ORIGINAL ARTICLE

# Formulation and evaluation of novel collagen/ carboxymethylcellulose blend film wound dressing

# Příprava a hodnocení směsného filmu na rány z kolagenu a karboxymethylcelulosy

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**Summary**

Films are thin, flexible, and transparent wound dressings. They can be prepared from both synthetic and natural materials. In practice, synthetic polyurethane dominates, but research is mainly focused on substances of natural origin. An endogenous substance with excellent film-forming properties, which is involved in the wound healing process, is collagen. However, collagen films themselves have weak mechanical properties, which can be improved by, among other things, combining collagen with other materials. Such material could be carboxymethylcellulose, which has been shown to affect wound healing positively. Films consisting only of CMC also have weak mechanical properties, so combining both materials seems to be a suitable solution to the given problems, and a wound dressing with many beneficial properties for wound healing could be created. Therefore, our experiment aimed to prepare composite films for wound therapy consisting of a combination of collagen and CMC. The films were prepared by the solvent evaporation method, and their properties were compared with those formed only by CMC. In both cases, films with suitable organoleptic, physicochemical, and application properties for wound therapy were produced. The composite films showed lower absorption capacity and better mechanical resistance compared to those formed only by CMC. The combination of collagen and CMC in composite films intended for wound therapy has thus resulted in improved properties of the resulting dressing and holds potential for further research.

**Key words:** film wound dressing • collagen • carboxymethylcellulose • evaluation • blend films

**Souhrn**

Filmy jsou tenké, flexibilní a transparentní prostředky na rány. Mohou být připraveny jak ze syntetických, tak i přírodních materiálů. V praxi jednoznačně dominuje syntetický polyuretan, avšak výzkum se zaměřuje především na látky přírodního původu. Látkou tělu vlastní, s výbornými filmotvornými vlastnostmi, účastnící se procesu hojení ran je kolagen. Samotné kolagenové filmy však mají slabé mechanické vlastnosti, které lze mimo jiné vylepšit kombinací s dalším materiálem. Takovým materiálem by mohla být karboxymethylcelulosa s prokázaným příznivým účinkem na hojení ran. Filmy tvořené pouze CMC také naráží na slabé mechanické vlastnosti, tudíž by se kombinace obou materiálů mohla jevit jako vhodné řešení daných problémů a navíc by mohlo vzniknout směsné filmové krytí s mnoha výhodnými vlastnostmi pro hojení ran. Cílem našeho experimentu bylo vytvořit směsné filmy pro účely terapie ran tvořené kombinací kolagenu a CMC. Filmy byly připraveny metodou odpaření rozpouštědla a jejich vlastnosti byly porovnávány s vlastnostmi samotných filmů tvořených pouze CMC. V obou případech vznikaly filmy vhodných organoleptických, fyzikálně-chemických i aplikačních vlastností pro uplatnění v terapii ran. Směsné filmy měly v porovnání s těmi tvořenými pouze CMC nižší absorpční kapacitu a lepší mechanickou odolnost. Kombinace kolagenu a CMC pro vytvoření směsných filmů určených k terapii ran tak vedla ke zlepšení vlastností výsledného krytí a má potenciál pro další výzkum.

**Klíčová slova:** filmové krytí na rány • kolagen • karboxymethylcelulosa • hodnocení • směsné filmy

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## Introduction

With technological advances, various types of wound dressings and different materials are available for the treatment of chronic wounds[1, 2]. An ideal wound dressing must provide and maintain a moist environment, enhance epidermal migration, promote angiogenesis and tissue synthesis, allow gas exchange, maintain an appropriate temperature, provide protection against bacterial infection, must be sterile, nontoxic and nonallergic, and should be nonadherent to the wound and easy to remove[1, 3]. The selection of an appropriate dressing depends on the wound type and the healing phase and must respect the individual characteristics of each wound[4].

Films are thin, flexible, and transparent wound dressings[5, 6]. They are moisture vapor and oxygen permeable and impermeable to fluids and microorganisms. Their transparency allows for continuous inspection and visualization of the wound, and their flexibility enables a wide range of applications. Films have a minimal ability to absorb fluids, so they can be used for wounds where there is no/low exudate. These include minor burns and superficial wounds (e.g., scalds, cuts, abrasions, lacerations, superficial pressure ulcers, leg ulcers, and lightly exuding wounds). Because of their elasticity, they can also be used to cover sutures after surgery, protect the skin from shear forces in vulnerable areas, protect and secure cover for intravenous catheters, and protect donor sites following skin harvesting[1, 2, 6, 7]. Films can also be used as primary and secondary dressings to waterproof primary dressings such as foams and hydrocolloids[2]. Modern film dressings are mainly made of synthetic polyurethane[6, 8], but there is also a general effort to develop films from materials of natural origin and their derivatives due to the number of favourable properties such as biocompatibility, biodegradability, low antigenicity, non-toxicity, etc.[9–11]. However, these materials must have an excellent film-forming capacity[9]. In several studies, films have been prepared from chitosan, collagen, cellulose derivatives, or other materials or as a blend of different polymers[12–18].

Collagen is the major structural component of the extracellular matrix (ECM) and the most abundant protein in the human body[19]. It supports connective tissues such as tendons, ligaments, skin, and bone and is involved in physiological processes such as wound healing and scar formation[20]. Collagen is essential in all phases of wound healing. Therefore, it is used extensively in wound care and applications such as food, cosmetics, medicine, and tissue engineering[19–21]. Collagen wound dressings are available in various forms including sponges, powders, and hydrogels[21]. Collagen also has excellent film-forming properties; collagen films themselves are used extensively as edible coatings in the food industry[22]. However, the film wound dressings of this polymer are not yet used in clinical wound care. Their use as wound dressings is limited by their weak mechanical properties and poor handling after wetting (contact with wound exudate)[23, 24]. The use of collagen films as wound dressings would therefore require an improvement in the properties of collagen. There are several possibilities, such as crosslinking[25], but for our purposes, it is possible to combine collagen with another material that would improve the behaviour of the resulting dressing[26, 27]. One of these materials should be carboxymethylcellulose (CMC), a cellulose derivative widely used in medicine, pharmaceuticals, cosmetics, and tissue engineering[28]. CMC possesses optimal properties, including biocompatibility, biodegradability, low antigenicity, and non-toxicity, and is a suitable material for wound care. CMC is commonly used in various wound dressings, such as hydrogels, hydrocolloids, or hydrofibers. It also has an excellent film-forming capacity, but films made of this polymer are not yet used in practice[29, 30]. Many recent studies have looked at films of CMC as a potential wound dressing, including blends with polymers such as chitosan, gelatin, polyvinyl alcohol, etc.[31–33]. In our previous studies, collagen with the acidic form of carboxymethylcellulose in the form of a non-woven textile and bilayer carboxymethylcellulose-collagen films have been prepared. These modern dressings have satisfactory properties for their intended use[34, 35]. Another possibility is to prepare a film dressing based on a collagen/carboxymethylcellulose blend.

