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Allium cepa: A Natural Enhancer of Wound Closure and Cell Viability in O-Carboxymethyl Chitosan Films

Allium cepa: Potenciador Natural del Cierre de Heridas y Viabilidad Celular en Películas de O-Carboximetil Quitosano

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ABSTRACT

Wound healing and cicatrization after tissue damage are essential for the body's recovery process. Regenerative medicine has been focused on finding biocompatible polymeric materials reinforced with natural active substances, which have healing, anti-inflammatory, and antimicrobial properties. This study aimed to enhance O-carboxymethyl chitosan films with *Allium cepa* extracts, endemic to Chihuahua, Mexico. The extract's properties were analyzed, and the results show the saponin, flavonoid, and terpenoid contents and an antioxidant activity of 70 %. Cicatrization *in vitro* was also studied, and the results show that a 15 mg/mL concentration of the *Allium cepa* extracts improved cellular proliferation and migration in fibroblasts, which was further confirmed in a cellular viability study of the O-carboxymethyl chitosan films loaded with 7 and 20 wt. % of the extract, which showed a higher percentage of cellular viability after three days. The study's results suggest that low extract concentrations can be used as an active ingredient in polymeric biomaterials to aid skin cicatrization.

KEYWORDS: ***Allium cepa,*** **O-carboxymethyl chitosan, tissue regeneration, wound healing**

RESUMEN

La cicatrización de heridas y el daño en los tejidos son fundamentales para la recuperación del organismo. La medicina regenerativa se ha centrado en desarrollar materiales poliméricos biocompatibles, enriquecidos con principios activos naturales, que posean propiedades cicatrizantes, antiinflamatorias y antimicrobianas. Este estudio tuvo como objetivo mejorar el desempeño de películas de O-carboximetil quitosano utilizando extractos de *Allium cepa*, una planta endémica de Chihuahua, México. Se analizaron las propiedades del extracto, encontrando un contenido significativo de saponinas, flavonoides y terpenoides, así como una actividad antioxidante del 70 %. La cicatrización *in vitro* indica que una concentración de 15 mg/mL de extracto de *Allium cepa* potencia la proliferación y migración celular en fibroblastos. Esta mejora se confirmó en un estudio de viabilidad celular de las películas de O-carboximetil quitosano cargadas con 7 y 20 wt. % mg del extracto, las cuales mostraron un mayor porcentaje de viabilidad celular después de tres días. Los hallazgos sugieren que concentraciones bajas del extracto pueden ser utilizadas como ingredientes activos en biomateriales poliméricos, contribuyendo así a la cicatrización de la piel.

PALABRAS CLAVE: ***Allium cepa*, O-carboximetil quitosano, ingeniería tisular, cicatrización**

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INTRODUCTION

In recent years, there has been a growing interest in discovering new materials that can help heal skin wounds. This interest stems, in part, from the limitations of conventional medical products, which are often composed of inorganic materials that may pose the environment and health risks over time. Consequently, researchers are increasingly exploring alternative solutions, particularly natural bioactive substances with healing, anti-inflammatory, and antimicrobial properties.

Chitosan, a natural cationic polysaccharide derived from deacetylation of chitin, has garnered significant attention as a biomaterial. Its exceptional properties, including biocompatibility, bioadhesiveness, antimicrobial, and cicatrization capabilities, make it an ideal candidate for wound healing and tissue regeneration[1]. Among its derivatives, O-carboxymethyl chitosan (OCMC) has been widely studied due to its water solubility and enhanced performance[2]. For example, in 2002, Chen and his colleagues demonstrated that OCMC promotes fibroblast collagen proliferation and secretion[3]. Additionally, Weng *et al.* reported that hydrogels composed of oxidized dextran and an OCMC derivative accelerated the healing of skin wounds in mice[4]. Natural extracts have also been extensively investigated for their potential therapeutic applications. One such extract, derived from *Allium cepa*, has been used in traditional medicine due to its rich composition of bioactive compounds. *Allium cepa* exhibits diverse pharmacological effects, including antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic, antidiabetic, and cardiovascular benefits[5]. The plant primarily consists of water, lipids, carbohydrates, fibers, potassium, sulfur, phosphorous, calcium, and vitamins such as C, E, B6, folic acid, glutamic acid, arginine, lysine, and leucine. Furthermore, its secondary metabolites (phenolic acids, terpenes, alkaloids, phenylpropanoids, and flavonoids) play a vital role in its medicinal properties[6].

