UNIVERSITY OF MEDICINE AND PHARMACY

"CAROL DAVILA", BUCHAREST

DOCTORAL SCHOOL

PHARMACY FIELD

Development of topical biopolymeric supports with controlled drug release for healing of some cutaneous lesions

ABSTRACT OF THE DOCTORAL THESIS

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INTRODUCTION

The increasing incidence of acute traumatic and thermal skin injuries generates considerable costs for healthcare systems, highlighting the need for modern, efficient, and minimally invasive therapeutic solutions for wound management [1].

The novelty of this doctoral thesis lies in the development of an advanced therapeutic dressing, which, through a single intelligent formulation, integrates both a drug delivery system based on naproxen (a non-steroidal anti-inflammatory drug) and biopolymers with proven potential in tissue regeneration (collagen, methylcellulose, and hydroxyethylcellulose) in a well-defined ratio ensuring maximum biocompatibility, as well as pH-sensitive materials for monitoring wound healing.

The thesis is structured into two main parts. *The general-theoretical section* (Chapters 1-2) provides a comprehensive overview of acute skin injuries and modern therapeutic approaches, with particular emphasis on the use of collagen and cellulose derivatives - methylcellulose and hydroxyethylcellulose as biopolymeric supports in the formulation of advanced dressings. Furthermore, it describes modern experimental statistical design methods, including factorial designs, response surface methodology, and Taguchi technique, used for formulation optimization.

The personal contributions section (Chapters 3-6) presents the experimental stages related to: (1) the preparation and characterization of methylcellulose and hydroxyethylcellulose-based hydrogels to select suitable grades and concentrations of these polymers; the further formulation of efficient naproxen delivery systems based on hydroxyethylcellulose and various penetration enhancers for application to acute soft tissue injuries; (2) the development of advanced biopolymeric supports in the form of hydrogels and their lyophilized forms (spongious matrices) based on collagen and methylcellulose or hydroxyethylcellulose, for determining the optimal mixing ratio; the design, optimization, and subsequent characterization of collagen-hydroxyethylcellulose spongious matrices incorporating naproxen, using Box–Behnken experimental design coupled with response surface methodology and Taguchi technique, followed by *in vivo* testing of the optimal formulations to evaluate their anti-inflammatory and wound-healing effects in post-burn injuries; (3) the development of intelligent pH-sensitive films with a dual role: *therapeutic* (accelerating healing through the controlled release of naproxen from the biopolymeric support) and *diagnostic* (visual monitoring of post-burn wound status through color changes of a pH indicator - phenol red).

I. GENERAL SECTION

1. CUTANEOUS LESIONS. WOUND DRESSINGS AS THERAPEUTICAL OPTIONS

Skin injuries represent a major public health issue, with a significant socio-economic impact [2]. The multidisciplinary management of skin injuries is essential for restoring the functionality and appearance of the skin. Recent technological advances have enabled the development of modern dressings tailored to various types of wounds [3].

A classification of dressings includes *passive* and *active* dressings, characterized by the presence or absence of one or more pharmacologically active substances, as well as *traditional* and *modern* dressings [4]. Modern bioactive dressings are considered advanced drug delivery systems (DDS). Topical DDS address several limitations of conventional formulations by delivering active agents directly to the site of skin injury, thereby ensuring a localized therapeutic effect [5]. Modern dressings include hydrogels and their lyophilized forms (spongious matrices), hydrocolloids, films, semipermeable foams, and alginate-based dressings [4]. Alongside DDS, the development of intelligent dressings represents a critical advancement in recent medical technology innovations [6]. The pH value at the wound site plays a crucial role in wound management, as it serves as an accurate indicator of the healing process [7]. In recent years, pH-sensitive dressings, such as multifunctional biopolymeric films, have been developed. These combine essential properties for post-burn wound healing: a *therapeutic* function (controlled release of active substances, promoting tissue regeneration) and a *diagnostic* function (real-time monitoring of pH changes at the wound site). This dual functionality offers a significant advantage for guiding therapy, with major potential for efficient and personalized wound management.

Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently prescribed to alleviate pain and inflammation associated with wounds. Because of the side effects associated with oral administration, a viable alternative is the topical application of these drugs [8]. Despite its analgesic and anti-inflammatory properties, naproxen has been less extensively explored for the development of topical drug delivery systems. In this context, among the NSAIDs, naproxen was selected in this thesis as a model drug to assess its therapeutic potential in wound healing through the administration of various topical formulations for acute lesions of different etiologies.

2. BIOPOLYMERS AS TOPICAL SUPPORTS IN THE DESIGN OF MODERN CONTROLLED DRUG RELEASE DRESSINGS

In this study, *collagen* and *semi-synthetic cellulose derivatives* were selected for the development of topical polymeric systems intended for the treatment of acute skin injuries.

Collagen is a natural biopolymer [9] widely used in the medical field due to its role in wound healing and its versatility in being processed into various forms [10]. However, its low mechanical strength and limited stability are major drawbacks, which can be overcome by combining it with other biopolymers, such as cellulose derivatives, to enhance its physicochemical properties, thereby enabling the obtaining of modern biopolymeric systems for advanced wound therapy [11].

Ethereal cellulose derivatives (sodium carboxymethylcellulose - NaCMC, hydroxypropylmethylcellulose - HPMC, methylcellulose - MC, hydroxyethylcellulose - HEC, and hydroxypropylcellulose - HPC) are employed in wound treatment due to their advantageous properties [12], as demonstrated by numerous studies. Among these, NaCMC and HPMC are the most frequently used as biopolymeric materials for the development of potential dressings. Meanwhile, MC, HEC, and HPC, though less extensively studied, show promising potential for the design of such biopolymeric systems for wound treatment [13].

The development of biopolymer-based dressings for the topical delivery of drugs is a process that can be optimized through the application of an experimental strategy focused on modeling and controlling formulation parameters, known as statistical experimental design [14, 15]. Various experimental designs are employed to investigate the simultaneous effects of formulation factors and their interactions on system responses, as well as to generate predictive mathematical models. In the development of topical biopolymeric drug delivery systems aimed at regenerating tissue damaged by skin injuries, multiple experimental designs combined with response surface methodology have been used [16], enabling efficient decision-making that improves the quality, efficacy, and safety of the pharmaceutical formulation [17]. A highly advanced method for robust experimental design that allows the product and process optimization, while simultaneously improving product quality and ensuring process reliability, is Taguchi technique. This method is based on the use of a specific performance indicator, known as the signal-to-noise (S/N) ratio [18-20]. Integrating classical experimental design with data analysis by response surface methodology and Taguchi technique using the S/N ratio enables the identification of formulation factor combinations that not only yield optimal results but also ensure stable responses that remain resilient to variations caused by noise factors [21, 22].