Based on the above facts, this work aimed to prepare and evaluate a novel film wound dressing that combines two natural polymers – collagen and carboxymethylcellulose. The films were prepared by the solvent casting method. The physicochemical properties of the films were evaluated, particularly regarding the practical application of the dressing to the wound, and the suitability of the polymer combination was investigated.

## Experimental part

### *Materials*

Bovine collagen in the form of a 1.73% gel was supplied by Collado spol. s. r.o. (Brno, Czech Republic). Sodium carboxymethylcellulose (NaCMC) in the form of viscose fibers (degree of substitution 0.593) was purchased from Holzbecher spol. s. r. o. (Zlíč, Czech Republic). Macrogol 300, glycerine, and hydrochloric acid (all Ph. Eur. grade) were obtained by Fagron a. s. (Olomouc, Czech Republic). All other reagents and chemicals used in the experiment were of analytical grade or according to the European Pharmacopoeia.

### *Methods*

*Preparation of composite films*

The films were prepared by the solvent evaporation method. Initially, films just from carboxymethylcellulose were prepared; afterward, the second set of blend films from CMC and bovine collagen was prepared for comparison. In the beginning, the dispersion was com-

posed of NaCMC (small fibers) and a plasticizer (glycerine or macrogol 300 in different ratios – NaCMC : plasticizer 1 : 1; 1 : 1,5; 1 : 2) in purified water. The dispersion was left to swell at ambient laboratory temperature for 1 hour and was then acidified using a 5% and 1% solution of hydrochloric acid (HCl) under continuous stirring until a pH value of 3 was achieved. In the case of blend collagen films, the collagen gel was added to the dispersion after acidification, and the dispersion was homogenized by dispersing device Overhead Stirrers Hei-TORQUE 100 Value (Heidolph Instruments GmbH & Co. KG, Germany) at a speed of 500 rpm for 30 minutes. The thus prepared dispersions were cast on plastic trays and the solvent was left to evaporate. Blend films were dried at ambient conditions for approximately 48–72 hours. CMC films were dried first in a hot air drier Heratherm OMS60 (Thermo Scientific, Germany) for 3 hours at 60 °C and then at the laboratory for 24–48 hours. In the case of blended collagen films, a concentration of 0.003 g/cm$^2$ was maintained. Dry films were peeled off the trays, and the samples of required dimensions were punched using steel punches and stored in closed plastic bags to await testing. The composition of prepared samples is summarized in Table 1.

*Evaluation of prepared films*

*Organoleptic and microscopic evaluation*

All prepared films were assessed by visual examination in the dry and wet states. Their appearance, transparency, surface, and cohesiveness in the wet state were evaluated. In addition, the structure and film surface properties were characterized by SEM. The samples were placed on aluminum stubs with double-sided adhesive carbon tape and observed using a scanning electron microscope MIRA 3 (Tescan Brno, s.r.o., Czech Republic). Obtained signals of the samples were produced by secondary electrons (SE), at 3 kV voltage and 100× and 500× magnifications.

*Film thickness*

Film thickness was measured using an Elcometer 456 (Elcometer Limited, UK), a coating thickness gauge. The sample was laid on a solid base, and the thickness was measured at 30 different locations. The results were used to recalculate data obtained from the texture analysis to a value of 100 μm to facilitate further comparison. Results are presented as a mean value ± SD.

*Film weight and mass content uniformity*

Weights of all films (20 rigorously cut pieces of a size of 25 × 25 mm from random locations of the prepared films) were measured using a KERN 440-45 (Gottl. KERN & Sohn GmbH, Germany) analytical scales. Results are presented as a mean value ± SD. The uniformity of mass of films for wound application is not specified in the European Pharmacopoeia. For this reason, the evaluation of the films was done accordingly to the adapted test (2.9.5. Uniformity of mass of single-dose preparations) described in the European Pharmacopoeia[36]. All the masses of the films were compared with the average mass and the percentage of deviation was then calculated. Limits for uncoated and film-coated tablets with an average weight under 80 mg were applied. Not more than 2 of the individual masses could deviate from the average mass by more than 10 % and none by more than twice that percentage. Results are presented as average values with minimum and maximum values expressed in mg and % of film weight and are accompanied by a statement of the European pharmacopeia limit meeting[36].

*Surface pH and pH alterations in time*

The surface pH was measured using a contact pH meter (Flatrode, Hamilton, USA) after wetting with a drop of purified water. The measurement was repeated four

*Table 1. Labeling and composition of 100 g casting dispersions*

| Sample | Amount of CMC (g) | Collagen | Amount of collagen (g) | Plasticizer | CMC : plasticizer ratio | Amount of plasticizer (g) |
|---|---|---|---|---|---|---|
| **G-1** | 1.0 | – | 1.0 | glycerine | 1 : 1 | 1.0 |
| **G-1,5** | 1.0 | – | 1.0 | glycerine | 1 : 1.5 | 1.5 |
| **G-2** | 1.0 | – | 1.0 | glycerine | 1 : 2 | 2.0 |
| **M-1** | 1.0 | – | 1.0 | macrogol 300 | 1 : 1 | 1.0 |
| **M-1,5** | 1.0 | – | 1.0 | macrogol 300 | 1 : 1.5 | 1.5 |
| **M-2** | 1.0 | – | 1.0 | macrogol 300 | 1 : 2 | 2.0 |
| **G-1-C** | 1.0 | bovine | 1.0 | glycerine | 1 : 1 | 1.0 |
| **G-1,5-C** | 1.0 | bovine | 1.0 | glycerine | 1 : 1.5 | 1.5 |
| **G-2-C** | 1.0 | bovine | 1.0 | glycerine | 1 : 2 | 2.0 |
| **M-1-C** | 1.0 | bovine | 1.0 | macrogol 300 | 1 : 1 | 1.0 |
| **M-1,5-C** | 1.0 | bovine | 1.0 | macrogol 300 | 1 : 1.5 | 1.5 |
| **M-2-C** | 1.0 | bovine | 1.0 | macrogol 300 | 1 : 2 | 2.0 |

times per sample, with results presented as mean values ± SDs of each sample. The measurement of surface pH alterations in determining time intervals (1, 3, 8, 24 h) was made with the use of an artificial wound model developed by Vinklárková et al.[18] The model consists of a Petri dish and an absorptive sponge with a rough surface soaked in a 20 ml buffered salt solution of pH 7.2 (BSS) according to the Ph. Eur. The model aims to simulate the natural wound environment conditions. Film squares measuring 25 × 25 mm were cut and put on the wound model surface. The Petri dish was covered with a lid to prevent water evaporation, and the surface pH was measured at determined time intervals. The measurement was repeated four times, and the results are presented as each sample's average value and SD.

