PREPARATION AND RESEARCH OF PROPERTIES OF COMBINED ALGINATE/GELATIN HYDROGELS

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Abstract

The work presents the studies on the synthesis of hydrogel material based on the natural biopolymers (sodium alginate and gelatin) for medical applications. Sodium alginate and gelatin are biocompatible, non-toxic, biodegradable polymers and renewable raw materials. Combined alginate gelatin hydrogels are formed due to the formation of a hydrogel network by simultaneous cross-linking of calcium ions of sodium alginate macromolecules, gelatin, and macro-chains of rarely cross-linked polyacrylic acid. The optimal synthesis conditions (reagent concentrations, the molar ratio of calcium ions to the number of carboxyl groups Ca²⁺/COOH⁻) of the combined hydrogels with satisfactory physicochemical and mechanical properties are determined. The dependences of the mechanical properties of alginate-gelatin hydrogels on the degree of swelling indicate a wide range of their satisfactory performance characteristics. The studies on sorption and release of analgesics (lidocaine and novocaine) show long-term release of drugs and allow predicting the possibility of their prolonged delivery. In vitro cytotoxicity analysis proved the absence of toxic effect on living cells. The results suggest that the obtained combined alginate-gelatin hydrogels are a promising material for producing hydrogel dressings for wound care.

Keywords: hydrogel; sodium alginate; gelatin; polymerization; wound healing; calcium.

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Introduction

Recently the issue of new means for the treatment and protection of various wounds has attracted much attention. Along with traditional gauze, a variety of wound dressings are increasingly used. A large number of these dressings are obtained using biocompatible natural and synthetic polymers [1]. There is a wide range of requirements for polymeric materials used in medicine to obtain wound dressings, and the first selection criteria are the absence of cytotoxicity, biocompatibility, biodegrability [2-4]. Effective wound dressings should possess high sorption activity, the ability of effectively removing excess wound exude and its toxic components. They also should be permeable to air and water vapor; to prevent dehydration of the wound surface; to prevent wound infection; to be modeled on wound surfaces with a difficult relief, and possess sufficient mechanical strength. In general, the wound dressing should provide the optimal microenvironment for wound healing at the absence of local irritants and allergic effects.

Recently scientists paid attention to hydrogel-based wound dressings as hydrogels, to some extent, mimic living tissues [5]. Hydrogel materials can be easily produced; they are amenable for targeted changes in their properties in a wide range by varying the chemical composition of gelling polymers (ratio and nature of comonomer units, crosslink density, etc.) [6-8]. The main trends in the world practice of forming modern hydrogels are hydrogels based on natural polymers that can combine the known advantages of hydrogel materials (biocompatibility, non-toxicity and non-allergenicity) with satisfactory physical and mechanical properties. Another advantage of hydrogel-based wound dressings is the possibility of including necessary drugs (antibacterial, antiseptic, anti-inflammatory drugs that affect the regenerative processes) in the hydrogel volume. When hydrogel dressing is applied to the wound, drugs are prolonged to provide a therapeutic effect.

Despite the advantages, using hydrogels as wound dressings has certain limitations because a number of problems. This includes low mechanical properties, which significantly decrease with swelling of the bandage (long-term using) and limited ability to absorb specific wound fluids (exudates, transudates, etc.) [9].

The latest advance in the development of hydrogel wound dressings is a two-layer or combined material (sheet) with one layer containing collagen (human collagen is used for the most expensive dressings) and cells grown by tissue engineering from human body tissue [10; 11]. In addition, such materials contain human growth factors, cytokines, fibroblasts and keratocytes [12; 13]. These means are very expensive; they are used for wounds that do not heal for a long time, and in the case all other treatments do not give a positive result [14; 15].

Combined or hybrid hydrogel materials based on natural polymers such as pectin or alginate can be considered as alternatives for the mass consumer. These materials should include important elemental amino acids – proline, oxyproline, and oxylsine, which are specific for building collagen macromolecules [16]. Thus, these combined hydrogels can be accepted as a model of hydrogels that mimic the composition of collagen and epithelial cells.

