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Topical HPMC/Carbopol 934 Gel for Wound Healing: Formulation and in-Vivo Evaluation

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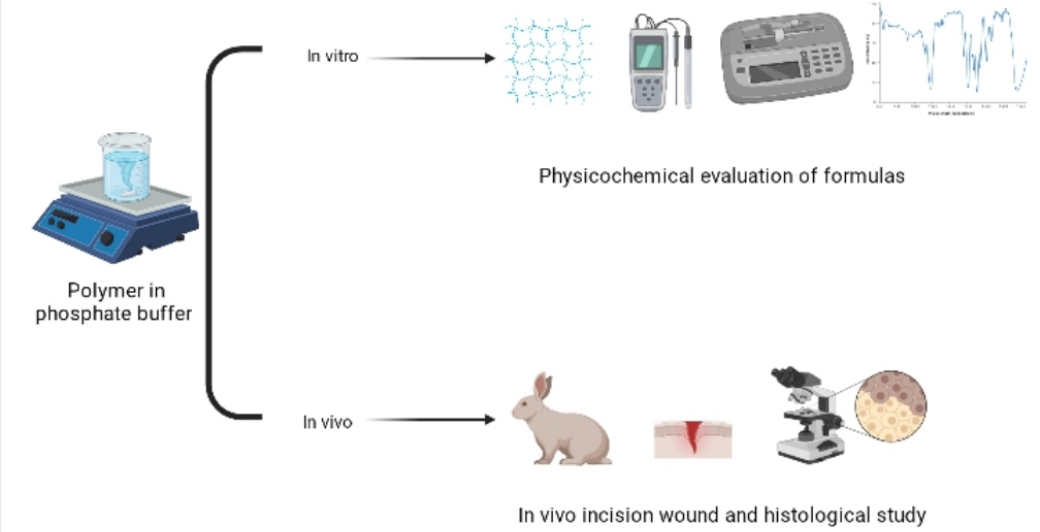
ABSTRACT

Wound healing is a vital physiological process for preserving the integrity of the skin following trauma caused by an accident or an intentional surgery. The characteristics of hydrogels, such as their high moisture content, ability to act as a barrier against bacterial infections, and ability to provide a moist environment for wound healing, can be used for the management of oozing and granulating wounds. Moreover, hydrogel may accelerate wound healing by stimulating cell proliferation. The present study aimed to formulate and evaluate topical hydroxypropyl methylcellulose (HPMC)/carbopol 934 gels for wound healing purposes. Different formulas were prepared using each polymer alone as in F1 and F3 and a combination of them (F2). The gels were evaluated by physical characterization, pH measurement, spreadability test, scanning electron microscopy, rheological testing, and Fourier transform infrared (FTIR) spectroscopy. Furthermore, the wound healing in rabbit skin was evaluated. All formulas showed good results relating to visual appearance, pH, FTIR study, and spreadability. However, the results of the viscosity and histological studies revealed that the best formula is the one composed of 1% HPMC and 1% carbopol 934. This product will eventually meet all standards for evaluation in preclinical and clinical studies.

Graphical Abstract



Introduction

Skin plays a crucial role in the immune system of humans since it serves as a protective barrier between internal organs and the outside environment. Burns, surgical procedures, or trauma that harm the skin can result in significant bacterial infections and other health issues, including inflammation, dermal maturation, and even the potential for blood poisoning.[1] Wound healing is a required response to tissue injury. The complex physiological processes of re-epithelialization, granulation tissue formation, and tissue remodeling are sequential steps of inflammation and tissue repair.[2] These complex processes of cellular and biochemical interactions involve a variety of cells, including keratinocytes, fibroblasts, and endothelial cells.[3]

Clinically, wounds are typically dressed with fibers, gauze, hydrogel dressings, medical films, etc. Hydrogel dressings, among them, have impacts on extra secretion absorption, facilitate gas exchange, and produce a moist environment for wound healing.[4] Because of their remarkable biodegradability, nontoxicity, and biocompatibility, hydrogels are widely used in the pharmaceutical and biomedical industries. More interest has been given to hydrogel systems based on polysaccharides of natural polymers, such as chitosan, alginate, cellulose, dextran, hyaluronan, and carrageenan.[5] There are also numerous hydrogels for use on wounds that are derived from cellulose and its byproducts. The cellulose- and chitosan-based biopolymer hydrogels have proven to have excellent properties for the treatment of wounds. Intriguing mechanical characteristics, such as good elasticity, flexibility, compressive stresses, an improvement in Young's modulus, and tensile stresses, were demonstrated by the hydrogels cross-linked with synthetic polymers. These characteristics are helpful in wound management.[6] In addition to having a 3D network structure and a high-water absorption rate when swelling in water, hydrogels are hydrophilic polymers.[7] Because of their distinct physical characteristics, hydrogels can shelter labile medicines from deterioration, enable controlled disintegration, and control the release of a variety of actives, including cells, macromolecules,

and small-molecule drugs. Therefore, hydrogels are suitable for use in various systems for drug delivery, wound dressings, hygiene products, and regenerative medicine due to their unique capacity to resolve several formulation- and drug-related challenges.[8]

Cellulose derivatives have a healing impact on wounds whether they are used alone or in combination with other natural, semi-synthetic, and synthetic polymers.[9] The fundamental advantage of mixing two or more biopolymers is the improvement of the resultant dressing's physicochemical properties, which also aid damaged tissue. These biopolymers can be used successfully as a base for a variety of formulations due to their high gelation properties. The exudates at the site of the lesion can be efficiently absorbed and retained by cellulose derivatives.[9] Hydroxypropyl methylcellulose (HPMC) is a cellulose ether that has been used as a hydrophilic gel matrix in drug formulation and delivery due to its non-toxic nature, good bio-adhesive properties, viscosity enhancement, and tolerance to high levels of drug loading.[10]

A class of synthetic polymers known as carbomers that are manufactured from acrylic acids are readily available in the market. Carbomer has long been employed as a primary medication carrier for transdermal administration. It offers the benefits of high viscosity, high drug compatibility, strong heat stability, and great tissue compatibility.[11,12]

The present study was conducted to create wound healing hydrogel utilizing carbopol 934 and hydroxypropyl methylcellulose (HPMC) for better therapeutic results.