*Swelling properties of the films*

The swelling properties were determined using an artificial wound model described before. Film samples (25 × 25 mm) were weighed on an analytical balance (KERN 870–13, Gottl. KERN & Sohn GmbH, Balingen, Germany) under dry conditions ($W_d$). After that, the samples were placed on the surface of the artificial wound model. The Petri dish was covered with a lid to prevent water evaporation and swollen films were then evaluated. At certain time intervals (1, 3, 8, 24 h), films were weighted ($W_s$). The degree of swelling $S_w$ in the film was calculated using the following equation:

$$S_w = (W_s - W_d)/W_d.$$

The measurement was repeated three times, with results presented as mean values and SDs of each sample.

*Mechanical properties of the films*

The mechanical properties of the films were evaluated by texture analysis. Both measurements – tensile and puncture testing were made in a dry state and wet state after swelling for one hour on an artificial wound model soaked with PBS. A CT 3 Texture Analyzer (Brookfield, Middleboro, MA, USA) equipped with a 4.5 kg load cell and TexturePro CT software was used for tensile testing. Rectangular-shaped film strips (40 × 10 mm) were mounted between the upper and lower grips of the TA-DGA probe. The lower grip was held stationary, and the films were stretched by the upper grip moving at a speed of 0.5 mm/s to pull apart until it broke. The force and work done during the process and the deformation (elongation) of the films at the moment of tearing was measured. For the puncture test, a TA39 cylindrical probe (2 mm diameter, probe motion speed 0.5 mm/s) was used. Film squares (25 × 25 mm) were fixed in the JIG TA-CJ holder, and the force needed to puncture the film, work done during this process, and the sample deformation at the moment of penetration were measured. Both measurements were repeated five times for each sample, and the results are presented as tensile (puncture) force, tensile (puncture) work, and deformation of the films recalculated for a uniform thickness of 100 µm.

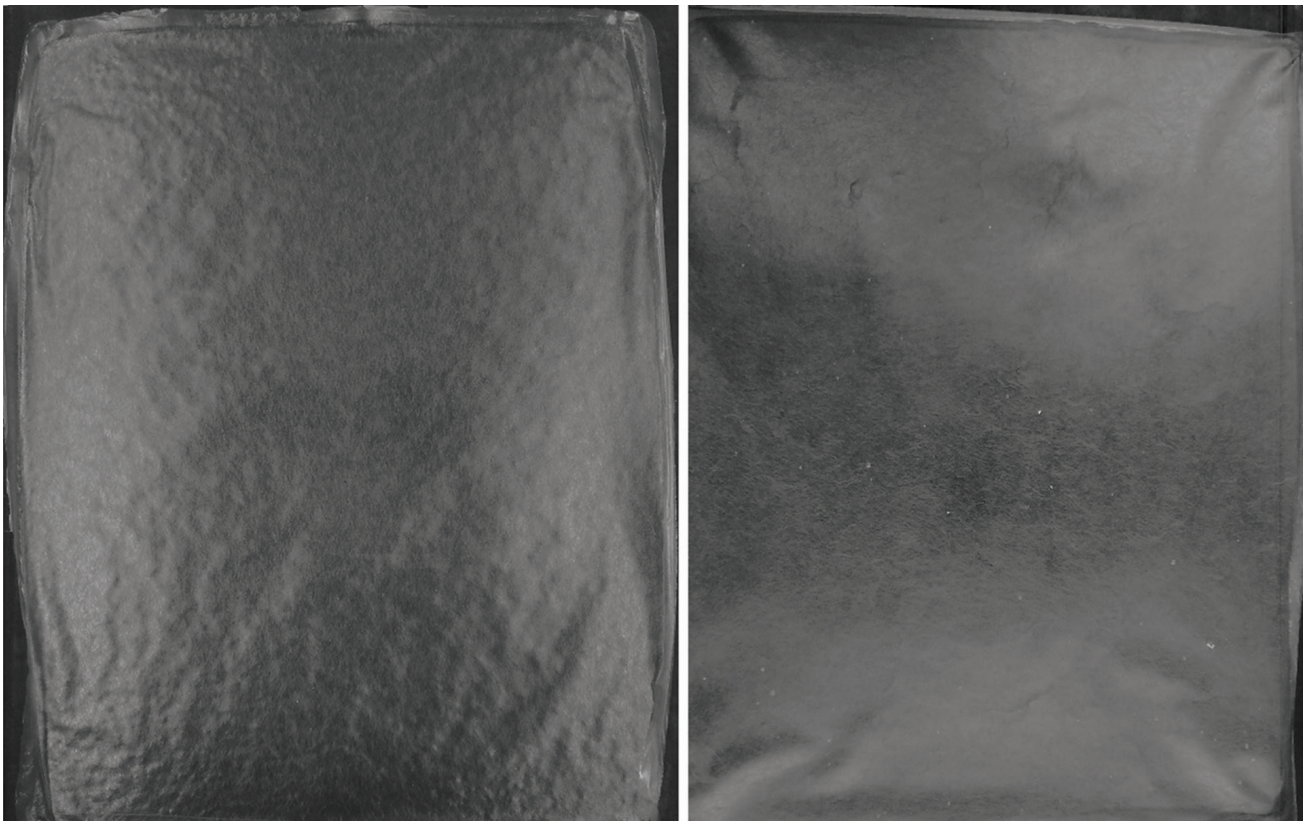

*Fig. 1. The appearance of the films: G-2 at the left, G-2-C at the right*

## Results and discussion

An ideal film wound dressing should be similar to the skin, homogenous with a smooth structure, and free of voids and cracks. The specific property of films is their transparency, which endorses wound inspection without removal. The flexibility of films enables an easy conformation to the patient's body and is also essential for good manipulation. Such an important property is also the cohesiveness of the resulting film, especially when wet[37, 38]. However, film wound dressing should also have antibacterial, antioxidant, or anti-inflammatory properties and can be loaded with various substances[37, 39, 40]. The organoleptic evaluation confirmed our assumptions mentioned above (Fig. 1). All the films were homogenous, transparent, and flexible. Although there were no significant differences between the films, glycerine ones were more flexible and softer than macrogol ones. Blend films were firmer in comparison with CMC samples.