This paper presents a study on the incorporation of *Allium cepa* extract into OCMC films to create a bioactive material for skin regeneration. The innovative combination leverages the bioactive compounds of *Allium cepa* and the inherent properties of OCMC, offering a promising solution for wound healing applications.

MATERIALS AND METHODS

Allium cepa extract

The bulbs of *Allium cepa* were sourced from Delicias, Chihuahua, Mexico. The bulbs were sectioned and frozen for 24 hours before being lyophilized and ground into a fine powder. A 10 g sample of the powdered *Allium cepa* was mixed with 130 ml of 80% methanol (Methanol Certified ACS, Fisher Chemical) and stirred for two hours at room temperature. The resulting mixture was centrifuged at 3400 rpm for 10 minutes to collect the supernatant. This process was repeated three times to obtain a concentrated supernatant. To remove the solvent, the extract was evaporated at room temperature, yielding the *Allium cepa* extract (ACE). The chemical composition of the extract was analyzed using Fourier-transform infrared spectroscopy (FT-IR). Samples were prepared by cutting and drying the material and analyzed at room temperature in the spectral range of 4000 to 500 cm^{-1}. Each spectrum was recorded using 100 scans at a resolution of 16 cm^{-1}.

Metabolite quantitative identification

The Salkowski test was conducted to identify the presence of saponins. In a Pyrex test tube, 5 mL of ACE was combined with 2 mL of chloroform and stirred for 15 seconds. Subsequently, 3 mL of sulfuric acid was added, and the

mixture was shaken for 10 minutes. The presence of saponins was indicated by the formation of foam on the surface, accompanied by a reddish-brown coloration. The saponin content was classified as follows, abundant with a foam height >14 mm; moderate content, foam height between 10 and 14 mm; low content, foam height <10 mm. Flavonoid identification involved mixing 3 mL of ACE with 15 drops of a 50% NaOH solution. The mixture was left to stand until a yellow coloration appeared, after which concentrated HCl was added dropwise until the solution became clear. The intensity of the yellow color in the alkaline medium reflected the flavonoid concentration. An intense yellow color indicates a high concentration of flavonoids, while a fainter color indicates a lower concentration. When HCl is added at high concentrations, a rapid and noticeable disappearance of yellow color further confirmed the flavonoid presence. Alkaloid identification was performed using Dragendorff reagent. For each test, 3 mL of liquid extract was used, with the test conducted in triplicate. The reagent was added dropwise until a precipitate formed. The color of precipitate indicated alkaloid concentration: a reddish precipitate indicated a high concentration, while a cream-colored precipitate indicated a low or moderate concentration. The criteria for the qualitative assessment of these metabolites are summarized in Table 1[7].

TABLE 1. Defined Criteria for Qualitative Metabolite Testing.

Criteria	Yardstick
Abundant presence	+++
Moderate presence	++
Low presence	+
Absence	-

Quantification of antioxidant activity

A 150 μmol/L methanolic dilution of DPPH was prepared. Aliquots of 100 μL of methanolic ACE at a 75 mg/mL concentration were collected in triplicate, and 200 μL of the prepared DPPH dilution was added to each. The mixture was incubated in the dark at room temperature for 30 minutes. After the incubation time, the absorbance was measured at 517 nm using a Benchmark Plus Microplate Reader spectrophotometer at various time intervals. A blank was prepared by mixing an equal volume of DPPH solution with methanol.

The antioxidant activity of the ACE was assessed by calculating the percentage of DPPH radical inhibition at a concentration, using the following equation 1:

$$\% \text{ Inhibition} = \frac{Ac - As}{Ac} \times 100 \quad (1)$$

Ac= Control's absorbance t=0

As= Sample's absorbance t=30 minutes

Cell viability

ACE at concentrations of 15, 50, and 75 mg/mL was cultured with 50,000 3T3 murine fibroblast cells in 24-well plates, and 3 mL of DMEM containing 10 % fetal bovine serum (FBS) and 3 % antibiotics was added. The culture

was incubated at 37°C and 5% CO_2 for 24 and 72 hours. Afterward, the medium was removed from the wells, and 200 μL of DMEM and 50 μL of MTT solution (5 mg/mL in 1X PBS) were added. The plate was incubated at 37 °C and 5 % CO_2 for 1 hour. After incubation, the solution was removed from the wells, and 50 μL of Dimethyl Sulfoxide (DMSO) was added to each well to dissolve the formazan crystals synthesized by the cells. The absorbance readings were taken using a Benchmark Plus Microplate Reader spectrophotometer at 570 nm. The tests were performed in triplicate, and images were captured during the evaluation. The process was conducted using a Carl Zeiss Optical Microscope. The percentage of cell viability is given by Equation 2:

$$\text{Cell viability } (\%) = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \tag{2}$$