Gelatin obtained from collagen is the direct functional analog of collagen according to the amino acid composition. It is widely used for pharmaceutical and medical applications due to its biocompatibility, ability of biodegrading and accelerating tissue regeneration and wound healing [17-21]. At the same time, alginate is known for its antibacterial properties and its ability to stimulate the processes of epithelialization and development of granulation tissue in wounds treatment. Thus, the development of combined alginate-gelatin hydrogels is an urgent task for scientists [22; 23],

groups)-based hydrogels are favored by the three-dimensional coordinates bonded by di- or tri-valent metallic ions [6; 18-20]. Thus, calcium salts (chloride and gluconate) are prevalently employed, however they suffer from yielding structural inhomogeneity in hydrogels due to rapid gelation. Despite achieving products with certain geometry remains non-trivial, in the present work, a calcium salt, rarely crosslinked polyacrylic acid (RPAA,Ca), was used as the structural agent (Fig. 1.). Rarely crosslinked polyacrylic acid with carboxyl groups partially neutralized by Ca2+ ions is able to form a stable dispersion in non-aqueous media. Using such a dispersion with particles as a source of sustained release of calcium ions, hydrogel wound coatings of homogeneous structure can be obtained. Calcium salts of rarely crosslinked polyacrylic acid with different degrees of substitution of carboxyl groups were used in this study.
Fig. 1. The structure of the structuring agent (RPAA_Ca)

Fig. 2. The scheme of alginate-gelatin hydrogel formation

Alginate and pectin (containing carboxyl groups) interact with calcium ions to form alginate-gelatin hydrogels. Cross-links between different macromolecules create a homogeneous polymer network of combined alginate-gelatin hydrogel, which also includes ionically linked chains of rarely crosslinked polyacrylic acid (Fig. 2).

Also, hydrogel dressings for wound treatment should be elastic, not cause pain, and not overdry the wound. Hydrogel materials can lose elasticity (hydrogel becomes hard) if too much water is lost, and a patient feels discomfort. To prevent this, additional water-retaining components are introduced into the hydrogel composition; these components act also as a plasticizer.

The aim of the present study was to develop hydrogels with satisfactory mechanical properties and a high ability to absorb wound fluid based on the combined use of biopolymers (sodium alginate and gelatin).

Experimental part

Sodium alginate, gelatin, glycerin, polypropylene glycol 2000 (purity 98.5 %) obtained from Aldrich were used without further purification.

Model exudate to assess hydrogel ability to absorb wound secretions was obtained according to the method described in [5] and it contained: 20.0 mmol CaCl$_2$, 400 mmol NaCl, 80 mmol 2-amino-2-(hydroxymethyl) propane-1,3-diol (TRIS), and 2 % bovine serum albumin in distilled water, and its pH was maintained at 7.5.

Alginate-gelatin hydrogels were obtained by the following method. 10 % solution of gelatin, 3 % solution of sodium alginate in deionized water, and 10 % dispersion of calcium salt of rarely structured polyacrylic acid in a mixture of glycerol and polypropylene glycol with MM = 2000. Calcium salts of rarely structured polyacrylic acid with the following degrees of substitution – 0.35; 0.53; 0.85; 1.0 were used to create the necessary concentration of calcium ions in alginate-gelatin hydrogels.

Solutions of gelatin and sodium alginate were mixed in a given ratio and stirred for 5 minutes at a temperature of 40 °C. A given amount of...
dispersion was introduced to the resulting homogeneous solution and stirred for 2 minutes. The resulting mixture was poured into plane-parallel molds, sealed, and heated at 40 °C for 1 hour.

The flexibility and appearance of alginate-gelatin hydrogel were assessed visually. The thickness was estimated using a digital caliper at five points (one in the center and four at the edges).

Drugs (lidocaine, novocaine) in alginate-gelatin hydrogels were introduced via exhaustive absorption of a given amount of their aqueous solution [27; 28]. The content of drugs in the hydrogel was 2–2.2 %.