SEM images showed and confirmed the presence of fibers which is given by the use of the partially substituted CMC (Fig. 2). The microfibrillar structure is more noticeable in CMC films than in blend films because

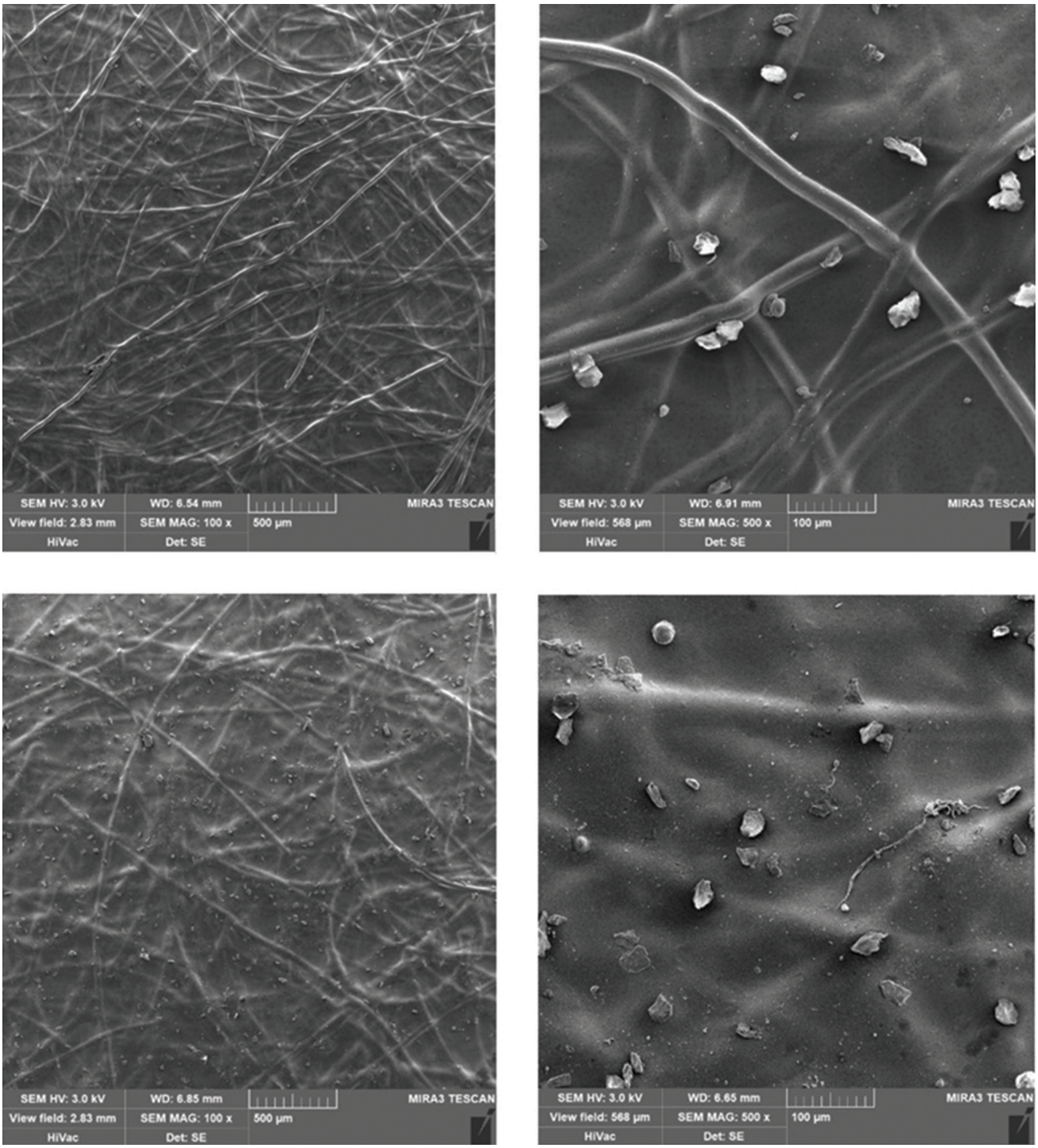


*Fig. 2. Microscopic appearance of the films. Comparison of G-1 (top images) and G-1-C (bottom images). Magnifications 100× and 500× from left to right.*

of collagen presence. Microfibrous CMC leads to the strengthening and improvement of the mechanical properties of the resulting film wound dressing[16]. Moreover, acidification of dispersion is also a critical factor for the CMC film preparation because soluble NaCMC is converted to the insoluble acidic form of carboxymethylcellulose (HCMC) after acidification. The resulting film remains cohesive and maintains suitable mechanical properties even after wetting (contact with wound exudate)[41].

Film thickness is an essential parameter from the technological point of view because uniform thickness provides uniform mechanical properties and means a correct preparation method, and in the case of medicated films, it also provides a homogenous drug distribution. Film thickness is influenced by the preparation method, amount of casting gel, and flatness of the drying surface[37, 42, 43]. Table 2 shows that the thickness of all the films ranged from 47.0 ± 5.9 μm to 66.6 ± 3.7 μm. Blend films were thicker than CMC films because of the collagen addition. As the amount of a plasticizer increased, the thickness of the films was higher. All the samples with macrogol were also thicker in comparison with glycerine ones.

The average weight of the square film samples ranged from 47.6 ± 3.6 mg to 75.4 ± 2.3 mg with an uptrend in plasticizer concentration (ratio) increase (Table 3). Blend films weighed more, as expected. For each sample, deviations from the mean weight were calculated and the uniformity mass test was done. All the films met the test requirements according to the European Pharmacopoeia[36]. Table 3 shows the minimum and maximum deviations from the mean weight of samples, expressed in mg and %.