Cicatrization activity *in vitro*

50,000 3T3 fibroblasts were added to 6-well plates with 3 ml of DMEM containing 2 % FBS. The plates were then incubated at 37 ^{0}C with 5 % CO2 until the cells attached. Once the cells covered the entire surface, a wound was made under sterile conditions with a micropipette. Next, 3 ml of DMEM mixed with ACE at varying concentrations was added to each condition. The plates were then incubated at 37 ^{0}C with 5% CO2. Images were taken at 0, 2, 4, 24, and 26 hours using a Zeiss Axio Vert A1 microscope. This entire process was performed in triplicate for extracts. The concentration of mitomycin used was 10 μg/mL to prevent cell migration. Wound closure was calculated using the following equation 3:

$$\text{Percentage of wound closure } (\%) = \frac{\text{At0} - \text{At}}{\text{At0}} \times 100 \tag{3}$$

At0 represents the wound length at time zero, while At represents the wound length later in the analysis.

Cell adhesion was observed and assessed using confocal microscopy. After culturing the cells for up to 72 hours, the cell nuclei were stained blue by incubating them in a PBS solution containing 4′,6-Diamidine-2′-phenylindole dihydrochloride (DAPI) (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA). Samples were incubated in the staining solution for 1 hour and then observed using fluorescence microscopy (Axio Vert. A1, Carl Zeiss), with the ZEN Blue software used to capture the images.

OCMC synthesis

OCMC was produced using Valencia-Gomez's method[1]. Firstly, 50 mL of isopropanol (Sigma-Aldrich) was placed under continuous stirring at room temperature. Then, 5 g of chitosan (Medium Molecular Weight Sigma-Aldrich) was added and stirred at a constant speed for 2 hours at room temperature. After that, 20 mL of a 40 % w/v solution of sodium hydroxide NaOH (J.T. Baker) was added every 15 minutes to 80 mL. The mixture was stirred for 30 more minutes. Then, 100 mL of a monochloroacetic acid solution (Sigma-Aldrich, 99 %) was added, previously prepared by dissolving 25.5 g of monochloroacetic acid in 100 mL of isopropanol. The reaction continued for another 24 hours at room temperature and constant stirring. Then 200 mL of ethyl alcohol was added to the mixture, and the resulting solid was filtered out. The solid was then washed six times with methanol (Methanol Certified ACS, Fisher Chemical), using 200 mL portions between each wash to eliminate the NaCl salt formed as a byproduct of the reaction. The resulting OCMC was placed inside a laminar flow hood at room temperature until completely dry.

OCMC/ACE Film

Two grams of OCMC were mixed in 100 milliliters of deionized water (w/v) while stirring constantly at room temperature for 20 minutes. Following this, 1 milliliter of glycerin (Sigma-Aldrich) was added under constant stirring for another 10 minutes. Finally, 2 milligrams of genipin (Challenge Bioproducts Co., Ltd.) were added. Immediately, 25 milliliters of the solution were poured into each petri dish and left at room temperature until the solvent had evaporated entirely. For the OCMC films, the ACE was added after the OCMC had been added to the solution. The freeze-dried extract concentrations used for functionalization were 7 and 20 % by weight (Table 2). A film containing 20 wt. % of quercetin was used as a control, as it is one of the most relevant flavonoids in ACE.

TABLE 2. **Concentrations of film components**

Film	OCMC (wt. %)	ACE (wt. %)	Quercetin (wt.%)
OCMC	100	0	0
OCMC/ACE/7	93	7	0
OCMC/ACE/20	80	20	0
OCMC/Q	80	0	20

Film characterization: FT-IR and SEM

The FT-IR characterization of the films was conducted at room temperature in the range of 4000 to 500 cm^{-1}. The samples were cut and dried before analysis using a Thermo Nicolet 6700 FT-IR spectrometer with germanium glass, in ATR mode. All spectra were recorded using 100 scans and 16 cm-1. SEM (Scanning Electron Microscopy) is a valuable technique for surface analysis. The test utilized a Hitachi Field Emission Scanning Electron Microscope (FESEM) with an accelerating voltage of 5 KV. The sample size used for this technique was 2 X 2 cm.

