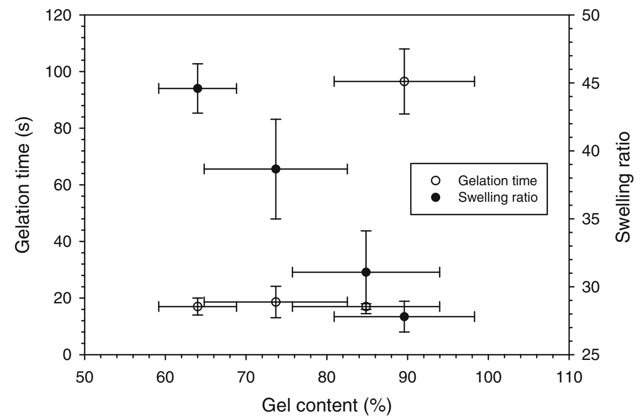

Fig. 5 Gelation time and swelling ratio against gel content. The hydrogels were prepared from Odex and PAA at different concentrations in pH 8 and pH 10.4 buffers, respectively. Data represent mean ± standard deviation ($n = 3{-}6$)

The Odex-Oligochi system showed the largest gel content and lowest swelling ratio, while the Glycolchi and PAA hydrogels showed the lowest gel content and highest swelling ratio, respectively. There were larger differences in the swelling ratio than the gel content. The former ranged approximately from 9.5 to 21.2, while the latter ranged only from 61.5 to 71.5%.

3.3 Thromboelastography (TEG)

Figure 6 presents representative thromboelastographs for citrated whole human blood with a blank (i.e., a pH buffer) or polymer solutions to elucidate their individual and gelation effects on blood coagulation. Different parameters can be derived from the graph. Figure 6 shows the key TEG parameters used in our study: (1) time to detectable clot formation (R) (the amplitude = 2 mm in the TEG tracing); (2) maximum amplitude depicting clot strength (MA); and (3) time to reach MA (TMA). The TEG tracing shows that gelation took place immediately when both Odex and PAA were mixed with blood. As far as blood coagulation is concerned, the presence of both Odex and PAA led to the earliest onset of clot formation (i.e., R time, Fig. 6) and the strongest clot (as measured by the maximal amplitude, MA), followed by the presence of PAA, Odex and the blank in terms of clot strength.

Table 3 summarizes the R, MA, TMA values of blood alone and in the presence of the polymers. Compared to blood alone, a significant reduction in R time by all gelling systems was observed except for Odex-Oligochi. Gelation between Odex and PAA led to an increase in MA, while the Odex-Oligochi and Odex-Glycolchi resulted in a decrease in MA. Only the Odex-Oligochi affected TMA with an increase. Therefore, the gelation of Odex-PAA produced the most significant procoagulant effects.

Table 2 Effects of amine-containing polymers on gelation time, gel content and swelling ratio, with a constant Odex concentration of 10 w/v% in pH 8 buffer. The amine polymers were dissolved in pH 10.4 buffer at 20 w/v% for PAA, LoMw PAA, Oligochi, and at 4 w/v% for Glycochi, respectively

Type of gelation system	Odex-PAA	Odex-LoMw PAA	Odex-Oligochi	Odex-Glycolchi
Gelation time (s)	17.0 ± 3.1 ($n = 6$)*	26.7 ± 7.4	36.0 ± 4.4	17.0 ± 2.8 ($n = 4$)
Gel content (%)	64.0 ± 4.8 ($n = 6$)	67.3 ± 2.4	71.5 ± 5.7	61.5 ± 2.6 ($n = 4$)
Swelling ratio	21.2 ± 1.8 ($n = 6$)	16.4 ± 1.4	9.5 ± 2.2	17.1 ± 2.4 ($n = 4$)

* Data are expressed as mean ± standard deviation ($n = 3$, unless specified)