Materials and methods

Source of chemicals. The polyacrylic acid polymer (carbopol 934) was purchased from HIMEDIA Laboratory (India), and HPMC was obtained from Merck. All of the chemicals were of analytical grade and were not further purified before usage.

Formulation method. Formulas were prepared by dispersing either carbopol 934 (F1) or HPMC (F3) in a certain volume of phosphate buffer solution (pH 7.5) followed by mixing using a magnetic stirrer until uniform dispersion of final concentration of 1% was obtained. The resultant mixture was left for 24 hours to ensure complete swelling of the polymer. For F2, which contain 1% of carbopol 934 and 1% of HPMC, the amount of carbopol 934 was added to the HPMC dispersion, mixed into the solution, and then allowed to sit for an additional 24 hours to completely swell and dissolve. Using a glass rod, the final translucent formula was simply mixed to create a homogeneous mixture. Each formula was packed into a tightly closed container and stored between 5-8°C until subjected to further evaluation. The formulations are depicted in Table 1.

Table 1. Composition of wound healing gel formulas

Formulas	Carbopol 934 %	HPMC%
F1	1	--
F2	1	1
F3	--	1

Table 2. pH measurementof the formulas

Formula	pH (mean±SD)
F1	4.64±0.04
F2	4.15±0.03
F3	3.96±0.02
mean±SD, (n=3)	

Table 3. Spreadability of selected formula at different speed

Formulas	Spreadability (cm)
F1	6.5±0.68
F2	7.45±0.36
F3	-
mean±SD, n =3	

Evaluation

Physical characterization. All formulations were evaluated for clarity, colour, and organoleptic properties.

pH measurement. One gram of each formula (F1 or F2) was diluted with purified water up to 10 ml and then pH was measured using a pH meter, while F3 was directly measured.

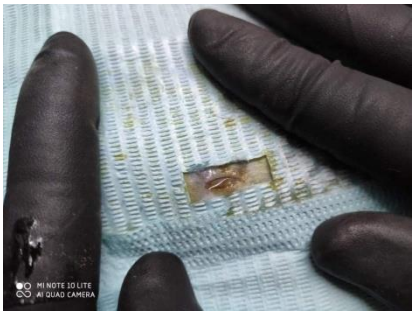


Figure 1. *Wound incision in rabbit*

Spreadability test. To test the spreadability of the hydrogel formulations (F1 and F2), 0.5 g of hydrogel was sandwiched between two 20 x 20 cm horizontal plates. Then, 5 g of a standardized weight was placed on the upper plate and left for roughly 5 minutes, after which no further spreading was anticipated. Spread circle diameters were measured in centimetres and used as benchmarks for spreadability. Measurements were made in triplicate.

describe the flow behaviour of the formulas.

Fourier transform infrared (FTIR) spectroscopy. Samples of each pure polymerin addition to a combination of carbopol and HPMC in 1:1 ratio were examined using FTIR spectroscopy to exclude any chemical interactions in the preparations.

Determination of wound healing potential in rabbit skin. The care of the animals used in this study and all treatment protocols were carried out according to the University of Mosul's animal ethics guidelines. At the University of Mosul's animal house, 12 healthy domestic male rabbits, each weighing 2 ± 0.5 kg were obtained. In an adequately air-conditioned area with 12-hour light and dark periods, all the animals were properly caged, fed a standard meal, and given unlimited access to water. An intramuscular injection of 40 mg/kg ketamine and 4 mg/kg xylazine was given to each rabbit to induce anaesthesia.[13] Throughout the procedure, more sedation was given as needed. After 5 minutes, total anaesthesia was achieved. Each

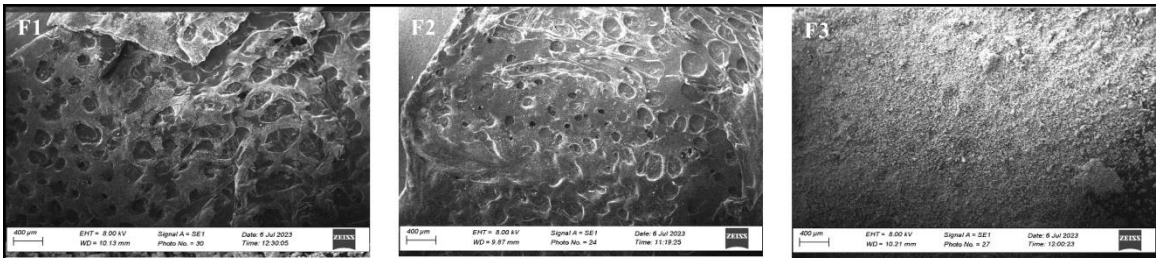


Figure 2. *Scanning electron microscopy images of prepared formulas*

Scanning electron microscopy. The gel formulations (F1 and F2) and the F3 liquid formulation were lyophilized by a freeze dryer and then the surface morphology of the formulas were examined using an 8 KV scanning electron microscope (EVO 10 ZEISS GERMANY).

