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ABSTRACT
Surgical sealants are an integral part of the surgical toolbox. They are used mainly to seal tissues or suture lines. In the current research, we examined a novel surgical sealant based on the natural polymers gelatin and alginate, crosslinked with carbodiimide and loaded with hemostatic agents. Incorporation of the hemostatic agent montmorillonite significantly improved the ex vivo burst strength and the other physical properties (swelling ratio, weight loss, curing time, and viscosity) of the basic surgical sealant, due to the formation of nanocomposite structure. This novel system presents a new approach for improving the physical properties of surgical sealants.

1. Introduction

1.1. Surgical sealants
Various medical bioadhesives which provide solutions in the medical field were developed in the past few decades. These bioadhesives can be divided into three groups: (a) hemostats that form blood clots through different mechanisms, generally only in the presence of blood in the target tissue; (b) glues that attach lacerated and traumatic tissue together; (c) sealants that create a sealing barrier which prevents the leakage of gas or liquid from a structure. Adhesives and sealants undergo self-polymerization in situ and are most effective in a dry area. The three types of bioadhesives usually have similar properties, although it is necessary to adjust their properties to the specific desired clinical situation. Hemostats are available as sponges, granular powders, patches, and polymeric hydrogels, whereas the sealants are necessarily in the form of polymeric hydrogels which polymerize in situ [1–3]. A summary of FDA-approved surgical sealants for different clinical applications is presented in Table 1. The requirements from polymeric hydrogel sealants are first of all biocompatibility as well as other mechanical and physical properties such as adequate burst strength, curing time, viscosity, swelling degree, and degradation [4,5]. All of these were investigated in the current study.

Novel tissue-adhesive formulations based on a combination of gelatin with alginate as a polymeric additive and crosslinked with carbodiimide (EDC) have recently been developed and studied in our lab [6,7]. The current research focuses on the development of surgical sealants based on the same components, to combine high burst strength with biocompatibility and other desired properties (suitable viscosity, curing time, etc.). Since these sealant formulations contain natural polymers and hemostatic agent fillers, they are termed “composite sealants.” Such composite sealants have not been developed and studied to date.
1.2. Composition of the sealant

Gelatin is a natural water-soluble polymer derived from collagen. It has become one of the most extensively investigated materials for surgical sealants due to its suitable natural properties. Gelatin is considered to be biocompatible, biodegradable, and nonimmunogenic [8]. It can form physically crosslinked hydrogel structures [9], has a natural tacky behavior in solution, and is abundant in nature [10]. In spite of its promising qualities, the mechanical strength of physically crosslinked gelatin surgical sealants is not sufficient as an adhering substance by itself [10]. A chemical crosslinking agent and a polymeric additive (with suitable available functional groups for the crosslinking reaction) can be added to the gelatin solution to create gelatin-based hydrogel formulations with mechanical properties that are suitable for surgical sealants [10–14]. Using this concept, our study focused on novel surgical sealants based on a combination of gelatin and a polymeric alginate additive, crosslinked with carbodiimide. Carbodiimide, which is mainly used for modification and conjugation of proteins and other biological macrostructures, was chosen as the crosslinking agent because carbodiimides and their crosslinking by-products have been reported to be less cytotoxic than other conventional crosslinking agents such as formaldehyde and glutaraldehyde [15].

Alginate is a natural polysaccharide which is extracted from marine algae. It was chosen as the polymeric additive for the gelatin sealant in the current research due to its natural source and high concentration of carboxylic groups which are essential for the crosslinking reaction of carbodiimides. Carbodiimide couples to a carboxylic group (originally from the gelatin or the alginate) to form an o-iso-acetyurea intermediate which is highly reactive and has an extremely short life. This intermediate undergoes a nucleophilic attack by a primary amino group (originally from the gelatin) to form an amide bond, with the release of a urea molecule (derivative of the carbodiimide type) as a by-product [16].

Since lacerated tissues contain exposed amino and carboxylic groups which can participate in the crosslinking reaction, our new gelatin–alginate–carbodiimide surgical sealants have the potential to be especially attractive for tissue adherence and indeed demonstrated very good ex vivo bonding strength and high biocompatibility [7].

1.3. The hemostatic agents used in the current study

Two types of hemostatic agents, sodium montmorillonite (MMT) and kaolin, were loaded into the gelatin–alginate (Gel-Alg) hydrogel in the current research, to improve the adhesive abilities in the hemorrhagic environment of the wound and to increase the cohesion strength. These hemostatic agents were chosen due to evidence from other studies that indicate their hemostatic properties in topical applications [17,18].