The pH value of healthy skin is around 4–6; however, the pH value during the healing process, especially in infected or chronic wounds, is changed. A widely accepted concept is that chronic wounds show pH higher than 7, although there are still debates on it[44]. Nevertheless, no matter what pH is, it is generally proven that pH affects the process of wound recovery, so evaluation of the ability of the dressing to influence the wound pH during its healing is an essential test. We used the buffered salt solution with a pH of 7.2 because it is similar to wound exudate in terms of the pH value and ion content[45]. The ability of films to maintain acidic pH even after 24 hours was tested because it defines whether films can modulate the pH values even after a longer interval. Table 4 shows the results of the evaluation of pH after wetting and pH alterations in determined time intervals. After wetting with a drop of water, the surface pH of all the samples was acidic, maintaining values

*Table 2. Film thickness*

| Sample | Thickness (μm) | Sample | Thickness (μm) |
|---|---|---|---|
| **G-1** | 47.0 ± 5.9 | **G-1-C** | 55.8 ± 6.8 |
| **G-1,5** | 49.3 ± 6.0 | **G-1,5-C** | 59.0 ± 5.4 |
| **G-2** | 49.5 ± 7.8 | **G-2-C** | 62.3 ± 5.8 |
| **M-1** | 57.7 ± 6.8 | **M-1-C** | 61.8 ± 7.4 |
| **M-1,5** | 57.2 ± 5.7 | **M-1,5-C** | 65.2 ± 6.3 |
| **M-2** | 60.1 ± 7.4 | **M-2-C** | 66.6 ± 3.7 |

*Table 3. Uniformity of mass*

| Sample | Average weight (mg) | Min. weight | | Max. weight | | Compliance with the European Pharmacopoeia limit |
|---|---|---|---|---|---|---|
| | | mg | %[a] | mg | %[a] | |
| **G-1** | 48.1 ± 3.2 | 41.5 | –13.8 | 52.7 | +9.5 | Yes |
| **G-1,5** | 54.8 ± 3.1 | 49.6 | –9.4 | 59.9 | +9.4 | Yes |
| **G-2** | 65.2 ± 3.8 | 58.9 | –9.7 | 71.5 | +9.7 | Yes |
| **M-1** | 47.6 ± 3.6 | 42.0 | –11.7[b] | 52.0 | +9.3 | Yes |
| **M-1,5** | 57.7 ± 2.7 | 51.9 | –10.1 | 61.1 | +5.9 | Yes |
| **M-2** | 64.9 ± 4.2 | 58.6 | –9.7 | 70.6 | +8.7 | Yes |
| **G-1-C** | 59.7 ± 2.3 | 55.9 | –6.3 | 63.5 | +6.4 | Yes |
| **G-1,5-C** | 61.9 ± 3.4 | 55.8 | –9.8 | 67.6 | +9.3 | Yes |
| **G-2-C** | 69.9 ± 1.8 | 66.9 | –4.3 | 74.2 | +6.1 | Yes |
| **M-1-C** | 55.9 ± 4.0 | 50.8 | –9.2 | 61.2 | +9.4 | Yes |
| **M-1,5-C** | 70.4 ± 3.1 | 63.9 | –9.2 | 77.1 | +9.5 | Yes |
| **M-2-C** | 75.4 ± 2.3 | 71.0 | –5.8 | 79.0 | +4.8 | Yes |

[a] deviation from the average; [b] maximum two samples were out of the limit ± 10%

around 2.5–3.0. All the films preserved acidic or neutral pH even after 24 hours, and the pH values ranged from 4.96 ± 0.24 to 6.35 ± 0.11. This indicates the ability of the films to keep the pH in the acidic range, so it is possible to consider them as pH-modulating wound dressings.

The swelling behaviour of wound dressing should be considered one of the most important parameters because it reflects the ability of the dressing to absorb wound exudate and provide a moist environment in the wound. High absorption values are not expected from films, but appropriate efficiency in this area is also desirable[6, 37]. Figure 3 summarizes the swelling degrees of all the samples. Blend films significantly exhibited a lower degree of swelling compared to CMC films. Samples with macrogol showed a slightly higher swelling than glycerine ones. All the swelling values increased gradually up to 24 h. Blend films have a lower absorption capacity compared to CMC films.

An ideal wound dressing should have good mechanical properties such as flexibility and resistance related to the practical application to the wound. Films should adapt to different body parts so they can be durable, soft, and elastic[37]. Mechanical properties of films depend on the polymer, solvent, solution pH, presence of plasticizer, and mixing process. Synthetic polymers usually have better mechanical properties than natural ones, but their adherence, absorption, and permeability are low, so combinations of synthetic and natural compounds were designed as well as modifications and improvements[46]. The deformation values express the flexibility of films. The higher