Rheological testing. An NDJ-8S viscometer was used to assess the rheological properties of each formulation using L4 and L1 spindle types at different shear rates ranging from 1.5 – 30 rpm. Each measurement was carried out in triplicate. At increasing shear stress, the dynamic viscosity was measured to

linear incision on the back of each rabbit measured 1 cm in length and 0.5 cm in depth as illustrated in Figure 1. The wound was visible. The animals were divided into four groups of three rabbits each. Group 1 animals acted as the negative control group and were not handled. Groups 2, 3, and 4 received F1, F2, and F3, respectively. After the incision was made, the formulas were applied to it twice daily. The potential of wound healing was determined by comparing histologically the wound area healed on corresponding days with the negative control groups. The duration of epithelialization was noted.

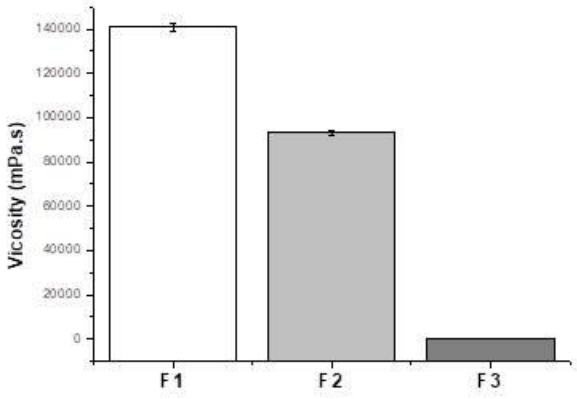


Figure 3. *Viscosity of formulas at 1.5 rpm*

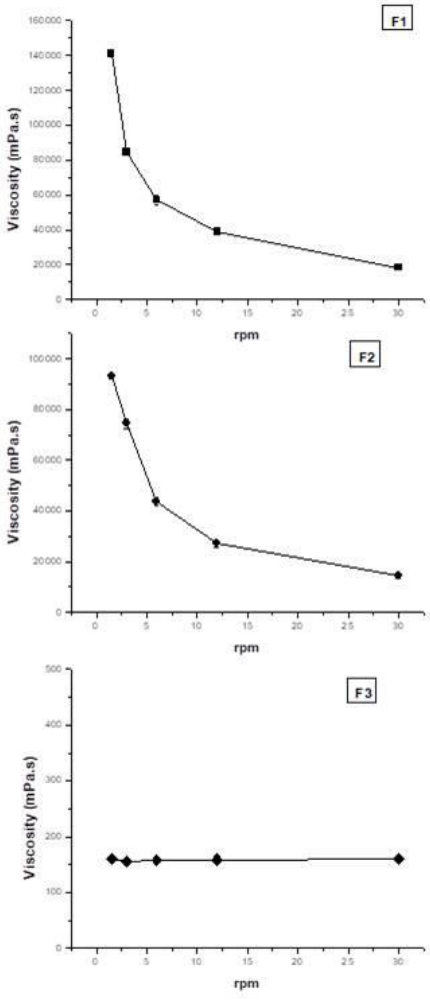


Figure 4*. Viscosity versus shear rate of formulas*

Histological assessment. One rabbit from each group was sacrificed, and skin samples were taken at 1-, 3-, and 7-days post-wound induction. Hematoxylin-eosin was used to stain the central region of the underlying tissue after it had been preserved in 10% buffered formalin. A magnification power of 40x and 100x by light microscope were used to examine the impacts of treatments on histological structure. Two pathologists conducted a double-blind histological investigation.

Statistical analysis. The experimental data were analyzed using the Sigma Plot software program for statistical analysis. Histological scores of the inflammatory cell's infiltrations, granulation tissue formation, and re-epithelialization were done by pathologists and analyzed statistically by Kruskal-Wallis One Way Analysis of Variance on Ranks test and Pairwise Multiple Comparison Procedures (Tukey test) at $p \leq 0.05$.

Results and Discussion

Physical characteristics of prepared formulas. Two of the prepared formulas (F1 and F2) were gels, while F3 was liquid. This is because F1 and F2 contain carbopol, which in an aqueous solution exhibits a sol-to-gel shift as the pH is increased above its pKa.[14] The formulas were examined macroscopically for the existence of structures that could be seen with the naked eye. The formulas were homogenous, lump-free, and without any roughness. The prepared formulas were clear and transparent.

pH of prepared formulas. Table 2 shows the pH of each formula. The un-neutralized carbopol 934 gel dispersions had an acidic pH, as was to be predicted. To improve healing, it was determined that lowering the pH of wounds would be a useful strategy to reduce protease activity.[15] Acidification of skin wounds can accelerate epithelialization and healing.[16] Because they successfully lower the pH of the wound surface, the use of these formulas can have a beneficial effect on the wounds healing process.

Spreadability test outcome. Table 3 displays the results of the spreadability test for two different F1 and F2 gel formulas for wound healing. Both formulas showed good spreadability results. Spreadability,