Kaolin is a clay mineral which has a 1:1 layered silicate structure and the formula Al₂(Si₂O₅)(OH)₄ (presented in Figure 1) in which the silica tetrahedral layer (Si₂O₅)²⁻ is rendered electrically neutral by an adjacent Al₂(OH)₄³⁻ layer. The midplane consists of O²⁻ anions from the (Si₂O₅)²⁻ layer as well as OH⁻ ions that are a part of the layer. A kaolinite crystal is made of a series of these double layers or sheets that are stacked parallel to each other and form small flat plates which are typically less than 1 µm in diameter and nearly hexagonal [19–22]. The surface charge properties of kaolin are important for the interaction with the polymer solutions. Good affinity between the polymeric matrix and kaolin leads to reinforcement effect of the composite. In case of incompatibility between the two, a decrease in the composite properties may occur. The permanent charges are due to imperfections in the kaolinite crystals which result in a negative charge in the faces (basal planes). The conditional charges are pH dependent and are due to the broken bonds at the edges that exist as a result of exposed Al and Si at the edges which can yield positive charges in acidic solutions and negative charges in alkaline solutions [23,24]. Kaolin is a strong contact pathway activator agent that initiates rapid clot formation in wounds. It is used as an activator in different laboratory evaluations of hemostasis. Quikclot® (Z-Medica) is one of the many applications of kaolin that received FDA approval as a topical hemostatic gauze with

![Figure 1](image-url). The chemical structure of (a) kaolin and (b) sodium montmorillonite.
kaolin impregnated as a hemostatic agent in nonwoven standard medical gauze. This application is widely used by military forces and hospitals around the world [17,18].

Montmorillonite is a 2:1 layered silicate with hemostatic properties and a structure of two-dimensional layers where a central octahedral sheet of alumina is fused to two external silica tetrahedral layers such that the tips of the tetrahedral of each silica sheet and one of the hydroxyl layers of the octahedral sheet form a common layer. MMT has the structure of (NaCa)0.3 (Al, Mg)2− (Si4−Al2)3(OH)2·nH2O (presented in Figure 1), which consists of partial substitution of the trivalent Al cation in the octahedral layer by the divalent Mg cation. Due to this substitution, the mineral is characterized by a negative surface charge which is balanced by interlayer cations, commonly sodium or calcium. A particular feature of the resulting structure is that since these ions do not fit in the tetrahedral layer and the layers are held together by relatively weak Van der Waals forces, weak hydrogen bonds between the layers restrict the expansion of formed in a 1:1 layered silicate such as kaolin, where strong hydrogen bonds between the layers cause the lattice to expand [25,26].

Incorporation of layered silicate into a polymer matrix may result in a classic microcomposite with a phase separation between the silicate and polymer matrix. Such a structure is formed in a 1:1 layered silicate such as kaolin, where strong hydrogen bonds between the layers restrict the expansion of silicate layers and thus the polymer intercalation. On the other hand, nanocomposite structures can be formed due to the expansion of layers. Partial expansion allows polymer chain intercalation between the silicate layers, i.e., an intercalated structure. More extensive expansion is expressed as full delamination into singular layers, i.e., an exfoliated structure [27].

The major milestone in the field of polymer-layered silicate nanocomposites is attributed to Toyota’s research group who in 1993 studied the incorporation of layered silicates with nylon-6. The key aspects were superior strength, modulus, heat distortion temperature, and water/gas barrier properties, with an impact strength that is comparable to neat nylon-6. Since then, nanocomposites based on layered silicates have been studied when incorporated in various synthetic polymers as well as natural polymers such as gelatin, chitosan, and alginate [28–31].

In the current study, we investigated the effects of the composite sealant’s components on the physical properties of our unique gelatin–alginate–carbodiimide sealant system when loaded with the hemostatic agents MMT and kaolin. The burst strength, sealing ability, viscosity, swelling behavior, and weight loss of the loaded surgical sealants were characterized. These properties are the most relevant for the surgical sealants’ application. Biocompatibility was studied as well.