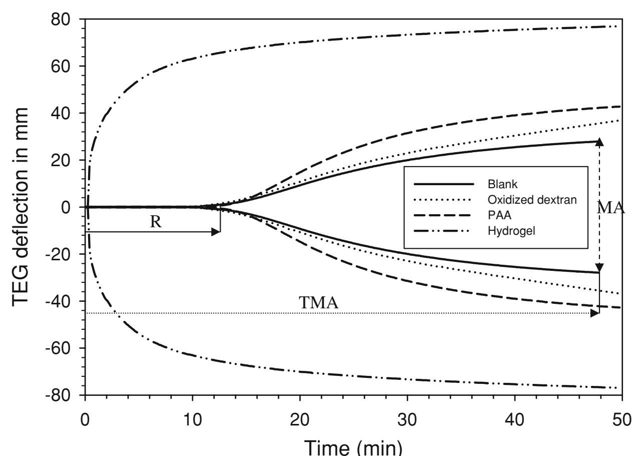

Fig. 6 Typical TEG tracings of citrated whole blood with polymer solutions and pH buffers for dissolving the polymers. All tracings of pH buffers are similar and only one tracing of pH buffer was included for clarity. The dashed arrows indicate the maximal amplitude (MA) for maximum clot strength of blood with PAA while the solid and dotted arrows point to the R and TMA time when the amplitude is 2 mm for initiation of clot formation and reaches maximum of clot strength, respectively

4 Discussion

In addressing the aim of this study to develop in situ-forming hydrogels using oxidized dextrans and amine-containing polymers, we first prepared dextrans oxidized to various extents by adjusting reactant concentrations and reaction time. Compared to reported results [20], our oxidization is higher based on FTIR results. The qualitative FTIR spectroscopy is in agreement with the quantitative titration method. The small increase in the oxidization with increasing reaction time from 4 to 24 h (82.6–87.5% calculated from titration) is consistent with the report indicating only a slight increase in oxidation after 4 h [34].

Gelation took place through a Schiff base reaction between the two functional groups on each polymer. Given an excess amount of amine over aldehyde, the hydrogel was likely formed by the chemical crosslinking of PAA with Odex as a polymer crosslinker, as indicated by the PAA-like FTIR spectrum of the hydrogel. The presence of an aldehyde band in the IR spectrum suggests an incomplete gelation reaction. We also tested the gelation of other oxidized polysaccharides (alginate and carboxymethylcellulose) with PAA, where a gel was immediately formed, but none was able to stop stirring within 3 min. When the gelation of oxidized dextran with other amine-containing polymers and small molecules, such as gelatin, chitosan, and adipic acid dihydrazide was tested, the resulting gel formation was not as fast as reported [20, 35]. Since rapid gelation is a prerequisite for use as a wound sealant, we focused on the gelling systems with a gelation time of less than 3 min.

An optimum polymer concentration is essential for gelation, because the polymer solution could become too viscous at high concentrations for the reactive groups to react with each other. This is in contrast to reports indicating a continuous decrease in gelation time with increasing polymer concentrations in other systems [8, 21, 35]. For the Odex-PAA system, mixing 10 w/v% Odex and 20 w/v% PAA solutions at equal volume was found to be optimal, corresponding to a molar ratio of 0.25 between the aldehyde group on Odex to the amine group on PAA. Optimum concentrations were also observed for other

Table 3 TEG measurements of citrated whole human blood alone or in the presence of gelling polymers. All Odex solutions were prepared at 10 w/v% in pH 8 aqueous buffer. All amine-containing polymer solutions were prepared in pH 10.4 aqueous buffer at 20 w/v% for PAA, LoMw PAA, Oligochi, and at 4 w/v% for Glycolchi, respectively

Type of gelation system	Blood alone	Odex-PAA	Odex-LoMw PAA	Odex-Oligochi	Odex-Glycolchi
R (min)	13.3 ± 4.3	0.22 ± 0.04*	1.8 ± 0.7*	12.2 ± 3.1	1.2 ± 0.9*
MA (mm)	56.1 ± 3.7	73.9 ± 3.1*	72.6 ± 2.8*	42.3 ± 4.5*	45.6 ± 1.5*
TMA (min)	36.7 ± 6.2	29.1 ± 10.1	31.1 ± 5.4	55.2 ± 6.9*	40.3 ± 2.2

* Data are expressed as mean ± standard deviation ($n = 3$ except $n = 20$ for blood alone). Significant difference from blood alone ($P < 0.05$)

polymers, except for Glycolchi which became too viscous in 4–6 w/v% to mix with the Odex solution.