II. PERSONAL CONTRIBUTIONS

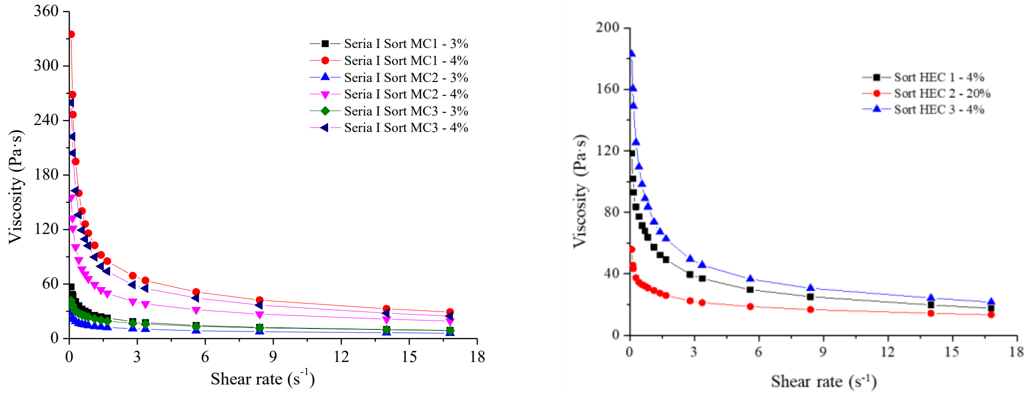
3. RESEARCH HYPOTHESIS AND OBJECTIVES

The **primary objective** of this doctoral thesis is the development of active topical biopolymeric supports (hydrogels, spongious matrices, and films) based on collagen and cellulose derivatives, designed to enable the controlled release of naproxen in order to reduce inflammation and pain, as well as to accelerate tissue regeneration in acute traumatic and thermal skin injuries. The general objectives include: (i) selection of cellulose derivatives for the formulation of hydroxyethylcellulose-based hydrogels containing penetration enhancers, followed by *in vitro/in vivo* characterization; (ii) evaluation of the compatibility of collagen with methylcellulose and hydroxyethylcellulose through physicochemical and biological characterizations; (iii) design, optimization, and physicochemical, biopharmaceutical, and biological characterization of collagen-hydroxyethylcellulose spongious matrices loaded with naproxen; (iv) *in vivo* validation in animal models of the performance of the optimized spongious matrices selected, using a modern experimental design; (v) development of intelligent pH-sensitive films capable of visually monitoring the healing of post-burn wounds while promoting skin tissue regeneration; (vi) dissemination of results through the publication of scientific articles, book chapters, the filing of a patent application, and participation with oral communications / posters at national and international scientific events.

4. DEVELOPMENT OF BIOPOLYMERIC HYDROGEL SUPPORTS FOR THE RELEASE OF AN ANTI-INFLAMMATORY DRUG FOR HEALING OF ACUTE TRAUMATIC LESIONS

This chapter focuses on the development of biopolymeric hydrogel supports for the controlled release of naproxen, aimed at promoting the healing of acute traumatic soft tissue injuries characterized by a highly complex inflammatory process. In the ***first stage of this study***, methylcellulose and hydroxyethylcellulose hydrogels were prepared at various concentrations and rheologically characterized in order to identify formulations with suitable flow properties, which would serve as a starting point for the development of new biopolymeric supports for the controlled release of the anti-inflammatory drug.

Rheological analysis revealed that the viscosity of MC hydrogels (divided into two series, I and II) and HEC hydrogels decreased with increasing shear rate, indicating pseudoplastic, non-Newtonian behavior at 23°C. This flow behavior is a key quality requirement for topical semisolid systems, as it facilitates hydrogel spreading and proper application to the skin surface [23]. Figure 4.1a,b illustrates, as examples, the viscosity-shear rate rheological profiles for a selection of MC and HEC hydrogels.



a) b)

Fig. 4.1. Viscosity versus shear rate rheograms for the: a) MC hydrogels from series I; b) HEC hydrogels

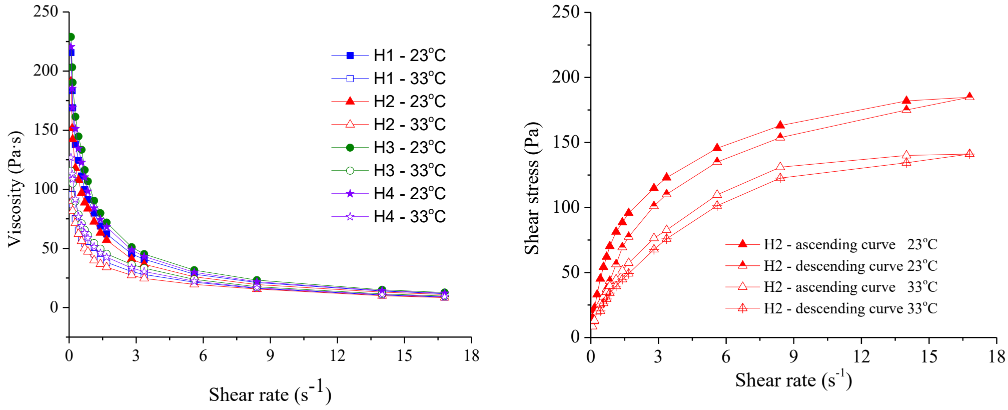
The MC hydrogels from both series, as well as the HEC hydrogels at the selected concentrations, exhibited an adequate degree of pseudoplasticity. Another quality criterion targeted in the design of semisolid formulations was thixotropic behaviour, observed in several of the prepared hydrogels, specifically, in all MC hydrogels from series I, part of those from eries II, and in all HEC hydrogels.