Studies of the drug release (novocaine, lidocaine) from hydrogel samples were performed as follows. Samples of hydrogels (sized 2 cm x 2 cm and thickness 2 mm) saturated with the drug were placed in eight times (compared to the sample weight) excess of fluid (water or model exudate), and at regular intervals, samples of fluid were taken. The optical density of these samples was measured at λ = 262 nm for lidocaine and λ = 290 nm. Quantitative assessment of the drug content in the solution was performed using the appropriate calibration dependence Cn = 17.263·D – 5.34·10^{-2} for lidocaine and Cl = 688.82·D + 8.6·10^{-3} for novocaine. Then, the percentage of the drug that remained in the hydrogel was calculated according to the formula given in [29].

The degree of swelling of the hydrogel samples (the ratio of the mass of absorbed fluid to the mass of polymers in the hydrogel sample) was determined by the gravimetric method at 20 °C in distilled water and the model wound fluid. The calculation of the degree of swelling was performed according to the formula given in [30; 31].

To determine the mechanical properties, a standardized sample of hydrogel with a diameter of 12 mm and a thickness of 7 mm was tested using a stepwise uniaxial compression load in increments of 500 μm. The maximum value of the load at which the hydrogel still retained its integrity was considered the load maximum.

Cytotoxicity study. To assess the biocompatibility and cytotoxicity of hydrogel matrices (combined hydrogels) a test culture of bull sperm was used [32]. Ejaculates with the following physiological characteristics were selected for studies: volume 3–4 ml, sperm concentration 6–11·10^8 cells/ml, number of live germ cells 80–85 %. Sperm samples were divided into groups: control – diluted with phosphate-buffered saline (PBS): NaCl - 800 mg, KCl - 20 mg, Na₂HPO₄ - 110 mg, KH₂PO₄ - 20 mg, MgCl₂ - 10 mg, distilled water - up to 100 ml) and research. Hydrogels were added to the test samples in doses of 10, 20, and 50 mg/ml of a diluted sperm sample. The biocompatibility of hydrogels was investigated by culturing sperm in direct contact with hydrogel samples. Indicators characterizing the course of redox processes in germ cells were determined, in particular: succinate dehydrogenase activity (SDH, units) using 2,3,5-triphenyl tetrazolium chloride and cytochrome oxidase (CHO, units) with "Nadi" reagent.

The cytotoxicity of the obtained combined hydrogels was evaluated by the duration of sperm survival (h) using a microscope (× 200) until linear translational motion in semen stored at a temperature of 2–5 °C is observed.

Results and discussions

Figure 3 shows the results of studies on the change in the elasticity modulus of hydrogel plates with the introduction of glycerol, polyethylene glycols, and polypropylene glycols with different molecular weights. Hydrogels obtained using only glycerin as a plasticizer show satisfactory elasticity. However, the glycerol in the hydrogel can migrate rapidly from the dressing to the surrounding tissues and the plasticization effect is lost. Since glycerin is the most effective plasticizer in this system (other compounds did not possess a plasticization effect), combined systems of plasticizers were searched. It was found that it is possible to replace half of the required amount of glycerol with polyethylene glycol or propylene glycol without the loss of plasticization effect. The best results showed equal amounts of glycerin and polypropylene glycol (PPG-2000). The content of plasticizer components in the gel-forming composition is 15–25 %; it allowed ensuring sufficient elasticity of the hydrogel dressing, even when it is dried.

Previous experiments have determined the range of gel-forming polymer concentrations from 2.5 to 4.5 %. Below this concentration, the hydrogels were either not formed or were too weak, and above this concentration, they lost the ability to swell and were too tough.
Fig. 3. Dependence of the elasticity modulus of alginate-gelatin hydrogel on the type of plasticizer introduced into its composition

Hydrogel samples were investigated for gel fraction content, uniaxial compressive strength, the degree of swelling in water and the solution simulating the exudate to determine the optimal conditions for hydrogel formation. Table 1 shows the properties of the obtained hydrogels depending on the synthesis conditions.