2. Experimental

2.1. Materials

Porcine skin “type A” gelatin (G6144), alginic acid sodium salt (A1112), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), and kaolin (K1512) were purchased from Sigma–Aldrich, Rehovot, Israel. Sodium montmorillonite (Cloisite Na+) with particle size of <25 µm (d50) was purchased from BYK (USA).

2.2. Preparation of hemostatic agent-loaded surgical sealant

The preparation of the sealant is based on dissolving various amounts of gelatin and alginate (Gel-Alg) and hemostatic agent powders (kaolin or MMT) in distilled water, under heating up to 60°C. Various amounts of the crosslinking agent (EDC) were added to the Gel-Alg solution containing the hemostatic agents just before the sealant’s use. The influence of the gelatin and alginate on the bioadhesive properties were characterized at concentrations of 200–600 and 0–40 mg/mL, respectively. The effect of kaolin and MMT was studied in concentrations of 5, 10, 20, 50 and 2.5, 5, 10, 15, 20 mg/mL, respectively. The formulations are presented in the form of Gel-Alg-EDC, where Gel is the concentration of gelatin (mg/mL), Al is the concentration of alginate (mg/mL), EDC is the concentration of the carbodiimide-crosslinking agent (mg/mL).

In all experiments, the sealant was applied using double syringes with static mixer at a 4:1 volume ratio (Mixpac L-System, Sulzer, Switzerland) which provides a consistent mixing of the polymers and crosslinker solutions.

2.3. In vitro burst strength measurements

The burst strengths of various sealant formulations were tested using a custom-built mechanical burst tester following the standard test method for Burst Strength of Surgical Sealants ASTM F2392-04. The principle of this test is to measure the maximal pressure at the tissue leakage point that can be held by the sealant. A washed collagen casing (S1 Fibran, Spain) with a uniform 3.0-mm diameter hole was used as the tissue substrate. Approximately 0.5 mL of sealant was applied to the collagen casing substrate, sealing the defect with a measured thickness of approximately 1 mm. The sample was placed in the test unit and the pressure was applied (Figure 2). The pressure at which sealant failure occurred was recorded as the maximal burst pressure. A minimum of 10 repetitions were performed for each formulation.

2.4. Microstructure characterization

The microstructure of the sealants was investigated to characterize the dispersion of the hemostatic agent (kaolin or MMT).
in the sealant matrix. For this purpose, 0.5 mL of cubic hemostatic agent sealant specimens were air-dried in a chemical hood, freeze-fractured, and their cross section was observed using an environmental scanning electron microscope (Quanta 200 FEG ESEM) in a high vacuum mode, with an accelerating voltage of 10 kV.

2.5. Viscosity characterization

The initial viscosity of the polymeric (Gel-Alg) surgical sealant at the moment of application on the tissue is affected mainly by the viscosity of the aqueous Gel-Alg solution. Viscosity measurements of polymer solutions were performed using a controlled stress rheometer (model DHR3, TA Instruments Ltd.), fitted with a cone-and-plate geometry (1° cone angle, 40 mm diameter), at a constant temperature of 37°C, and a constant shear rate of 10 Hz, to investigate the effect of hemostatic agents on the sealants’ initial viscosity.

2.6. Swelling and weight loss

The surgical sealants were poured into 7.0 \( \times \) 7.0 \( \times \) 3.5 mm\(^3\) silicon molds and after gelation they were carefully removed and dried for 24 h. The surgical sealants were then weighed (\( W_i \)) and immersed in 3 mL PBS (pH 7.0), placed in a static incubator at 37°C and 100% relative humidity for 2, 24, and 72 h. They were then weighed (\( W_f \)) by removing the PBS and blotting using Kimwipes and then dried for 24 h and weighed again (\( W_f \)). The swelling degree and the weight loss were calculated according to the following equations:

\[
\text{Swelling degree}: \frac{(W_i - W_f)}{W_i} \times 100\% \quad (1)
\]

\[
\text{Weight loss}: \frac{(W_i - W_f)}{W_i} \times 100\%. \quad (2)
\]

Then 3–4 repetitions were performed for each formulation at each point of time.

2.7. Curing time

Crosslinking time, i.e., curing time, indicates the time required for the sealant to reach the desired state when applied on a wound. Curing time was determined as the time required for a magnetic bar to stop moving after mixing the polymer solution with the crosslinker solution. Approximately 1 mL of sealant, not loaded or loaded with a hemostatic agent, was poured into a 1.6-cm diameter plate under mixing at 300 rpm with a 1.4-cm magnetic bar at room temperature.