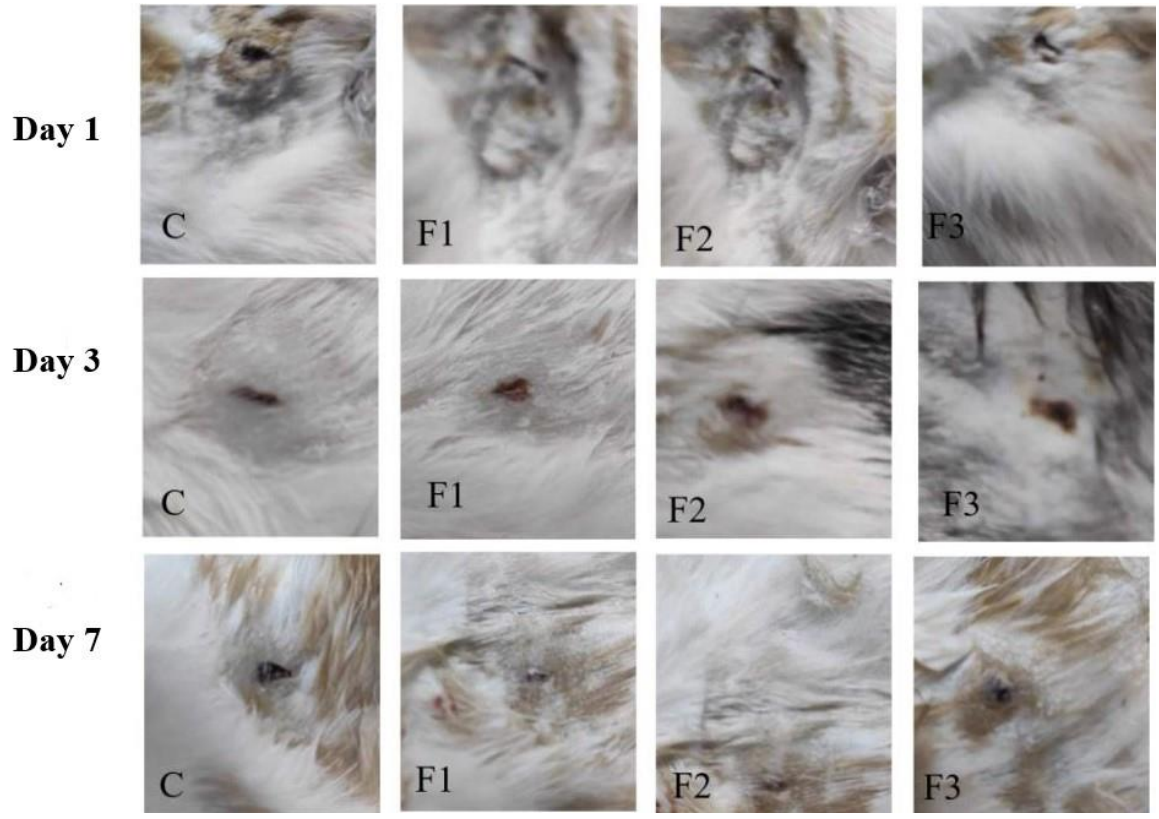


Figure 5. *Photographic shots of wounds in control group (C) and treated groups results at days 1, 3, 7 post-wounding using a digital camera*

which influences how readily the preparation can be spread to the skin, is crucial in skin treatments. Greater viscosity, which results in less spreadability, will make the preparation more challenging to apply. Unwantedly, a viscosity or spreadability that is too low or high, respectively, will shorten the retention time on the skin's surface.

Scanning electron microscopy analysis. The SEM micrographs of F1, F2, and F3 are shown in Figure 2. Both carbopol and the combined HPMC/carbopol revealed a clearly defined and connected porous structure. It was found that the combination of carbopol and HPMC created a structure like a sponge with uneven pore sizes ranging between 100 and 200 μm. Pore size is regarded as an indication of the gel's ability to absorb more exudate and so prevent fluid collection at the wound site. This effect results from the ability of larger pores to accommodate more fluid.[17] F3 on the other hand seems to have a compact structure devoid of a network of pores.

Rheological test outcome. Viscosity is an important factor in the quality of topical products, as it affects their retention time. Figure 3 shows the results of viscosity experiments for the formulations for wound healing. F1 and F2 demonstrated satisfactory viscosity properties. Low viscosity is undesirable since it will reduce the retention time on the skin's surface. Therefore, it is imperative to have optimal viscosity values.[18] The test results show that all formulations except F3 have good viscosity properties. F3 has low viscosity because it contain 1% of hpmc alone. Lack of occlusive coating, difficult spreading, and insufficient spreading are all effects of the preparation's insufficient viscosity. The longer the preparation is