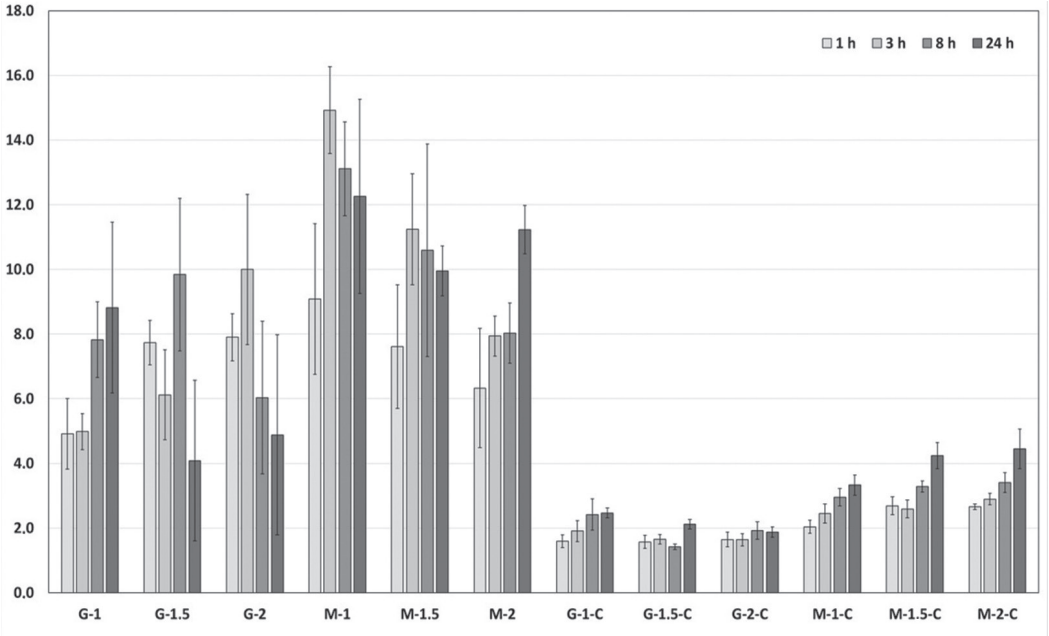


*Fig. 3. Swelling properties of the films*

*Table 4. Surface pH*

| Sample | pH | | | | |
|---|---|---|---|---|---|
| | After wetting | pH alterations in determined time intervals | | | |
| | | 1 h | 3 h | 8 h | 24 h |
| **G-1** | 2.46 ± 0.03 | 4.10 ± 0.05 | 4.12 ± 0.17 | 4.69 ± 0.55 | 6.25 ± 0.09 |
| **G-1,5** | 2.80 ± 0.03 | 3.85 ± 0.13 | 4.72 ± 0.52 | 4.34 ± 0.33 | 6.00 ± 0.26 |
| **G-2** | 2.79 ± 0.03 | 3.99 ± 0.07 | 4.39 ± 0.29 | 4.54 ± 0.63 | 6.15 ± 0.14 |
| **M-1** | 2.69 ± 0.03 | 3.84 ± 0.20 | 4.36 ± 0.25 | 4.58 ± 0.38 | 4.96 ± 0.24 |
| **M-1,5** | 2.89 ± 0.02 | 4.05 ± 0.24 | 4.52 ± 0.22 | 4.88 ± 0.94 | 5.86 ± 0.52 |
| **M-2** | 2.96 ± 0.01 | 4.19 ± 0.45 | 4.48 ± 0.38 | 4.87 ± 0.51 | 6.50 ± 0.13 |
| **G-1-C** | 2.83 ± 0.02 | 4.04 ± 0.23 | 4.57 ± 0.15 | 4.85 ± 0.61 | 5.66 ± 0.24 |
| **G-1,5-C** | 2.68 ± 0.05 | 4.19 ± 0.22 | 4.80 ± 0.24 | 6.07 ± 0.21 | 6.35 ± 0.11 |
| **G-2-C** | 2.59 ± 0.02 | 4.09 ± 0.17 | 4.72 ± 0.29 | 5.43 ± 0.13 | 5.69 ± 0.17 |
| **M-1-C** | 2.51 ± 0.05 | 3.79 ± 0.14 | 3.91 ± 0.02 | 4.89 ± 0.32 | 5.77 ± 0.22 |
| **M-1,5-C** | 2.46 ± 0.01 | 3.81 ± 0.10 | 4.02 ± 0.16 | 4.66 ± 0.15 | 5.22 ± 0.34 |
| **M-2-C** | 2.53 ± 0.02 | 3.82 ± 0.11 | 4.07 ± 0.07 | 4.85 ± 0.32 | 5.51 ± 0.26 |

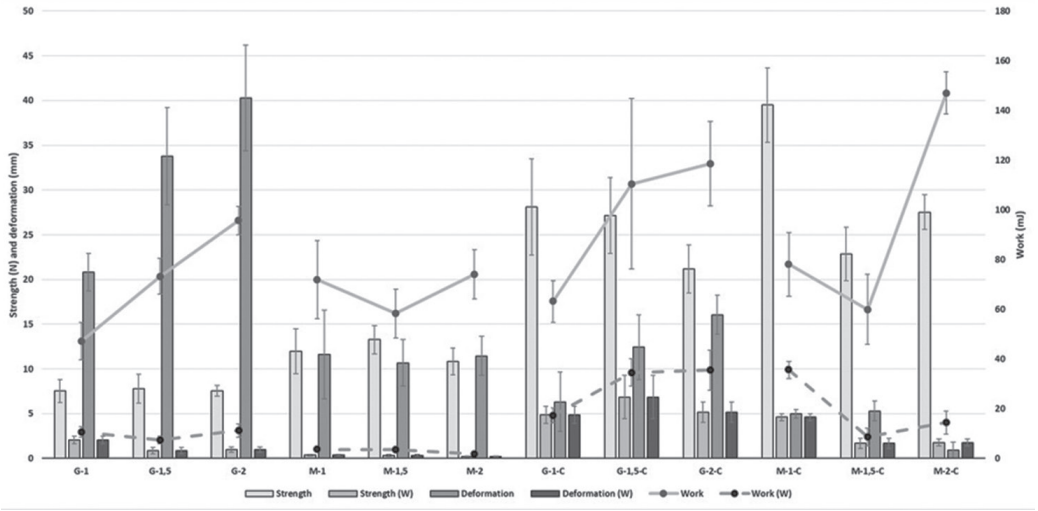


*Fig. 4. Comparison of tested parameters observed through tensile testing (strength, work, deformation) in dry and wet states (W)*

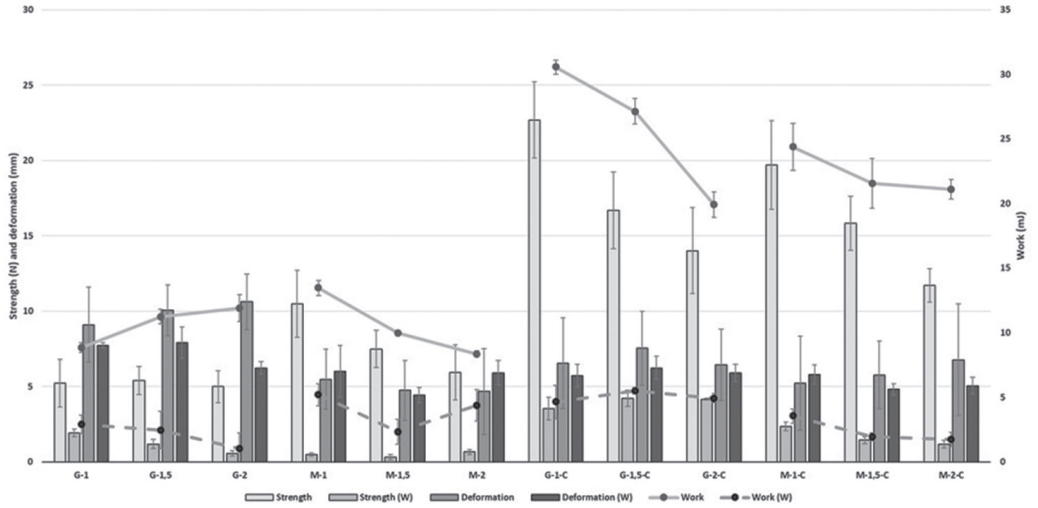


*Fig. 5. Comparison of tested parameters observed through puncture testing (strength, work, deformation) in dry and wet states (W)*

values, the more adaptable and flexible the film is. The film dressing should remain cohesive even after contact with wound exudate for easy removal[47]. The mechanical properties of the films were tested in a dry and wet state (after one hour on an artificial wound model), and two different approaches were used to assess their mechanical properties: tensile testing (strength, work, deformation) and puncture testing (strength, work, deformation). All values were recalculated for a uniform thickness of 100 µm.