2.8. Statistical analysis

All data were processed using the Excel software. Statistical comparison between more than two groups was performed using the ANOVA (with Tukey Kramer post hoc) method through the XLSTAT software. A value of \( p < 0.05 \) was considered statistically significant. Error bars indicate the standard error.

3. Results and discussion

In the current study, we investigated the effect of gelatin and alginate concentrations on the function and physical properties of a surgical sealant crosslinked with carbodiimide (EDC). The loading of layered silicates kaolin and MMT as bioactive fillers (hemostatic agents) was studied as well.

3.1. In vitro burst strength

3.1.1. Effect of the gelatin and alginate concentrations

Burst strength values are good measures of the sealing ability of materials. The effect of the gelatin and alginate concentrations on the burst strength of the neat Gel-Alg-EDC solutions is presented in Figure 3. We chose to first characterize formulations with an EDC concentration of 20 mg/mL, which is relatively high and was found in our previous work to be on the threshold of cytotoxicity [7].

Although gelatin and alginate are easily dissolved in water, the concentrations of alginate and gelatin are limited by the viscosity of the hydrogel. A very high viscosity is not suitable for surgical sealants and will restrict the loading of the hemostatic agents. As seen in Figure 3, the alginate concentration has practically no effect on the burst strength of the sealants in the studied concentration range (0–40 mg/mL) when crosslinked with 20 mg/mL EDC. However, alginate has a substantial effect on the viscosity of the surgical sealant. Furthermore, it is clearly seen that an increase in the gelatin concentration leads to a large increase in the burst strength up to 400 mg/mL gelatin. Further increases in the gelatin concentration only slightly increase the burst strength. It can be assumed that the crosslinking reaction reached saturation at a gelatin concentration of approximately 500 mg/mL, i.e., the reactivity of EDC is fully occupied by the gelatin–alginate-functional groups. Interestingly, at relatively low concentrations of gelatin (200 and 300 mg/mL), the burst strength was relatively low despite the fact that the EDC: gelatin ratio was high, meaning a high ratio between the crosslinker and the functional groups (amine and carboxyl). It can therefore be assumed that the concentration of the functional groups in the aqueous solution has a greater effect on the crosslinking reaction than the ratio between the crosslinker and the functional groups.

3.5 mm\(^3\)
3.1.2. Effect of the crosslinking agent

The effect of crosslinker (EDC) concentration was studied on two selected formulations, based on 400 and 500 mg/mL gelatin and a 400:10 gelatin:alginate ratio (Figure 4). As mentioned in the introduction, although carbodiimide crosslinkers are considered much less cytotoxic than other crosslinkers, such as glutaraldehyde and formaldehyde, the motivation for this study was to minimize the crosslinker concentration. For sealant formulations based on 400 and 500 mg/mL gelatin (without alginate), the burst strength was not affected by an EDC concentration in the range of 10–20 mg/mL. However, in the presence of alginate (10 mg/mL), the effect of reducing the EDC concentration was significant. The 400:10 Gel-Alg formulation exhibited a 45% decrease in the burst strength (from 408 to 226 mmHg) following a decrease in the EDC concentration from 20 to 10 mg/mL. It can be assumed that the EDC is found in saturation in a formulation containing gelatin only. However, in the presence of alginate, the number of carboxylic groups involved in the carbodiimide-crosslinking reaction increases and a reduction in the EDC concentration has a significant effect.

There was no significant difference (p > 0.05) between crosslinking with 20 or 15 mg/mL EDC in all tested formulations. It can be assumed that the EDC is found in excess when using the 20 mg/mL concentration. We therefore, the authors used an EDC concentration of 15 mg/mL for the following experiments.

3.1.3. Effect of the hemostatic agent’s concentration

The loading of kaolin resulted in some increase in the burst strength, as seen in Figure 5. However, it was significant only at the highest concentration of 50 mg/mL. The general trend of increase in the burst strength with an increase in kaolin content can be explained as follows:

- Kaolin has a high water absorbance capacity and forms a hydrate layer on its surface. This results in a decrease in water availability of the adhesive, and the crosslinking efficiency therefore increases. It is known that free water disturbs the carbodiimide reaction. It can also be learned from Figure 3 that sealant formulations containing 200–300 mg/mL gelatin result in a relatively dilute solution.
- A kaolin–polymer interaction is due to surface charges of kaolin edges and basal planes. Reactive hydroxyl groups on kaolin’s basal surface exhibit negative charges and can form hydrogen bonds with the positive charges of the gelatin’s amines. In addition, the edge surface yields positive charges which can interact with the alginate’s carboxylic groups.
- Kaolin–kaolin interactions due to kaolin’s above-mentioned surface charges could reinforce the structure by adding new bonds formed in three different modes of particle interactions: edge to face, edge to edge, and face to edge. It is important to note that loading kaolin at amounts exceeding 50 mg/mL resulted in a nonhomogenous crosslinked hydrogel, which led to the formation of an irregular gel. The sealant failed to resist the fluid flux in those nonhomogenous areas.

The effect of MMT was studied on the basic 400:10:15 Gel-Alg-EDC formulation as well as on the 400:0:15 formulation (the basic formulation without alginate) and is presented in Figure 6. A direct correlation with significant differences was found between enhancement of the burst strength and the MMT concentration when loaded in the 400:10:15 formulation. Loading 10 mg/mL MMT in the 400:10:15 formulation improved the burst strength by more than 40% compared to the nonloaded formulation. Moreover, the incorporation of 20 mg/mL MMT improved the maximal burst strength by approximately 2-fold compared to the nonloaded basic formulations.
Montmorillonite was loaded to a maximal concentration of 20 mg/mL, which resulted in a very condensed hydrogel solution. In contradiction to MMT, kaolin was loaded up to 50 mg/mL and it can be loaded at even higher concentrations according to the solubility of the hydrogel. Even 50 mg/mL kaolin was less effective than 20 mg/mL MMT.

Although both hemostatic agents, kaolin and MMT, have a similar crystalline structure, the formed composite structures differ greatly in the burst strength mechanical properties. This behavior is probably due to the fact that MMT is an expanding layered silicate which can promote intercalation of cationic substrates when reacting under suitable conditions, whereas kaolin is a nonexpanding layered silicate with less interaction with the polymers. Kaolin therefore tends to form microcomposites, while MMT tends to form nanostructures.

The effect of MMT concentration on the formulation without alginate was tested to enable loading a relatively high concentration of MMT without being restricted by the high viscosity of the hydrogel formulation. However, in this formulation, the MMT sank to the bottom of the sealant vials when its concentration exceeded 10 mg/mL. At this MMT concentration, the burst strength was approximately 20% higher when the formulation did not contain alginate (482.3 and 404.3 mmHg, respectively).

### 3.2. Microstructure

The bulk cross sections of the 400:10 Gel-Alg formulations, nonloaded and loaded with kaolin and MMT, were observed using ESEM and are presented in Figure 7. The nonloaded reference sample did not demonstrate any phase separation. Some cracking was observed, which probably resulted from the fracturing process (Figure 7a). The fractographs of the sealant loaded with kaolin demonstrated uniform kaolin dispersion in the surgical sealant at both low (20 mg/mL) and high (50 mg/mL) concentrations (Figure 7b). The kaolin layers are clearly seen surrounded by the polymer bulk. In fact, the incorporation of kaolin in the sealant formed a microcomposite structure of kaolin layers integrated in the polymeric matrix. This microstructure indicates partial phase separation between the kaolin and surgical sealant matrix which demonstrated kaolin’s characteristic as a nonexpanding layered silicate.

In contradistinction, when MMT was loaded in the bioadhesive, the polymer matrix seemed much denser and fully occupied with the layered silicate even at a low concentration of 10 mg/mL (Figure 7c). Moreover, the MMT-layered silicate particles can barely be seen compared to the kaolin-loaded sealants. This observation can be explained by MMT’s above-mentioned expanding ability. These ESEM observations indicate that some intercalation probably occurred in the MMT-loaded systems due to the cationic groups of the gelatin, leading to better dispersion in the matrix polymer. These two different dispersion characteristics of kaolin and MMT in the gelatin–alginate matrix reflect formation of microcomposites and nanocomposites, respectively.

### 3.3. Curing time

The curing time is a crucial property in surgical bioadhesives, and in some cases, it is the feature which makes a difference between a glue and a sealant. To resist the fluid flux from the wounded tissue and to form the desired sealing effect, the sealant must achieve its mechanical strength faster than the flux. Optimal curing time depends on the clinical procedure and is usually in the range of 5–60 s [5].