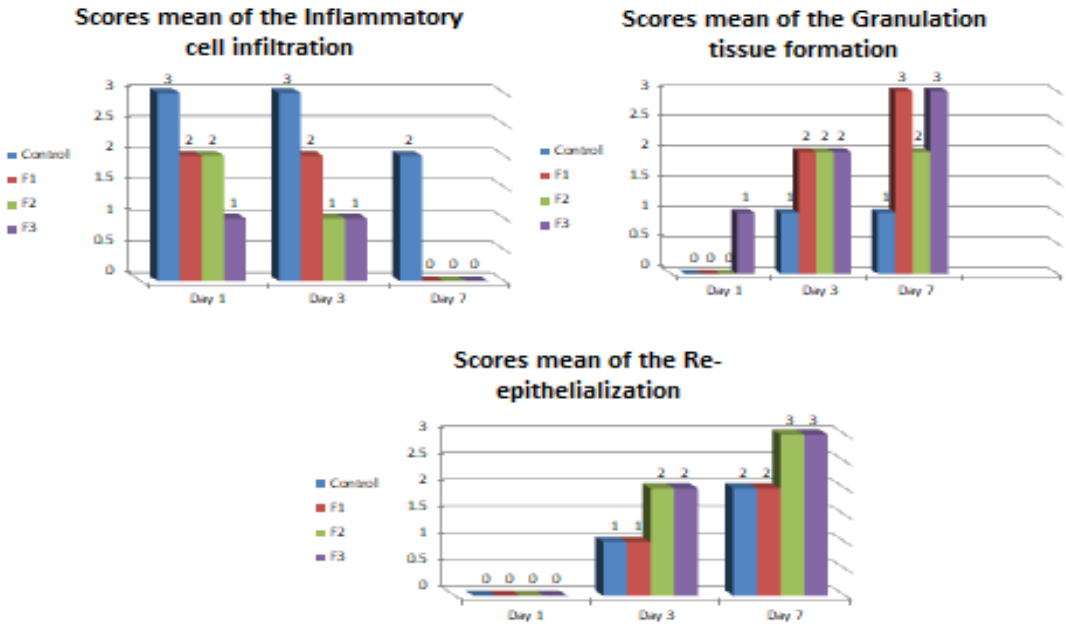


Figure 6. *The histological Scores mean at day 1, 3 and 7 of the study period*

left on the injured area, the more the region is shielded from outside influences, and the preparation itself can create a barrier.[19] It is important to detect whether the rheological behaviour of the gel formulas is Newtonian or non-Newtonian. The formulas encoded as F1 and F2 show a non-Newtonian shear thinning effect, while F3 exhibits Newtonian behaviour as presented in Figure 4. The shear thinning effect of F1 and F2 is important, which will facilitate the ease of removal of gel from the container by simple pressure application and also aid in dispersing the formulation to the skin.

FTIR spectroscopy analysis. The FTIR spectrum revealed HPMC-specific peaks. The peaks at 2867 and above 3450 indicate the existence of hydroxyl group (OH) stretching, and C-H group stretching. Also, there is a peak at 1048 that shows C-O group stretching. A peak in the FTIR spectra between 3000 and 2940 represents the OH stretching vibration. The carbonyl stretching (C=O stretching band) is shown as a prominent peak of about 1700–1701 cm^{-1}. The FTIR spectrum of the HPMC/carbopol mixture shows that HPMC maintains its peak at 1048 cm^{-1} while carbopol maintains its unique peak at 1702 cm^{-1}.[20] The lack of new peaks or a shift in the prominent characteristic peaks suggests that HPMC and carbopol are chemically compatible and can be combined into a single composition without any significant issues.[21]

Histological observations. The histological evaluation was carried out post-wounding on days 1, 3, and 7. The histological scores is shown in tables 4 and 5. Healing began to take place on the first day of post-wound induction in test groups. At the end of the treatment phase (7 days), as shown in Figures 5 and 6, the lesions treated with the formulations had a greater decrease in incision area than the control group.

On day 1 post-wounding (Figure 7), the control group showed a wide wound site (↔) with the destruction of the epithelium layer of mucosa, severe inflammatory exudate, and inflammatory cell infiltration (score 3) (→). The F1 group showed a deep

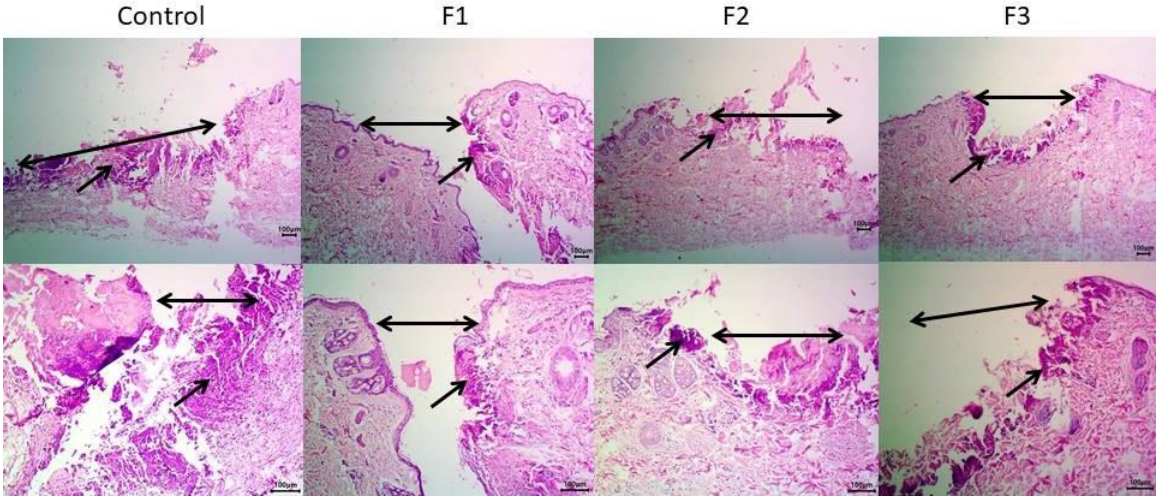


Figure 7. *Histological sections of rabbit skin for the negative control, F1, F2, and F3 groups at 1st day post-wound induction. H&E stain, (upper panel: 40X), (lower panel:100X).*