Considering visual aspects, the gelation concentration, degree of pseudoplasticity, thixotropic behaviour, as well as the technological and biopharmaceutical implications of the hydrogels (casting, handling, spreading), MC2 and HEC3 grades were selected, at concentrations ranging from 3 to 3.5%, as topical biopolymeric supports for the subsequent development of new dressings aimed at regenerating skin tissue affected by lesions of various etiologies.

In the ***second stage of the study***, six hydrogels (noted H1-H6) were designed for the first time, based on 3% HEC, incorporating naproxen at concentrations of 5 (H5), 7.5 (H6), and 10% (H1-H4), along with four penetration enhancers at different concentrations (Transcutol® P, PEG 200, ethanol, and isopropanol) in a mixture representing 40% of the total gel mass. This study resulted in a patent application, A / 00639 of 31.10.2023, published in BOPI no. 4/2024, RO138082A0 [24].

Figure 4.2a illustrates, as examples, the cumulative viscosity-shear rate rheograms corresponding to hydrogels H1-H4, tested at 23°C and 33°C. Rheological analysis demonstrated that the designed hydrogels exhibited pseudoplastic, non-Newtonian shear-thinning behaviour at both tested temperatures, as evidenced by a decrease in viscosity with increasing shear rate. All formulations displayed an adequate degree of pseudoplasticity at both temperatures, with the *n* flow index, determined using the Power Law model, ranging between 0.35 and 0.42. Regarding the *m* consistency index, the results highlight the influence of temperature on this rheological parameter, as values at 33°C were 1.57-1.75 times lower compared to those recorded at 23°C.

To evaluate thixotropic behavior, Figure 4.2b shows, as examples, the ascending and descending curves of the shear stress-shear rate rheological profiles for hydrogel H2 tested at 23°C and 33°C.

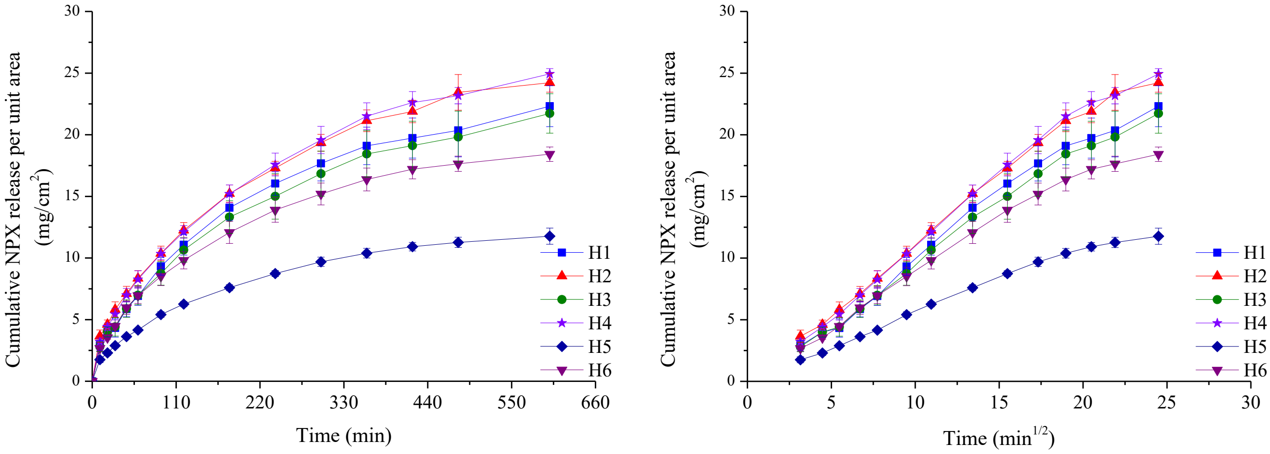


a) b)

Fig. 4.2. a) Cumulative viscosity-shear rate rheograms for hydrogels H1-H4, tested at 23 and 33°C; b) ascending and descending shear stress-shear rate curves for hydrogel H2

All formulations exhibited thixotropic behaviour at both tested temperatures, with the thixotropy index exceeding 5%. Furthermore, the effect of temperature on the thixotropy area was evident, with a decrease by approximately 1.46-1.54 times at 33°C.