OCMC/ACE film cell viability

Films were cut into 0.5 cm × 0.5 cm squares and sterilized under UV light for 15 minutes. The sterile films were placed in 24-well plates, and 50,000 3T3 fibroblasts were added to each well, along with 3 mL of DMEM containing antibiotics and FBS. The plates were incubated at 37 °C with 5 % CO_2. After 24 and 72 hours of incubation, the medium was removed, and 200 μL of DMEM and 50 μL of MTT solution were added. The plate was incubated for 1 hour, after which the solution was removed, and 50 μL of DMSO was added to dissolve the formazan crystals. Absorbance was measured at 570 nm using a Benchmark Plus microplate reader. The analysis was conducted in triplicate, using wells containing only cells without material as control. The cell viability percentage was calculated using Equation 2. The cells adhered to the films were fixed with a 3 % glutaraldehyde solution. The films were washed three times with deionized water, and hematoxylin was added for 6.5 minutes. After this time, the hematoxylin was completely removed, and the films were washed three times with deionized water, followed by acid alcohol, and finally washed with deionized water again. Following this, eosin was applied to completely cover the films, and the samples were left to rest for 6.5 minutes. Additional washes were then performed with 70 %, 96 %, and 100 % ethanol solutions. The fibroblasts were observed using a Zeiss AXIO Vert.A1 microscope.

RESULTS AND DISCUSSION

Allium cepa extract characterization: FT-IR

Figure 1 illustrates the infrared spectrum of the obtained extract. The bands at 3300 and 2928 cm^{-1} are attributed to the presence of O-H and C-H groups, respectively[8]. Additionally, a band appears at 1618 cm^{-1}, which may corre-

spond to the C=C stretching of the aromatic ring in combination with the C-C stretching attributed to the phenyl group. This suggests the presence of high levels of polyphenolic components[9]. The band at 1648 cm^{-1} is attributed to amide I, while the bands at 1405 and 1740 cm^{-1} are assigned to the asymmetric deformation of CH_3 and the stretching of the carbonyl group (C=O), respectively. The band at 1340 cm^{-1} is due to C-O stretching in combination with phenyl ring stretching, while the band at 1255 cm^{-1} is assigned to amide III (random coil conformation) in proteins[10]. The stretching band at 1105 cm^{-1} is attributed to C-OH groups, while the bands at 1025, 985, and 868 cm^{-1} are related to -CH_2OH, OCH_3, and CH groups, respectively[10]. These results suggest that ACE and the powder contain phenolic compounds, alkaloids, and terpenoids.

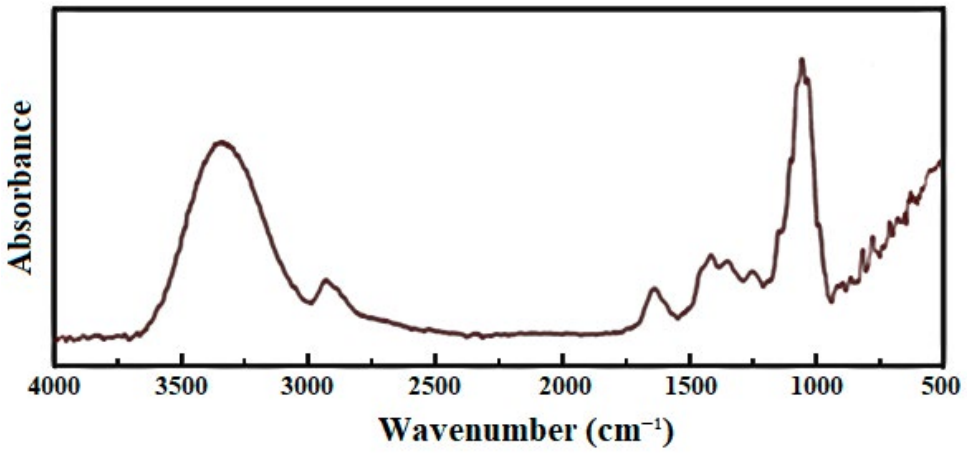

FIGURE 1. **Infrared spectrum of ACE.**

Qualitative analysis to identify saponins, flavonoids, and alkaloids in the ACE

Previous studies have indicated that certain plant families, such as monocotyledons including *Dioscoreaceae, Agavaceae*, and *Liliaceae* (including *Allium* plants), contain saponins[11], which are high-molecular-weight glycosides consisting of a sugar moiety attached to a triterpene or steroidal aglycone. Saponins have properties that are beneficial for skin regeneration, as they can act as antimicrobial, healing, and anti-inflammatory agents[11]. Table 3 summarizes the identification test results for saponins, alkaloids, and flavonoids. The qualitative test to identify alkaloids in the ACE yielded positive results, indicating a high concentration of alkaloids. R. Pérez *et al.*[12] have mentioned various benefits of alkaloids, including their considerable antioxidant action and antimicrobial, cytotoxic, and anti-inflammatory activity.