It is well documented that reaction pH affects the formation and stability of a Schiff base [36, 37]. Although the reaction is faster at a slightly acidic pH, the concentration of reactive primary amines increases with pH. In addition, the chemical linkage may become more stable due to a change of the imine bond to an amine [37]. For optimal gelation between Odex and amine polymers, pH-7 Odex and pH-10.4 PAA solutions were mixed at equal volume, producing a mixture with a final pH of 9.6. It is known that pH can affect blood coagulation and biocompatibility. For example, clotting time has been reported to increase by increasing the pH from 6.7 to 8.0 [38]. However, in a thromboelastographic study of fibrinogen-fibrin conversion by thrombin, clotting time and clot strength were found to decrease with an increase in pH of up to 7, but were unaffected by pH in the range of 7.0–10.0 [39]. In addition, hydrogels made from mixing 2 polymer solutions with a pH of 6 and 9 were shown to be biocompatible [9].

Swelling is of utmost importance in biomedical applications of a hydrogel. High water-absorbency may facilitate blood coagulation [40]. Swelling ratio was thus selected to evaluate the performance of different hydrogels. A decrease in swelling with an increasing Odex/PAA ratio is due to increased crosslinking density as implied by an increase in gel content. This is also in agreement with a reported dependency of swelling ratio on the Odex content of a hydrogel containing N-carboxyethyl chitosan [25]. On the other hand, a minimum swelling ratio is expected as the molar ratio between aldehyde and amine is close to 1.

The correlation between the gel content and swelling ratio is in agreement with the literature [14, 41] and our previous findings for another in situ-forming hydrogel [18]. A high degree of swelling implies limited crosslinking and is consistent with the low gel content. The swelling values are higher than the ones observed in Odex hydrogels crosslinked with adipic acid dihydrazide [20], but in the same range of hydrogels obtained from Odex and N-carboxyethyl chitosan [25], and oxidized hyaluronic acid and gelatin [42]. In comparison with our previously reported hydrogels composed of a multifunctional poly(ethylene glycol) (PEG) and PAA [18], the comparable gel content might imply the same extent of reaction. The lower swelling ratio observed was likely due to the lower water absorption of dextran compared to poly(ethylene glycol).

The effects of polymer molecular weight on Odex-PAA gelation are likely due to its influence on the viscosity of its solution and the mechanical strength of resulted hydrogels. The polymer with a higher molecular weight may result in a stronger hydrogel, and an increase in solution viscosity at a higher molecular weight may retard the chemical reaction between the polymer functional groups.

Chitosan was initially selected for in situ gelation with Odex due to its well-documented bioactivities, including its hemostatic properties [43, 44]. Chitosan-based injectable hydrogels have been reported [45] and used as a biological adhesive [46]. However, chitosan was primarily soluble in acidic solution and could not form a hydrogel rapidly (within 3 min) [47], but chitosan derivatives have been used to overcome the problem. Among these, glycol chitosan, a 6-(2-hydroxyethyl) ether derivative of chitosan, is soluble in both acidic and basic aqueous buffers because of the highly hydrophilic glycol group. Oligochitosan, converted from chitosan by hydrolysis, is also soluble in neutral and basic aqueous buffers and has distinctive chemical and biological properties from its parent chitosan [48]. Also, oligochitosan has been modified or combined with other materials for various biomedical applications [49]. It is noteworthy that Oligochi used in the study is in a β form, which may be more promising because of its higher reactivity as a result of weak intermolecular forces, compared to the one in an α form [50].