Figure 4.3a,b illustrates, as examples, the kinetic release profiles of naproxen from the designed hydrogels, as well as the *in vitro* release profiles of naproxen fitted to the Higuchi model (amount of naproxen diffused per unit area (mg/cm^2) versus the square root of time (min$^{1/2}$)).



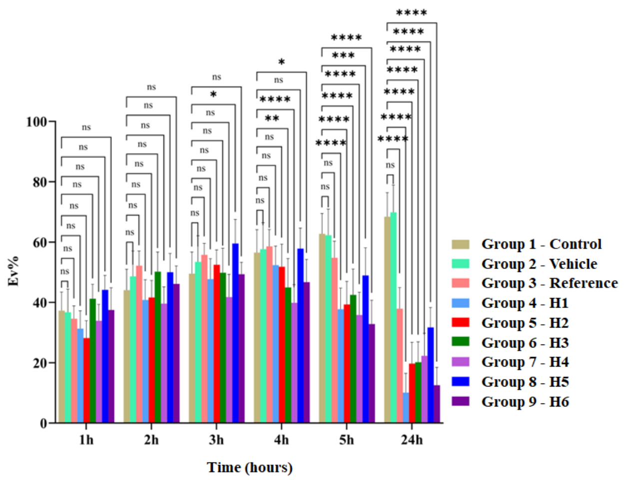
a) b)

Fig. 4.3. a) Naproxen release profiles from samples H1-H6; b) naproxen release profiles from samples H1-H6 fitted to the Higuchi model.

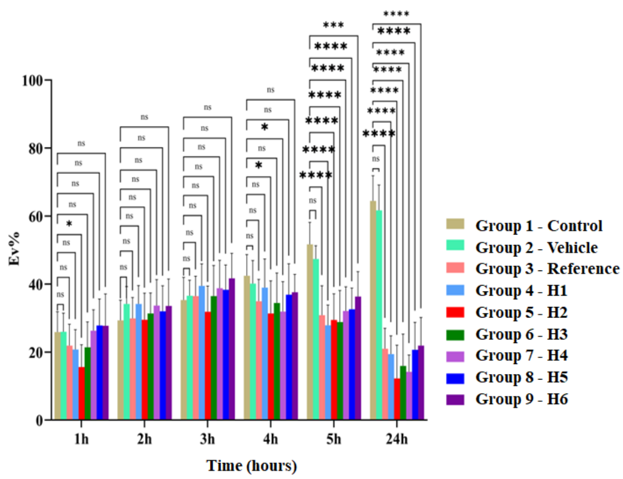
The *in vitro* kinetic analysis of NPX release from the hydrogels revealed similar kinetic profiles for the formulations containing 10% naproxen. This observation was supported by the amounts of naproxen diffused per unit area (mg/cm^2) per unit time (min) after 10 hours, which showed close values, ranging between 21.73 and 22.94 mg/cm^2. For the kinetic profiles recorded from the formulations containing different drug concentrations (10%, 5%, and 7.5%), the amounts of naproxen diffused per unit area (mg/cm^2) per unit time (min) after 10 hours differed, increasing proportionally with the NPX concentration in the samples: 11.77 (hydrogel H5, 5% NPX), 18.42 (hydrogel H6, 7.5% NPX), and 24.22 mg/cm^2 (hydrogel H2, 10% NPX) (Figure 4.5).

Application of the Higuchi model indicated a Fickian diffusion mechanism, with the drug diffusion rate being lower than the polymer relaxation rate.