TABLE 3. **Results of Metabolites in ACE.**

Metabolite	ACE
Saponins	++
Flavonoids	+++
Alkaloids	+++

Antioxidant activity by free radical DPPH

The ACE was studied for its antioxidant effect using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical method. This method uses a stable free radical with a maximum absorbance of 517 nm. Some plant derivatives can act as

hydrogen donors, preventing or delaying the development of degenerative diseases. Such derivatives include tannins, flavonoids, lignans, quinones, phenolic acids, catechins, and anthocyanins[13].

During the test, the degree of discoloration is an indication of the antioxidant potential of the extract. This study examined the free radical scavenging potential of the ACE at different time intervals ranging from 1 to 5 hours. The final result of the test is depicted in Figure 2. According to Figure 2, the ACE exhibits a high antioxidant effect from the first hour, with an antioxidant activity percentage of 66.02 %. This percentage increases significantly in the fifth hour when the percentage of antioxidant activity is 70.86 %.

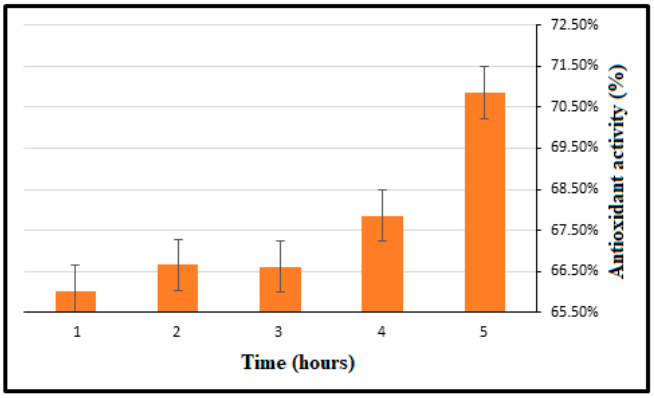

FIGURE 2. **Percentage of antioxidant activity of ACE at a concentration of 75 mg/L.**

Cell viability and *in vitro* wound closure of ACE

Figure 3 shows cell proliferation at different concentrations of ACE seeded with fibroblasts on the first and third days of culture. No significant difference in cell numbers was observed between these periods of time across the different concentrations of ACE. However, cell viability percentages greater than 100 % were observed. The cell viability study revealed that the different concentrations of extract maintained a viability percentage above 70 %, classifying it as non-toxic according to ISO 10993-5:2009 - Biological Evaluation of Medical Devices.

Figure 3 (II) presents images obtained after 72 hours of culture of fibroblast cells seeded with different concentrations of ACE. The images, captured using fluorescent staining (DAPI), show the nuclear material of viable cells in blue[14]. DAPI-stained nuclei display a well-defined outline, with shapes ranging from rounded to elongated, which is indicative of healthy cells, including those in the process of mitosis[15].

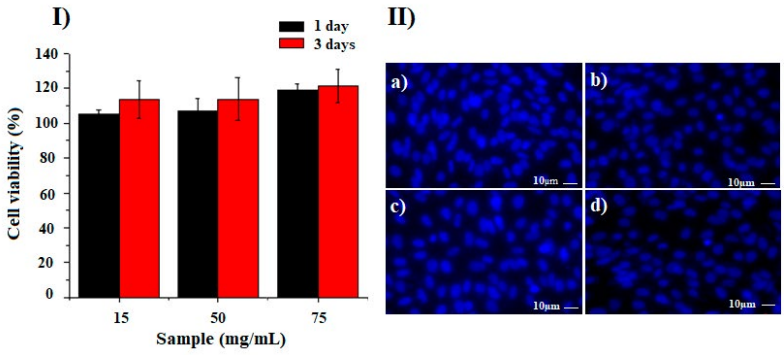

FIGURE 3. **Cell viability percentage at 24 and 72 hours, and II) Cell morphology at 72 hours of a) 15 mg/mL of ACE, b) 50 mg/mL of ACE, and c) 75 mg/mL of ACE seeded with fibroblastic cells.**

The effect of the extract on cell healing was evaluated *in vitro* using the wound healing assay. Figure 4 shows that the extract had no significant effect on wound closure at 2 hours, but after 4 hours, cell numbers were increased in the sample containing 15 mg/mL compared to the control. The wound was completely closed at 24 hours. While the control group showed noticeable wound closure after 24 hours, it still had a partially open wound after 26 hours. The percentage decrease in the wound area was calculated for each concentration and incubation time. The calculations showed that at 4 hours, the closure percentage was very similar in the presence of 15 mg/mL of extract (25 %) and in the control, which had no extract (18 %). At 24 hours, the highest closure percentage was observed with the sample containing 15 mg/mL of extract (90 %), compared to 50 and 75 mg/mL. The wound was first completely closed in the presence of the extract at 15 mg/mL, while in the absence of the extract, full closure occurred at 26 hours. These results demonstrate that the extract is not toxic to cells at concentrations up to 75 mg/mL.