The effect of gelatin concentration on the curing time of surgical sealants was investigated on formulations with 10 mg/mL alginate and on formulations without alginate. As seen in the data presented in Figure 8a, the higher the gelatin concentration, the faster the curing time. The addition of alginate to the gelatin solution had a minor accelerating effect on the curing time at all gelatin concentrations. This acceleration is probably related to the increase in the sealant’s viscosity when loaded with alginate (10 mg/mL). These results are consistent with the burst strength results (shown in Figure 3) in which it was found that higher concentration of gelatin increased the burst strength and a minimum gelatin concentration is necessary to achieve burst resistance by the gel formation of crosslinked polymer. The number of reactive groups has a major effect on the curing kinetics rather than the reactive groups:crosslinker molar ratio, when crosslinked with the medium quantity of EDC (15 mg/mL).

The EDC content also affects the curing time (Figure 8b). For example, an increase in EDC content from 10 to 20 mg/mL decreased the curing time from 9 to 5 s. It can therefore be concluded that the EDC exists in deficiency, such that gel formation is restricted by the amount of EDC. However, a concentration of 15 mg/mL was found to be sufficient for the desired burst strength and enabled the same burst strength as 20 mg/mL.
Kaolin had no effect on the curing time even when loaded at a high concentration of 50 mg/mL, while increasing the MMT concentration from 0 to 20 mg/mL slightly decreased the curing time (Figures 8c, d). This is in accordance with the mechanisms of the interaction of two different layered silicates with the above-described polymeric matrix. As seen in the burst strength tests, the kaolin-loaded sealants did not show substantial increases in curing time, indicating that no significant interactions were formed. The inert nature of kaolin probably did not affect the curing time in the same manner. On the other hand, the ionic interaction between the gelatin's positive side groups and MMT enables some decrease in the curing time.

The curing time of all studied formulations was in the desired range for sealing. It should be noted that the application by the double syringes with the static mixer had also some effect on the curing time. Thus, a shorter or longer curing time can be achieved by changing the geometry of the double-syringe system without changing the formulation.

3.4. Swelling and weight loss

Both the degree of swelling and weight loss of surgical sealants are very important physical parameters. They indicate the density of the network between the polymer chains formed by the crosslinking reaction. A high degree of swelling indicates a relatively weak and less dense network structure of the polymer. A denser hydrogel structure reduces the water molecules' ability to enter the hydrophilic parts of the polymer molecules and as a result less water can penetrate into the hydrogel structure. Similarly, high weight loss indicates that polymer chains are detached from the network easily or that the portion of the polymer involved in the connected network is smaller [32,33]. The weight loss and swelling degree of formulations loaded with kaolin and MMT are presented in Figures 9 and 10, respectively.

The swelling degree of the nonloaded formulation was 2.2 and 6.7 times higher than the initial dry weight of the sealant after 2 and 24 h, respectively. It is important to note that when using this method, the changes in these parameters are normalized to the dry weight of the sealant plugs to investigate

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**Figure 8.** Curing time of the surgical sealants as affected by each component: (a) effect of the gelatin concentration [crosslinked with 15 mg/mL EDC]; (b) effect of the EDC concentration on formulations with 400 mg/mL gelatin; (c) effect of the MMT concentration on the 400:10:15 Gel-Alg-EDC formulation; (d) effect of the kaolin concentration on the 400:10:15 Gel-Alg-EDC formulation (0 and 10 mg/mL alginate).

**Figure 9.** Effect of the kaolin concentration (0, 5, 10, 20, and 50 mg/mL) on: (a) weight loss and (b) swelling degree of a sealant based on 400:10:15 Gel-Alg-EDC.
the hemostatic agent’s effect on the hydrogel properties and on the crosslinking reaction. Normalization of the swelling ratio to dry weight provides a result with better significance than normalization to wet conditions (immediately after casting the sealant plugs). These values are therefore comparative.

Kaolin-loaded sealants did not exhibit any effect on the swelling degree after 2 h of incubation. However, after 24 h, the highest kaolin concentration of 50 mg/mL was completely dissolved (not shown in Figure 10b), whereas lower concentrations slightly reduced the swelling degree.

After 24 h, the kaolin-loaded sealants showed lower weight loss than the sealants without kaolin. The kaolin-layered silicate is hydrophilic and has a high ability to disperse in water and to form a suspension. In addition, kaolin acts as a filler which is not involved in the crosslinking reaction of the polymers. It can therefore be assumed that when the kaolin concentration is increased, the weight loss is influenced mainly by the detachment of kaolin from the adhesive rather than by detachment of the crosslinked gelatin and alginate polymer chains.