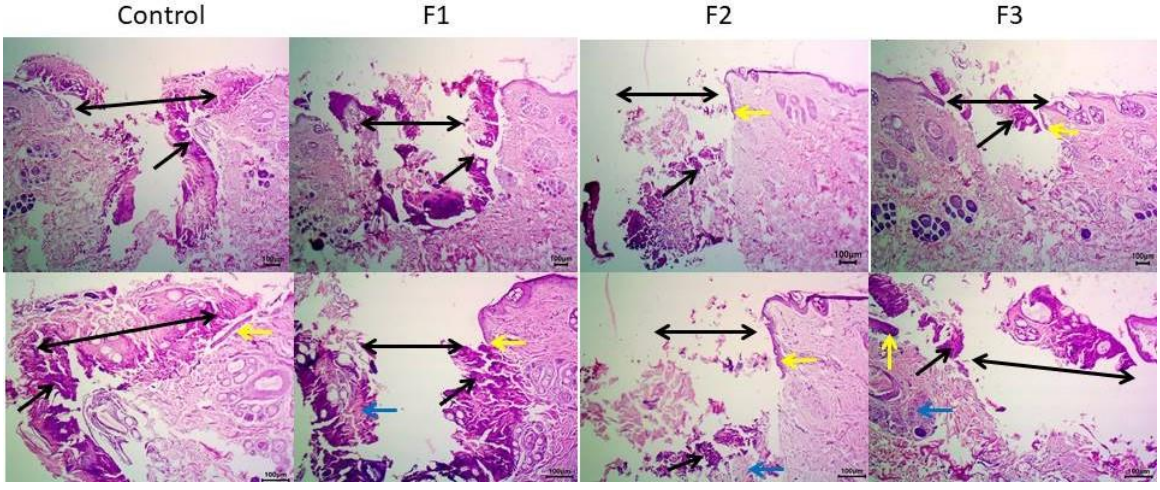


Figure 8. *Histological sections of rabbit skin for the negative control, F1, F2, and F3 groups at 3rd day post-wound induction. H&E stain, (upper panel: 40X), (lower panel:100X).*

wound site (↔) with moderate inflammatory exudate and inflammatory cell infiltration (score 2) (→). The F2 group showed a wide wound site (↔) with destruction of the epithelium layer of mucosa and moderate inflammatory exudate and inflammatory cell infiltration (score 2) (→). Meanwhile, the F3 group showed a narrow-wound site (↔) with destruction of the epithelium layer of mucosa, mild inflammatory exudate, and inflammatory cell infiltration (score 1) (→). As shown in Figure 8, on day 3 post-wounding, the control group had a wide wound site (↔) with the destruction of the epithelium layer of mucosa and severe inflammatory exudate, inflammatory cell infiltration (score 3) (→), and re-epithe-

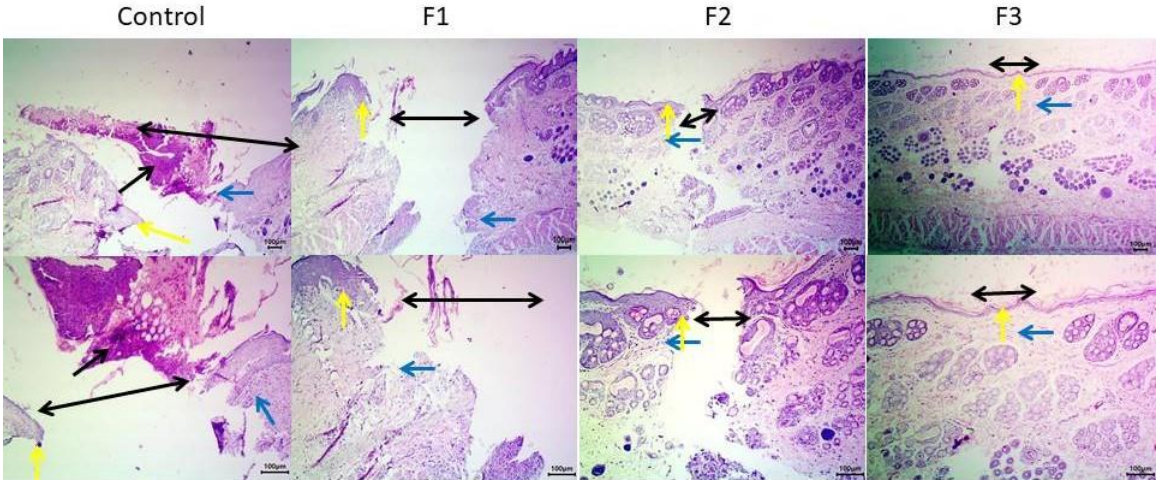


Figure 9. *Histological sections of rabbit skin for the negative control, F1, F2, and F3 groups at 7th day post-wound induction. H&E stain, (upper panel: 40X), (lower panel:100X).*

lialization (score 1) (→). The F1 group had a wide wound site (↔) with inflammatory exudate, inflammatory cell infiltration (score 2) (→), granulation tissue (score 2) (→), and re-epithelialization (score 1) (→). The F2 group had a wide wound site (↔), mild inflammatory exudate with inflammatory cell infiltration (score 1) (→), granulation tissue (score 2) (→), and re-epithelialization (score 2) (→). The F3 group had a narrow-wound site (↔) with destruction of the epithelium layer of mucosa and mild inflammatory exudate with inflammatory cells infiltration (score 1) (→), granulation tissue (score 2) (→) and re-epithelialization (score 2) (→).

Figure 9, at day 7 post wounding, the control group showed a wide wound site (↔) with destruction of the epithelium layer of mucosa, inflammatory exudate and inflammatory cell infiltration (score 2) (→), and re-epithelialization (score 2) (→). The F1 group showed a wide wound site (↔) without inflammatory cell infiltration (score 0), granulation tissue (score 3), and re-epithelialization (score 2) (→). The F2 group showed a narrow-wound site (↔), without inflammatory cell infiltration (score 0), granulation tissue (score 2) (→), and re-epithelialization (score 3) (→). The F3 group showed complete wound site occlusion (↔) without inflammatory cell infiltration (score 0), granulation tissue (score 3) (→), and re-epithelialization (score 3) (→).

Dermal reconstruction can be assessed through proliferation, remodeling, and maturation at the location of the wound. The tissues from the control group exhibited persistent inflammation, neutrophil, and polymorphonuclear cell infiltration until day 7. On the other hand, control tissues from day 7 showed fewer fibroblasts, blood vessels, and modest collagen deposition. Less blood vessel growth was observed in wounds that had received F1 treatment, along with a few of collagen bundles and a thin epithelial layer. On day 7, the tissues that were exposed to F2 showed a noticeable thick epithelial layer, large blood vessels, macrophages, and fibroblasts. On day 3, tissues from F3-treated wounds had more fibroblasts, more collagen being produced, and a thick epithelial layer. On day 7, the tissues had a high percentage of red blood cells and many blood capillaries, which suggested a better angiogenic process. At a later stage, the F2 and F3 groups showed the best re-epithelization, and the wound was almost completely closed by granulation tissue, with complete healing in some areas.