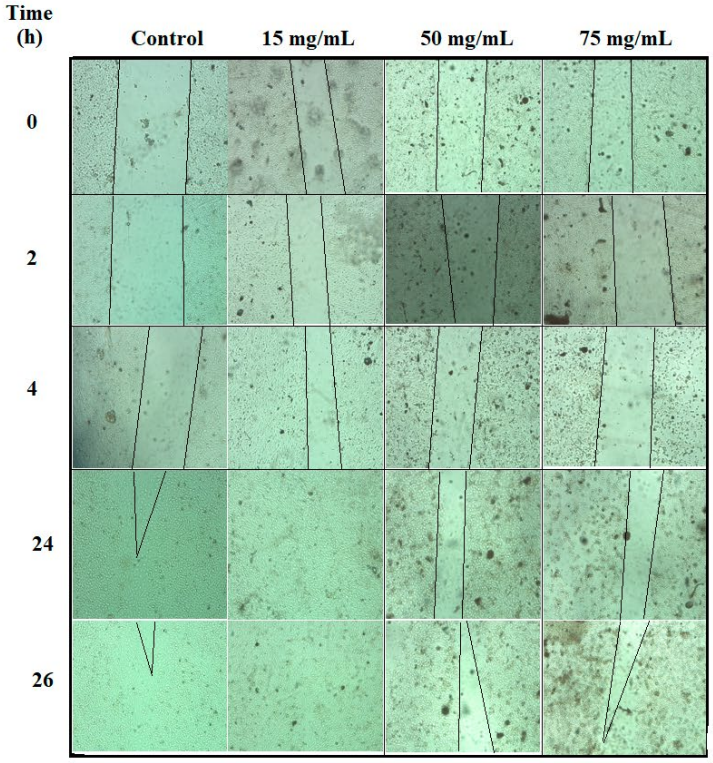

FIGURE 4. **Tear test with fibroblasts for 26 hours, a) control, b) 15 mg/mL of ACE, c) 50 mg/mL of ACE, and d) 75 mg/mL of ACE.**

Significant amounts of saponins, flavonoids, carbohydrates, and, to a lesser extent, alkaloids were found in ACE. Various studies have shown that flavonoids and phenolic acids can facilitate different stages of wound healing by stimulating collagen synthesis, cell proliferation, and angiogenesis[16][17][18]. This is due to their redox potential, which allows them to act as oxygen scavengers and reducing agents, thus reducing free radical stress and preventing oxidative damage, thereby offering the therapeutic potential to accelerate the healing process. Additionally, they modulate signaling pathways, activating endogenous mechanisms that increase antioxidant enzyme levels and progressively promote fibroblast growth, leading to faster wound closure.

During the healing process, complex cellular and molecular mechanisms regulate the inflammatory, proliferative, and maturation phases. Therefore, accelerated healing is attributed to the synergistic action of the various bioactive compounds present in extracts such as ACE[19]. Thus, the results obtained in this study indicate that the use of

a concentration of 15 mg/mL, may reflect the optimum combined effect of all the molecules present in ACE resulting in a faster wound closure process, likely due to its antioxidant, antibacterial, and anti-inflammatory properties. However, results obtained at higher concentrations, as reported in other studies, could be the result of an imbalance between free radical generation and antioxidant activity, leading to excessive oxidative stress in cells or the inhibition of signaling pathways involved in cell migration, factors that could slow or inhibit wound closure[17]. Additionally, other studies have reported cytotoxic effects of high saponin concentrations in certain cell lines[16].

Chemical characterization of O-carboxymethyl chitosan films with ACE

In Figure 5, the spectrogram displays bands corresponding to the stretching vibrations of O-H groups around 3350, at 2928 cm-1 to C-H stretching. Other bands can be seen around 1600 and 1410 cm^{-1}, which can be attributed to the asymmetric and symmetric stretching of C=O groups. Bands around 1320, 1050 cm^{-1}, and 850 cm^{-1} are also observed, related to C-H, C-O, and C-H groups, respectively[1][9]. No significant differences were observed between the spectra, regardless of the ACE concentration. This may be because the characteristic bands of the extract overlap with the absorption bands present in the OCMC.