Variations in gel contents and the swelling of various hydrogels may be attributable to the crosslinking density and hydrophilicity of each amine-containing polymer. For example, the higher swelling of the Glycolchi hydrogel, compared to the Oligochi hydrogel, is consistent with its lower gel content, implying a lower crosslinking density and a higher hydrophilicity of Glycolchi [23]. However, the higher degree of swelling of the PAA-based hydrogel, compared to the chitosan-based hydrogels, is inconsistent with their respective gel contents. This may be explained by the macromolecular interactions (e.g., hydrogen bonding and chain entanglement) between the gel-forming polymers and their different swelling capacity [51]. In addition, the minimal effect of PAA molecular weight on the gel content is in agreement with reported small differences in the solid content of 2-vinyl-4,4-dimethylazlactone-modified poly(vinyl alcohol) gels, despite variations in the molecular weight of the polymer ranging from 31,000 to 67, 000 [52].

The type of polymer can affect the gelation and gel content in a different manner. The faster gelation, but lower gel content resulting from different types of polymers, reflects different control mechanisms, the former being more controlled by gel structure and the latter by the extent of crosslinking. The different polymer structures (vinyl vs. saccharide unit) may further contribute to the observed differences between the hydrogels prepared from PAA and chitosan derivatives. Polysaccharides biodegrade relatively faster than synthetic polymers. Thus, Odex-PAA may be more durable than Odex-chitosan derivatives, but they still may break down through the hydrolysis of Schiff base and the degradation of Odex.

A number of in vitro tests of hemostasis and blood coagulation have been reported, but these tests are mainly concerned with the effects of solid materials on different parts of the coagulation system, such as thrombus formation and platelet function [53] and thus are not suitable for testing topical hemostatic materials soluble in blood. It is also difficult to relate findings from such tests to the overall effects on whole blood, as what actually occurs in clinical settings. To test the hemostatic properties of the gel-forming polymers in vitro prior to in vivo studies, a thromboelastography (TEG) method was used. TEG is recognized as a global assay of blood coagulation based on quantitatively measuring the elasticity of whole blood, from the beginning of coagulation, through clot formation, to the end stage with fibrinolysis [29].

Although designed for monitoring blood coagulation, the rheology-based mechanism would make TEG suitable for studying the gelation process. The TEG test uses only a small amount of sample material, but provides a real-time determination of the onset of gel formation, the rate of gelation, the maximum gel strength and the time to reach it, and even the degradation of the gel, if applicable. This has been demonstrated in a TEG study of the mechanical strength of fibrin sealants [54]. Therefore, TEG provides a convenient and relevant evaluation of in vitro coagulation effects of different gelling systems, thus providing justifications for possible in vivo follow-up studies.

Dextran has been used in fluid resuscitation, which could compromise blood coagulation at 50% hemodilution as measured by TEG [55]. On the other hand, Lee et al. have demonstrated that the effects of Oligochi on blood coagulation are minimal in TEG testing [24]. The reduced clotting time (R) by individual polymers could result from an increase in fibrin polymerization, and clot strength (MA) could be affected by each polymer because of its interference with fibrin crosslinking [56]. The observed effects of individual polymers, where calcium was added, could not be attributed to an increase in blood viscosity, because in a control study where no calcium was added, there was no detectable coagulation (i.e., tracing of a straight line) and thus no change in intrinsic viscosity due to the test polymers.

Blood coagulation has been considered as a liquid-solid transition similar to a sol–gel transition in polymers [57]. A decrease in R and an increase in MA values may thus be due to the combined effects of individual polymers and their gelation on blood coagulation.

5 Conclusions

A rapid gelation system was developed by using an oxidized dextran and synthetic or natural amine-containing polymers. For the gelling system composed of oxidized dextran and poly(allylamine hydrochloride), the optimal gelation time is dependant on the concentration of each polymer solution and the pH of each buffer used to dissolve the polymer. The hydrogels exhibited swelling properties related to their crosslinking densities as indicated by their gel contents. The molecular weight and the type of amine polymers also influenced the gelation and swelling of the hydrogels. Oxidized dextran and poly(allylamine hydrochloride) can reduce coagulation time and increase clot strength. These findings appear promising for the development of a biomaterial to reduce hemorrhage in vivo, possibly leading to a new hemostatic product by combining a procoagulating agent with a sprayable biomaterial.

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