Table 4. The histopathological scores of the inflammatory cells infiltration, granulation tissue formation, and re-epithelialization of the control and treatment groups as a Median mean at day 1,3 and 7of the study period

Histopathological criteria	Groups	Day 1	Day 3	Day 7
Scores mean of the Inflammatory cell infiltration	Control	3	3	2
	F1	2	2	0
	F2	2	1	0
	F3	1	1	0
Scores mean of the Granulation tissue formation	Control	0	1	1
	F1	0	2	3
	F2	0	2	2
	F3	1	2	3
Scores mean of the Re-epithelialization	Control	0	1	2
	F1	0	1	2
	F2	0	2	3
	F3	0	2	3
Rating for inflammatory infiltration: (1) few, (2) moderate, (3-4) plenty. **Granulation tissue quantity:** (0) absent, (1) scanty, (2) moderate, and (3) profound. **Re-epithelialization Scoring:** (0) the re-epithelialization of the wound's margin, (1) Less than 50% of the wound has re-epithelialization, (2) More than half of the lesion has been re-epithelialized, (3) Uneven width and complete re-epithelialization of the wound				

- Data expressed as Median of the scores
- The difference letters mean there are significant differences between groups at $p \leq 0.05$

Numerous cellular, physiological, and molecular processes must coexist for a scar to develop. Three phases of skin recovery take place: inflammation-induced wound debridement, formation of granulation tissue, and remodeling, which leaves a scar that resembles the surrounding skin and creates tear-resistant tissue.[22] The evaluation of inflammation, the first stage of wound healing, is essential in the study. Inflammation prevents infection brought on by the entry of foreign microbes. If the inflammatory period is extended and harms the nearby healthy tissues, the healing process will be postponed. Inflammation needs to be reduced for incisions to recover properly.[23] This observation is

Table 5: Comparisons of the histopathological scores as inflammatory cells infiltration ICI, granulation tissue formation GTF, and re-epithelialization RE between the control group and F1, F2, F3 treatment groups at the same day.

Time period	ICI (*P*-value)	GTF (*P*-value)	RE (*P*-value)
1st Day	0.047*	0.012*	1.000
3rd Day	0.033*	0.012*	0.012*
7th Day	0.031*	0.036*	0.012*

Kruskal-Wallis One Way Analysis of Variance on Ranks test was used for the comparisons between groups at $p \leq 0.05$. (*): Significant difference.

in agreement with many studies, that describe the anti-inflammatory and antibacterial effects of cellulose derivatives.[24] Azuma *et al* studied the anti-inflammatory effect of cellulose nanofibers against an inflammatory bowel disease model and their study revealed inhibition of colonic inflammation and reduction of tissue injury.[25] Granulation tissue is one of the parameters used to assess the wound healing process. It is a perfused, flexible connective tissue that develops from a wound's base and can cover incisions of almost any size. Type I collagen gradually replaces the type III collagen network that initially makes up granulation tissue. It forms and contracts as the lesion heals, and both processes are essential for wound recovery.[26]

Epithelialization of the wound, also known as re-epithelialization, is a crucial and distinctive aspect of wound healing. It involves migration, proliferation, and differentiation, which are three intertwined keratinocyte activities. The process begins with the breakdown of cell-cell and cell-substratum contacts. The newly formed epidermis is multilayered, and gene products are induced during differentiation to restore the epidermis' functionality.[27]

Conclusion

The findings of this study demonstrate that the combination of HPMC and carbopol in a gel composition for wound healing results in formulations that satisfy several quality standards for the medicinal product, including pH, spreadability, viscosity, and wound healing activity. While F3 displayed good wound healing activity but low viscosity, F1 demonstrated good physical and rheological qualities with low wound healing activity. F2, on the other hand, demonstrated good physical and rheological properties along with good wound healing activity. □

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

FYA and STI contributed to the conception and design of the study. FYA and STI conducted the experiments. STI analysis the data and writing of the manuscript draft. FYA gave final approval of the article. Both authors read and approved the final manuscript.

Ethics approval

The study was registered and approved by Department of Pharmaceutics, College of Pharmacy, University of Mosul.

Patient consent for publication

Not applicable.

Conflict of Interest: The authors declare that they have no competing interests.