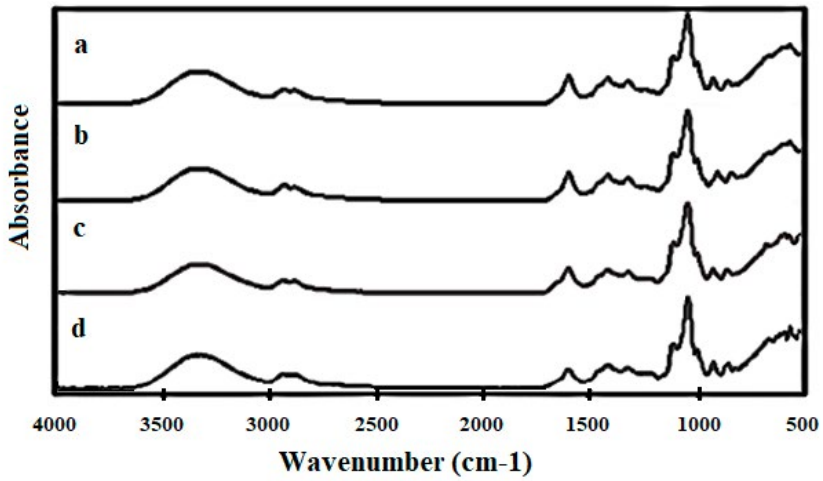

FIGURE 5. **Spectrogram obtained for the films of a) OCMC, b) OCMC/ACE/7, c) OCMC/ACE/20, and d) OCMC/Q.**

Morphological surface analysis by SEM

Four SEM images were taken using a scanning electron microscope as shown in Figure 6. The surface of different concentrations of ACE and commercial quercetin were observed. The results demonstrate that the OCMC films without ACE and those containing 7 wt. % of ACE and 20 wt.% of quercetin had a smooth surface without any irregularities. However, the film containing 20 wt. % of ACE displayed an irregular surface with extract particles.

Cell viability activity of films

Figure 7 shows the cell viability results for four synthesized films. The t-student test shows a statistically significant difference in cell viability after 24 hours between the OCMC film without ACE and the OCMC film with 20 wt. % of ACE and 20 wt. % of commercial quercetin. After 72 hours, there is a statistically significant difference in cell viability between the OCMC film with commercial quercetin and the other three films. Based on these results, it can be inferred that ACE or commercial quercetin improves cell viability in the first 24 hours. Still, after 72 hours, there is no significant difference in cell viability between the OCMC film with or without ACE. However, the

presence of quercetin at 72 hours does not have a positive effect on cell viability. It is important to note that none of the films were found to be cytotoxic.

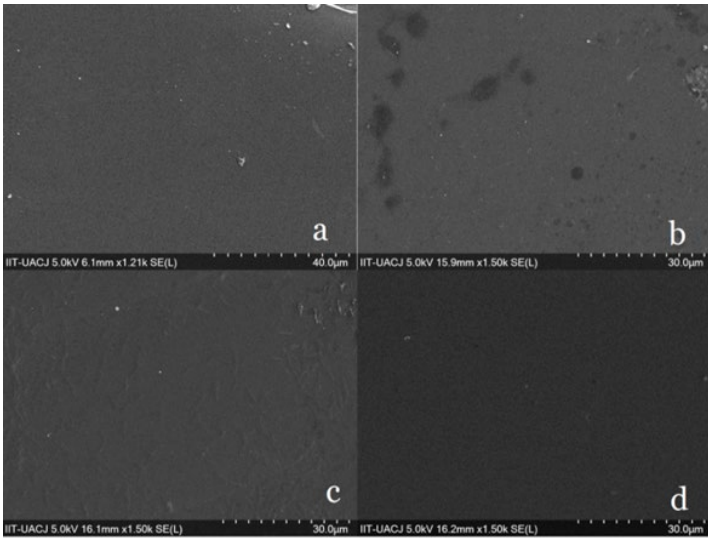

FIGURE 6. **SEM images were obtained from the surface morphology of the films of a) OCMC, b) OCMC/ACE/7, c) OCMC/ACE/20, and c) OCMC/Q.**