The presented data indicates that the value of the gel fraction of the obtained samples is in the range of 68–97%. The highest value is observed for samples without gelatin. With increasing gelatin concentration in the hydrogel sample at a constant concentration of other components, there is a decrease in the value of the gel fraction to 75%. The gel fraction content also decreases with an increase in the molar ratio of calcium ions to the number of carboxyl groups Ca\(^{2+}\)/COOH.

Figure 4a shows the analysis of the dependence of the hydrogel maximum load on the molar ratio of calcium ions to the number of carboxyl groups Ca\(^{2+}\)/COOH. Thus, with this ratio increasing, the maximum load value before destroying the gel increases, i.e. mechanical properties of the hydrogel largely depends on the number of bonds formed between the structuring agent and the polymers forming the network. There is also a direct proportional dependence of the maximum load on the total content of gelling polymers (Table 1).

As the molar ratio of calcium ions to the number of carboxyl groups Ca\(^{2+}\)/COOH increases, the degree of swelling in water naturally decreases (Fig. 4b). In addition, the dependences of the degree of swelling on the concentration of gelatin and sodium alginate in hydrogel samples are observed. It can be argued that both parameters have antibiotic effect on the degree of swelling in water - it decreases and with increasing concentration of gelatin and increases with increasing concentration of sodium alginate.

Gelatin macromolecules contain free amino groups that are capable of weak ionic interactions with carboxyl groups of other polymers (sodium alginate, rarely structured polyacrylic acid) forming additional mesh nodes; it, in turn, reduces the degree of hydrogel swelling.
### Table 1

Properties of the combined alginate-gelatin hydrogel depending on the production conditions

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<th>Sample no</th>
<th>Sodium alginate concentration, wt.%</th>
<th>RPA Ca concentration, wt.%</th>
<th>The degree of substitution of Ca in RPA</th>
<th>Total concentration of plasticizers wt.%</th>
<th>Gelatin concentration, wt.%</th>
<th>Total polymer content, wt.%</th>
<th>The ratio of sodium alginate to gelatin</th>
<th>The ratio Ca^{2+}/COOH^-</th>
<th>The content of the gel fraction, %</th>
<th>Maximum load, Pa</th>
<th>The degree of swelling on 3rd hour</th>
<th>water, g/g of polymer</th>
<th>exudate, g/g of polymer</th>
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Fig. 4. The dependence of the change in the maximum load a) and the change in the degree of swelling; b) on the molar ratio of calcium ions to the number of carboxyl groups Ca^{2+} / COOH^-.
An important characteristic of hydrogels as therapeutic materials is their ability to absorb wound fluid. It should be noted that too rapid absorption of wound fluid is not an advantage and, conversely, it should be avoided to prevent overdrying of the wound, as this may adversely affect the healing of wounds with the moderate release of fluids [33]. Figure 5 shows the changes in the amount of absorbed wound fluid compared to the absorbed water for alginate-gelatin hydrogels. The degree of swelling in water is several times higher than the degree of swelling in the model wound fluid because it contains additional substances (albumin, sodium and calcium chloride, etc.) that undoubtedly affect the swelling process.

![Figure 5](image1.png)

**Fig. 5. Dependence of amount of absorbed water and model exudate by combined alginate-gelatin hydrogels (sample 2 from table 1).**

The amount of absorbed model exudate is in the range of 30-35 g per 1 g of gel forming polymers or 1900 g per m² of the absorption area. According to the classification given in [34], the absorption in the range of 2000–2500 g / m² for the application on the wound up to 24 hours is considered perfect for effective wound healing. Therefore, it can be assumed that the obtained alginate-gelatin hydrogel can be the basis of an effective therapeutic dressing capable of maintaining water balance by preventing both excessive dehydration of the wound and the accumulation of exudate.