It is worth mentioning that in our previous work [7] the authors found that kaolin at a concentration as high as 50 mg/mL reduced the swelling degree of the polymeric matrix when crosslinked with a higher EDC concentration (20 mg/mL). This indicates the importance of density of the polymer matrix. In case of reaction of the polymers with higher crosslinker concentrations, the formed matrix is much denser and the kaolin–polymers interaction causes an even higher density. However, crosslinking with 15 mg/mL EDC, which was selected in this study for biocompatibility reasons, generated a less dense network in which kaolin was not held as much by the matrix and caused a rapid degradation of the sealant due to its hydrophilic nature.

After 24 h of incubation, the MMT-loaded sealants underwent less degradation and swelling than the nonloaded sealant formulation. Both degradation and swelling decreased with an increase in MMT concentration. This result is highly significant throughout the entire test. Moreover, the simultaneous decrease in swelling and degradation is considered a common effect which mainly reflects: (a) the densification of polymer matrix and (b) capping or prevention of the reaction of active groups in polymers with the surrounding medium.

These results are consistent with all other results presented here for MMT-loaded sealants. In contradistinction to kaolin, MMT-loaded sealants presented enhancement of the burst strength in all tested concentrations, with an increase in MMT content, and reduction in the swelling degree, weight loss, and gelation time. All this represents the formation of a stronger, denser, and tougher polymer matrix, providing an added value to surgical sealants based on the natural gelatin–alginate biopolymer.

3.5. Viscosity
Rheological tests were performed to elucidate the effect of kaolin and MMT on the sealant’s viscosity, which is a highly important characteristic and determines ease of use. The measurements were carried out at 37°C, on Gel-Alg solutions, i.e., the sealant solutions. The viscosity of the nonloaded 400:10 Gel-Alg solution is 1.1 Pa s and that of the kaolin-loaded 400:10 Gel-Alg is in the range of 0.8–1.5 Pa s (Figure 11).
Thus, our results show that the incorporation of kaolin in 400:10 Gel-Alg formulation has no significant effect on the viscosity of Gel-Alg solution. This means that the resistance of Gel-Alg to shear or tensile stress was not affected by the addition of kaolin to the solution and indicates that no significant bonds were formed between these natural polymers and kaolin in liquid solution. The loading of MMT in the surgical sealant increases the sealant’s viscosity up to six times that of the nonloaded sealant. Increasing the Gel-Alg solution’s viscosity with an increase in MMT concentration is obtained mainly due to the expanded nature of the MMT-layered silicate, as opposed to kaolin which is an expanded layered silicate and thus has no effect on the viscosity of polymeric solution.

4. Conclusion

In the current study, we developed and studied composite gelatin–alginate surgical sealants loaded with the hemostatic agents, kaolin and MMT. Our thought was that in addition to providing an attractive alternative for traditional sealant applications, the surgical sealant will also induce hemostatic effects and thus improve adhesion and overall function in a hemorrhagic environment.

The effect of hemostatic agents on the physical properties, i.e., burst strength, microstructure, viscosity, swelling degree, weight loss, and curing time were investigated.

Incorporation of kaolin resulted in the formation of a microcomposite structure of kaolin layers integrated in the surgical sealant matrix. It was found to moderately improve the in vitro burst strength of Gel-Alg-EDC surgical sealants when loaded at high concentrations. Incorporation of MMT into the surgical sealant formulation significantly improved the sealant properties, resulted in a higher burst strength, lower swelling degree and weight loss, and faster curing time compared to the nonloaded and kaolin-loaded sealants. The structure of sealant loaded with MMT is similar to the microcomposite structure achieved with kaolin. However, as an expanding layered silicate, it was found to have a higher dispersion rate which indicates a nanocomposite structure.

It is important to note that the characterization of our novel composite sealant system described here is unique, because it was performed before the system reached its final state, i.e., all properties were measured when the system was still in the gel state. Previous studies usually characterized composite systems after completion of dehydration and achievement of the solid state.

In conclusion, the incorporation of kaolin and MMT in our Gel-Alg surgical sealants seems to be a very promising novel approach for improving the properties of surgical sealants by enhancing the mechanical and physical properties and the reaction with the hemorrhagic environment.

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