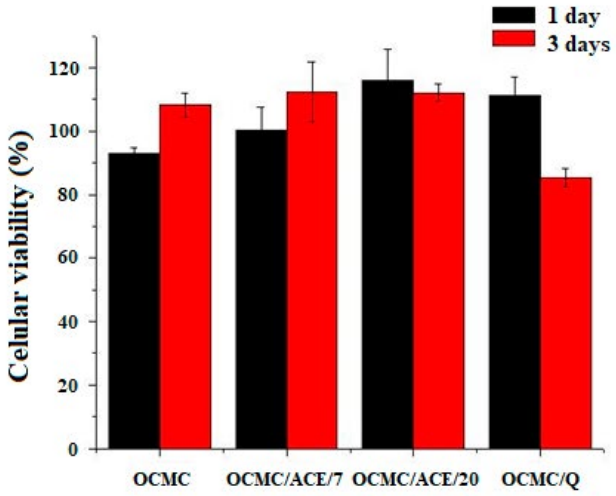

FIGURE 7. **The cytotoxicity of films synthesized from OCMC and ACE or commercial quercetin in 3T3 fibroblasts was measured through the MTT assay.**

Eosin and hematoxylin were used to stain cultured fibroblasts and observe their morphology. Figure 8 displays the cells cultured with synthesized film samples after 72 hours. The images show cells with an elongated shape, exhibiting pink cytoplasm and dark purple nuclei in all the samples. Uniform fibroblast growth is visible in the OCMC film (Figure 8a) and the OCMC film with 7 wt. % of ACE (Figure 8b). However, in the OCMC film with 20 wt .% of ACE (Figure 8c) and the OCMC film with 20 wt. % of commercial quercetin (Figure 8c), blank spots can be observed where cells do not grow. Furthermore, Figure 8b shows many junctions between cells, indicating that small concentrations of ACE can benefit cell proliferation.

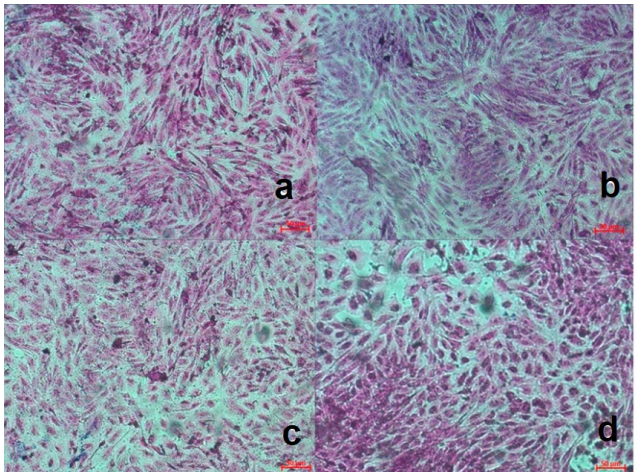

FIGURE 8. Observation of fibroblasts under an optical microscope after 72 hours of incubation. The fibroblasts were stained with hematoxylin and eosin and were seeded in four different conditions: a) OCMC, b) OCMC/ACE/7, c) OCMC/ACE/20, and d) OCMC/Q.

Previous studies have reported that the cellular viability of fibroblasts depends on both the incubation time and the concentration of EPA or quercetin. Low or moderate concentrations can promote fibroblast proliferation, while high concentrations may inhibit it. An increase in quercetin concentration is associated with a decrease in mTOR protein levels, a protein that plays a crucial role in cellular biological processes such as proliferation and apoptosis. Additionally, quercetin regulates the biological behavior of fibroblasts by inhibiting the expression of the migration-associated protein α-SMA and the production of collagen types I and III[20][21].

CONCLUSIONS

The results of this study demonstrate that *Allium cepa* extract at a concentration of 15 mg/mL significantly accelerated wound healing by promoting wound closure within 24 hours and enhancing fibroblast proliferation and migration during the assay. In contrast, higher concentrations of the extract (50 and 75 mg/mL) required more than 26 hours to achieve wound closure. The cell viability study revealed that the extract maintained a viability percentage greater than 70 %, classifying it as non-toxic. Additionally, films containing 7 % and 20 % (wt.) of the extract exhibited cellular viability exceeding 100 %, indicating enhanced cell growth and activity. These findings highlight the potential of *Allium cepa* extract and its polymeric films as promising materials for supporting tissue regeneration and wound healing applications.

AUTHOR CONTRIBUTIONS

L. E. V. G. conceptualization, methodology, validation, data curation, and writing—original draft; C. A. R.-G. methodology, investigation, and validation; M. A. V. methodology and investigation; F. M. C. methodology and investigation; K. R. L. methodology and investigation; J. F. H.-P. conceptualization, validation, and formal analysis; H. R. B. methodology and visualization; J. S. V. data curation and review & editing; I. O.-A. project administration, conceptualization, methodology, validation, resources, supervision, writing—review & editing.

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