![Figure 6](image2.png)

**Fig. 6. The dependence of the maximum load of the combined alginate-gelatin hydrogels (sample 2 table 1) swollen: a) - in water; b) - in the wound fluid. (° - The degree of swelling in the corresponding liquid is indicated above the bars)**

Hydrogel mechanical properties, especially during swelling are an important performance characteristic for alginate-gelatin hydrogels as wound dressings. Upon contact of the hydrogel with the wound, the wound fluid enters the hydrogel matrix, leading to the changes in the conformation of the polymer network and the stress of polymer chains; it can ultimately cause a decrease in strength and even the loss of structural integrity of the hydrogel [18; 35]. Therefore, studies of the mechanical performance of the hydrogel at uniaxial loading were performed according to the method of determining the maximum of hydrogel samples.
with different degrees of swelling (Fig. 6). The obtained results suggest that alginate-gelatin hydrogels naturally lose strength, which decreases in proportion to the increased degree of swelling in both water and model exudate. However, the combined hydrogels (compared to alginate hydrogels obtained under the same conditions) retain integrity even at maximum degrees of swelling withstanding the load only 2 times less than their initial maximum load.

In addition, as the obtained hydrogels are proposed to use as hydrogel dressings for therapeutic purposes, we conducted studies on absorption and release (into the aqueous medium) of the following drugs: novocaine (procaine, 2-(diethylamino)ethyl-4-aminobenzoate) and lidocaine (xycaine, xylocaine, 2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide). Fig. 7 shows the kinetic dependences of the release of these drugs from hydrogels. The factors regulating the rate of drug release from hydrogel dressings are their swelling and diffusion of the solute from the hydrogel composition. It is expected that a slower and more stable (constant) release of the drug (with maintained integrity of the bandage) will improve the wound healing process. It will also increase patient comfort during treatment due to reducing the frequency of bandage changes.

![Fig. 7. Kinetic curves of novocaine (1, 2) and lidocaine (3, 4) release from hydrogels of different composition.](image)

35–60 % of lidocaine (Fig. 7 curves 3 and 4) releases from the hydrogel into a solution in 1 hour and 50-80 % - in 5 hours. The maximal release of lidocaine (60–90 %) occurs in 24 hours. In the case of novocaine (Fig. 7 curves 1 and 2) 10–15 % of the drug is released in 1 hour, and 30–35 % - in 5 hours. In 24 hours the release of novocaine is 50–55 % of its initial content in the hydrogel.

The study showed that the release of these compounds takes a long time, which will ensure a prolonged delivery of drugs. The study showed that the release of these compounds takes a long time, which will ensure a prolonged delivery of drugs.

### Table 2

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<tr>
<th>Conditions</th>
<th>Dose, mg/ml</th>
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<th>SDH, units</th>
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An important property of hydrogel plates as wound dressings is their biocompatibility with the wound surface. Cytotoxicity assays were used to study biocompatibility in vitro [36, 37]. In this study, the biocompatibility of hydrogels was investigated by culturing bull sperm in direct contact with samples of hydrogels. The results of the studies are shown in Table 2. The standard governing the in vitro cytotoxicity study ISO 10993-5 states that "a decrease in cell viability of more than 25–30% is considered a cytotoxic effect." The viability of all cells in direct contact with hydrogels was more than 80% after 24 hours, and more than 75% after 48 and 72 hours of exposure. Thus, obtained hydrogels can be considered non-cytotoxic and safe.

Conclusions
Combined alginate-gelatin hydrogels with the ability to absorb wound fluid and retain mechanical properties after swelling have been developed. The conditions for hydrogel formation and the optimal ratio of biopolymers and the structuring agent in the hydrogel matrix to obtain maximal mechanical properties at different degrees of swelling were determined. In vitro cytotoxicity analysis revealed that this composition is non-toxic and biocompatible under direct contact with the living cells. The ability to sorb and release analgesic drugs suggested that obtained hydrogels could be promising as a wound dressing on different stages of healing.

Reference