Figure 4 demonstrates the tensile testing results. In most cases, CMC films reached lower tensile strength and work values, but higher deformation values compared with blend films. It seems that films with glycerol were firmer than macrogol ones. Not surprisingly, lower mechanical durability was observed in all wetted films. However, the mechanical properties in blend films did not decrease as rapidly after wetting as in CMC films. In particular, the deformation values did not decrease rapidly, which means good flexibility of the films even after wetting.

Figure 5 demonstrates the mechanical properties of the films after puncture testing. Blend films had slightly better properties in dry and wet states than CMC samples. Lower values of all the tested parameters were observed in the wet state (W). Although the work and strength had significantly decreased, the deformation values after wetting decreased minimally. That means good durability and adaptability of the films even in the wet state.

## Conclusion

This study aimed to prepare and evaluate a novel collagen-CMC blend film wound dressing and compare its properties with CMC films. The films were prepared by the solvent casting method; all of them had suitable organoleptic properties, which are characteristic

of film wound dressings with good cohesiveness and flexibility even after wetting. The microscopic evaluation confirmed the microfibrillar structure of CMC, which improved the mechanical durability of the films necessary for practical wound application. All the samples met the requirements according to mass content uniformity testing and maintained acidic or neutral pH even for 24 hours on an artificial wound model. Unlike CMC films, blend films had lower absorption capacity and better mechanical durability even in the wet state. This study confirmed the possibility of combining collagen and CMC in order to improve the properties of the resulting film wound dressing.

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## References

1. **Dhivya S., Padma V. V., Santhini E.** Wound dressings – a review. BioMedicine 2015; 5(4), 24–28.
2. **Rezvani Ghomi E., Khalili S., Nouri Khorasani S., Esmaeely Neisiany R., Ramakrishna S.** Wound dressings: Current advances and future directions. J. Appl. Polym. Sci. 2019; 136(27), 47738.
3. **Okur M. E., Karantas I. D., Şenyiğit Z., Üstündağ Okur N., Siafaka P. I.** Recent trends on wound management: New therapeutic choices based on polymeric carriers. Asian J. Pharm. Sci. 2020; 15(6), 661–684.
4. **Borda L. J., Macquhae F. E., Kirsner R. S.** Wound Dressings: A Comprehensive Review. Curr. Dermatol. Rep. 2016; 5(4), 287–297.
5. **Schmitz M., Mustafi N., Rogmans S., Kasparek S.** Pilot-study switchable film dressing & elderly skin/patients with chronic wounds: A non-interventional, non-placebo-controlled, national pilot study. Wound Med. 2020; 30, 100189.
6. **Sussman G.** Technology update: Understanding film dressings. Wounds International 2010; 1(4), 23–25.
7. **Peate I., Glencross W.** Wound care at a glance. Oxford: Wiley-Blackwell 2015.
8. **Gultekin G., Atalay-Oral C., Erkal S., Sahin F., Karastova D., Tantekin-Ersolmaz S. B., Guner F. S.** Fatty acid-based polyurethane films for wound dressing applications. J. Mater. Sci. Mater. Med. 2009; 20(1), 421–431.
9. **Vieira M. G. A., Da Silva M. A., Dos Santos L. O., Beppu M. M.** Natural-based plasticizers and biopolymer films: A review. Eur. Polym. J. 2011; 47(3), 254–263.
10. **Mogoşanu G. D., Grumezescu A. M.** Natural and synthetic polymers for wounds and burns dressing. Int. J. Pharm. 2014; 463(2), 127–136.
11. **Mir M., Ali M. N., Barakullah A., Gulzar A., Arshad M., Fatima S., Asad M.** Synthetic polymeric biomaterials for wound healing: a review. Prog. Biomater. 2018; 7(1), 1–21.
12. **Kathe K., Kathpalia H**. Film forming systems for topical and transdermal drug delivery. Asian J. Pharm. Sci. 2017; 12(6), 487–497.
13. **Wang K., Wang H., Pan S., Fu Ch., Chang Y., Li H., Yang X., Qi Z.** Evaluation of New Film Based on Chitosan/Gold Nanocomposites on Antibacterial Property and Wound-Healing Efficacy. Adv. Mater. Sci. Eng. 2020; 2020, 1–10.
14. **Kalayciğlu Z., Kahya N., Adimcilar V., Kaygusuz H., Torlak E., Akin- Evingür G., Erim F. B.** Antibacterial nano cerium oxide/chitosan/cellulose acetate composite films as potential wound dressing. Eur. Polym. J. 2020; 133, 109777.
15. **Attauil Sukumaran S., Kalimuthu B., Selvamurugan N., Mani P.** Wound dressings based on chitosan/gelatin/MgO composite films. Int. J. Polym. Mater. 2022; 71(16), 1252–1261.
16. **Rathod L., Bhowmick S., Patel P., Sawant K.** Calendula flower extract loaded collagen film exhibits superior wound healing potential: Preparation, evaluation, in-vitro & in-vivo wound healing study. J. Drug Deliv. Sci. Technol. 2022; 72, 103363.
17. **Juncu G., Stoica-Guzun A., Stroescu M., Isopencu G., Jinga S. I.** Drug release kinetics from carboxymethylcellulose-bacterial cellulose composite films. Int. J. Pharm. 2016; 510, 485–492.
18. **Vinklárková L., Masteiková R., Vetchý D., Doležel P., Bernatonienė J.** Formulation of novel layered sodium carboxymethylcellulose film wound dressings with ibuprofen for alleviating wound pain. Biomed. Res. Int. 2015; 2015, 892671.
19. **León-López A., Morales-Peñaloza A., Martínez-Juárez V. M., Vargas-Torres A., Zeugolis D. I., Aguirre-Álvarez G.** Hydrolyzed Collagen – Sources and Applications. Molecules 2019; 24(22), 4031.
20. **Rangaraj A., Harding K. G., Leaper D.** Role of collagen in wound management. Wounds UK. 2011; 7(2), 54–63.
21. **Hochstain A. O., Bhatia A.** Collagen: Its role in wound healing. Podiatry Management 2014; 103–110.
22. **Wu X., Luo Y., Liu Q., Jiang S., Mu G.** Improved structure-stability and packaging characters of crosslinked collagen fiber-based film with casein, keratin and SPI. J. Sci. Food Agric. 2019; 99(11), 4942–4951.
23. **Ding C., Zhang M., Li G.** Preparation and characterization of collagen/hydroxypropyl methylcellulose (HPMC) blend film. Carbohydr. Polym. 2015; 119, 194–201.
24. **Wang W., Wang Y., Wang Y., Zhang X., Wang X., Gao G.** Fabrication and characterization of microfibrillated cellulose and collagen composite films. J. Bioresources Bioprod. 2016; 1(4), 162–168.
25. **Adamiak K., Sionkowska A.** Current methods of collagen cross-linking: Review. Int. J. Biol. Macromol. 2020; 161, 550–560.
26. **Geanaliu-Nicolae R., Andronescu E.** Blended Natural Support Materials – Collagen Based Hydrogels Used in Biomedicine. Materials 2020; 13(24), 5641.
27. **Sionkowska A.** Collagen blended with natural polymers: Recent advances and trends. Prog. Polym. Sci. 2021; 122, 101452.