REFERENCES

1. Yu N., Wang X., Ning F., Jiang C., Li Y., Peng H., Xiong H. Development of antibacterial pectin from Akebia trifoliata var. australis waste for accelerated wound healing. *Carbohydr. Polym.* 217, 58-68, 2019.
2. Rousselle P., Braye F., Dayan G. Re-epithelialization of adult skin wounds: Cellular mechanisms and therapeutic strategies. *Adv. Drug Deliv. Rev.* 146, 344-365, 2019.
3. Agubata C.O., Okereke C., Nzekwe I.T., Onoja R.I., Obitte N.C. Development and evaluation of wound healing hydrogels based on a quinolone, hydroxypropyl methylcellulose and biodegradable microfibres. *Eur. J. Pharm. Sci*. 89, 1-10, 2016.
4. Yin H., Song P., Chen X., Huang Q., Huang H.A self-healing hydrogel based on oxidized microcrystalline cellulose and carboxymethyl chitosan as wound dressing material. *Int. J. Biol. Macromol*. 221, 1606-1617, 2022.
5. Kong B.J., Kim A., Park S.N. Properties and in vitro drug release of hyaluronic acid-hydroxyethyl cellulose hydrogels for transdermal delivery of isoliquiritigenin. *Carbohydr. Polym.* 147, 473-481, 2016.
6. Alven S., Aderibigbe B.A. Chitosan and cellulose-based hydrogels for wound management. *Int. J. Mol. Sci.* 21(24), 9656, 2020.
7. Kong W., Huang D., Xu G., Ren J., Liu C., Zhao L., Sun R. Graphene oxide/polyacrylamide/aluminum ion cross-linked carboxymethyl hemicellulose nanocomposite hydrogels with very tough and elastic properties. *Chem. Asian J.* 11(11), 1697-1704, 2016.
8. Jacob S., Nair A. B., Shah J., Sreeharsha N., Gupta S., Shinu P. Emerging role of hydrogels in drug delivery systems, tissue engineering and wound management. *Pharmaceutics*. 13(3), 357, 2021.
9. Tudoroiu E.E., Dinu-Pîrvu C.E., Albu Kaya M.G., Popa L., Anuța V., Prisada R. M., Ghica M.V. An overview of cellulose derivatives-based dressings for wound-healing management. *Pharmaceuticals*. 14(12), 1215, 2021.
10. Al-Bazzaz F.Y., Al-Kotaji M. Ophthalmic in-situ sustained gel of ciprofloxacin, preparation and evaluation study. *Int. J. App. Pharm*.10(4), 153-61, 2018.
11. Hayati F., Ghamsari S.M., Dehghan M.M., Oryan A. Effects of carbomer 940 hydrogel on burn wounds: an in vitro and in vivo study. *J. Dermatol. Treat.* 29(6), 593-599, 2018.
12. Ghanim Z.S., Alkotaji M., Qazzaz M.E. Insight into Topical Preparations for Wound Healing: Traditional and Modern Dressings. *Al-Anbar Medical Journal.* 1;19(2), 2023
13. Paknejad M., Rokn A.R., Eslami B., Afzalifar R., Safiri A. Evaluation of three bone substitute materials in the treatment of experimentally induced defects in rabbit calvaria. *Front. Dent.*171-176, 2017.

14. Gupta S., Vyas S.P. Carbopol/chitosan based pH triggered in situ gelling system for ocular delivery of timolol maleate. *Sci. Pharm*. 78(4), 959-976, 2010.
15. Greener B., Hughes A.A., Bannister N.P., Douglass J. Proteases and pH in chronic wounds. *J. Wound Care.* 14(2), 59-61, 2005.
16. Percival S.L., McCarty S., Hunt J.A., Woods E.J. The effects of pH on wound healing, biofilms, and antimicrobial efficacy. *Wound Repair Regen.* 22(2), 174-186, 2014.
17. Chen X.Y., Low H.R., Loi X.Y., Merel L., Mohd Cairul Iqbal M.A. Fabrication and evaluation of bacterial nanocellulose/poly (acrylic acid)/graphene oxide composite hydrogel: Characterizations and biocompatibility studies for wound dressing. *J. Biomed. Mater. Res. Part B Appl. Biomater.* 107(6), 2140-2151, 2019.
18. Veronica E.F., Dwiastuti R. Formulation and evaluation of wound healing gel of white Leadtree (leucaena LEUCOCEPHALA (lam.) de wit.) leaves extract. *Int. J. App. Pharm*. 14(1), 275-280, 2022.
19. Miastkowska M., Kulawik-Pióro A., Szczurek M. Nanoemulsion gel formulation optimization for burn wounds: Analysis of rheological and sensory properties. *Processes*, 8(11), 1416, 2020.
20. Coates J. Interpretation of Infrared Spectra, A Practical Approach. In: Meyers RA, editor. Encyclopedia of Analytical Chemistry [Internet]. Chichester, UK: John Wiley & Sons, Ltd; 2006 [cited 2023 Feb 26]. p. a5606. Available from: https://onlinelibrary.wiley.com/doi/10.1002/9780470027318.a5606
21. Alkotaji M., Ismail S.T., Alnori H. Nasal In-situ Gel of Inert Cellulose for Allergic Rhinitis. *Trop. J. Nat. Prod. Res.* 6(9), 2022.
22. Arif S., Attiogbe E., Moulin V.J. Granulation tissue myofibroblasts during normal and pathological skin healing: The interaction between their secretome and the microenvironment. *Wound Repair Regen.* 29(4), 563-572, 2021.
23. El Fawal G.F., Abu-Serie M.M., Hassan M.A., Elnouby M.S. Hydroxyethyl cellulose hydrogel for wound dressing: Fabrication, characterization and in vitro evaluation. *Int. J. Biol. Macromol.*111, 649-659, 2018.
24. Baranov N., Popa M., Atanase L.I., Ichim D.L. Polysaccharide-based drug delivery systems for the treatment of periodontitis. *Molecules*. 26(9), 2735, 2021.
25. Azuma K., Osaki T., Ifuku S., Saimoto H., Morimoto M., Takashima O., Minami S. Anti-inflammatory effects of cellulose nanofiber made from pear in inflammatory bowel disease model. *Bioact. Carbohydr. Diet. Fibre.* 3(1), 1-10, 2014.
26. Gurtner G.C., Neligan P.C. Plastic surgery E-Book; Volume 1: Principles. *London: Evier Health Sciences*, 2017.
27. Raja K.S., Garcia M.S., Isseroff R.R. Wound re-epithelialization: modulating keratinocyte migration in wound healing. *Front. Biosci.-Landmark.* 12(8), 2849-2868, 2007.