28. **Klunklin W., Jantanasakulwong K., Phimolsiripol Y. et al.** Synthesis, Characterization, and Application of Carboxymethyl Cellulose from Asparagus Stalk End. Polymers 2021; 13(1), 81.
29. **Kanikireddy V., Varaprasad K., Jayaramudu T., Karthikeyan Ch., Sadiku R.** Carboxymethyl cellulose-based materials for infection control and wound healing: A review. Int. J. Biol. Macromol. 2020; 164, 963–975.
30. **Basu P., Narendrakumar U., Arunachalam R., Devi S., Manjubala I.** Characterization and Evaluation of Carboxymethyl Cellulose-Based Films for Healing of Full-Thickness Wounds in Normal and Diabetic Rats. ACS Omega 2018; 3(10), 12622–12632.
31. **Hu D., Qiang T., Wang L.** Quaternized chitosan/polyvinyl alcohol/sodium carboxymethylcellulose blend film for potential wound dressing application. Wound Med. 2017; 16, 15–21.
32. **Trevisol T. C., Fritz A. R. M., De Souza S. M. A. G. U., Bierhalz A. C. K., Valle J. A. B.** Alginate and carboxymethyl cellulose in monolayer and bilayer films as wound dressings: Effect of the polymer ratio. J. Appl. Polym. Sci. 2019; 136(3), 46941.
33. **Jiang Z., Wang Y., Li L., Hu H., Wang S., Zou M., Liu W., Han B.** Preparation, Characterization, and Biological Evaluation of Transparent Thin Carboxymethyl-Chitosan/Oxidized Carboxymethyl Cellulose Films as New Wound Dressings. Macromol. Biosci. 2022; 22(2), 2100308.
34. **Tenorová K., Masteiková R., Jarábková J., Vetchý D., Bernatonienė J.** Kolagen v kombinaci s kyselou formou karboxymethylcelulosy v podobě netkané textilie jako moderní krycí prostředek pro terapii ran – formulace, příprava a hodnocení. Čes. slov. Farm. 2020; 69, 163–171.
35. **Tenorová K., Masteiková R., Kovárová N., Kostelanská K., Přikryl J., Vetchý D., Bernatonienė J.** Příprava a hodnocení dvouvrstvých filmů na bázi kolagenu a karboxymethylcelulosy za účelem terapie ran. Čes. slov. Farm. 2019; 68, 229–236.
36. **European Pharmacopoeia Commision.** European Pharmacopoeia, 9th ed. Stuttgart: Deutscher Apotheker Verlag 2017.
37. **Savencu I., Iurian S., Porfire A., Bogdan C., Tomuță I.** Review of advances in polymeric wound dressing films. React. Funct. Polym. 2021; 168, 105059.
38. **Weller C. D., Team V., Sussman G.** First-line interactive wound dressing update: a comprehensive review of the evidence. Front. Pharmacol. 2020; 11, 155.
39. **Walczak M., Michalska-Sionkowska M., Kaczmarek B., Sionkowska A.** Surface and antibacterial properties of thin films based on collagen and thymol. Mater. Today Commun. 2020; 22, 100949.
40. **Yaşayan G., Karaca G., Akgüner Z. P., Öztürk A. B.** Chitosan/collagen composite films as wound dressings encapsulating allantoin and lidocaine hydrochloride. Int. J. Polym. Mater. 2021; 70(9), 623–635.
41. **Vinklárková L., Masteiková R., Foltýnová G., Muselík J., Pavloková S., Bernatonienė J., Vetchý D.** Film wound dressing with local anesthetic based on insoluble carboxymethycellulose matrix. J. Appl. Biomed. 2017; 15, 313–320.
42. **Ahmad N., Tayyeb D., Ali I., Alruwaili N. K., Ahmad W., Ur Rehman A., Khan A. H., Iqbal M. S.** Development and Characterization of Hemicellulose-Based Films for Antibacterial Wound-Dressing Application. Polymers 2020; 12(3), 548.
43. **Alavi T., Rezvanian M., Ahmad N., Mohamad N., Ng S. F.** Pluronic-F127 composite film loaded with erythromycin for wound application: formulation, physicomechanical and in vitro evaluations. Drug Deliv. Transl. Res. 2019; 9(2), 508–519.
44. **Dong R., Guo B.** Smart wound dressings for wound healing. Nano Today 2021; 41, 101290.
45. **Power G., Moore Z., O'Connor T.** Measurement of pH, exudate composition and temperature in wound healing: A systematic review. J. Wound. Care 2017; 26, 381–397.
46. **Schmitz M., Mustafi N., Rogmans S., Kasparek S.** Pilot-study switchable film dressing & elderly skin/patients with chronic wounds: A non-interventional, non-placebo-controlled, national pilot study. Wound Med. 2020; 30, 100189.
47. **Simi C. K., Abraham T. E.** Biodegradable biocompatible xyloglucan films for various applications. Colloid Polym. Sci. 2010; 288, 297–306.