The anti-inflammatory effect of the hydrogels, evaluated through the combined application of two plantar edema induction models in animals, confirmed the superior therapeutic efficacy of the formulated hydrogels in reducing inflammation associated with plantar edema in Wistar rats over 24 hours, compared with the commercial gel and control. These findings demonstrated that the novel hydrogel compositions, with appropriately selected ratios of penetration enhancers, directly influenced the clinical performance of the developed formulations (Figure 4.4a,b).



a)



b)

Fig. 4.4. Anti-inflammatory effect of the designed gels H1-H6 on plantar edema induced in Wistar rats by: a) λ-carrageenan; b) kaolin

(*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, **$p < 0.0001$, ns: not significant)

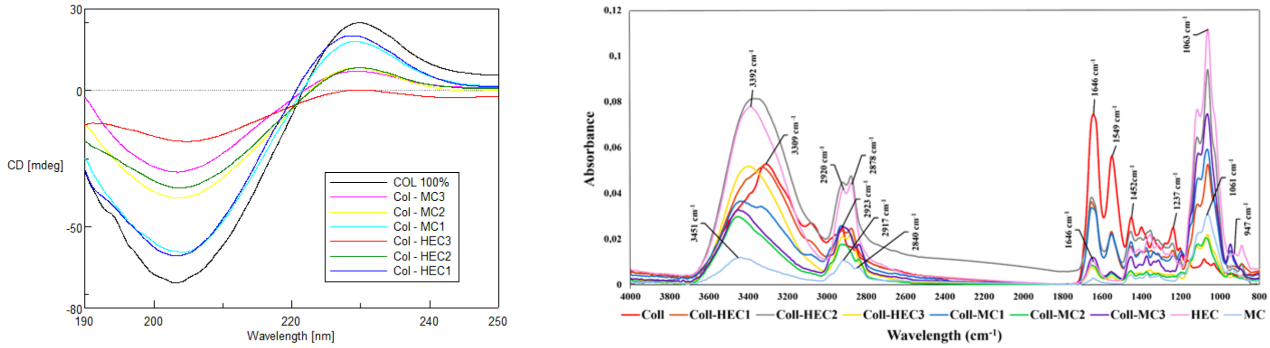
5. DEVELOPMENT OF BIOPOLYMERIC SPONGIOUS MATRICES SUPPORTS FOR THE DELIVERY OF AN ANTI-INFLAMMATORY DRUG FOR HEALING OF POST-BURN SKIN LESIONS

This chapter focuses on determining the optimal mixing ratio of collagen with methylcellulose and hydroxyethylcellulose for the development of spongious matrices designed for tissue regeneration, as well as their optimization for the controlled release of naproxen in the treatment of acute post-burn lesions.

The aim of the ***first stage of this study*** was to prepare and characterize novel hydrogels and their lyophilized forms (spongious matrices), obtained by combining bovine type I collagen with methylcellulose and hydroxyethylcellulose. The primary objective was to evaluate the physicochemical properties and biocompatibility of these polymers, with the purpose of employing the designed biopolymeric supports as potential dressings for the functional regeneration of wounds. The hydrogels and spongious matrices were coded as: Coll, MC, HEC, Coll-MC1, Coll-MC2, Coll-MC3, Coll-HEC1, Coll-HEC2, and Coll-HEC3 [25], according to the mixing ratio (30-70%) of collagen gels with MC or HEC.

Rheological analysis showed that all hydrogels exhibited pseudoplastic, non-Newtonian behaviour at 23°C, indicating that viscosity decreased with increasing applied shear rate. This result is advantageous for the preparation and casting process, as it may provide the manufacturer with greater control over the physical properties of the system, enabling the design of suitable products.

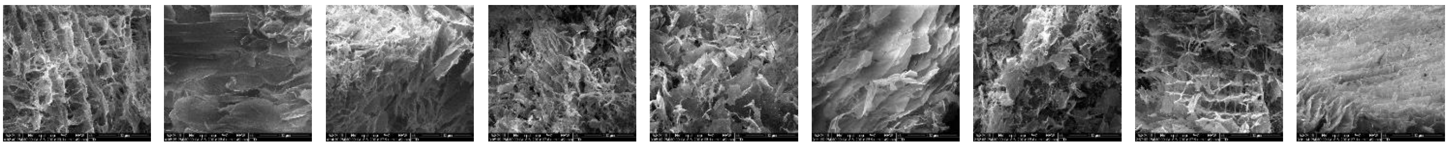
Circular dichroism and FT-IR spectroscopic analyses indicated that the triple-helix structure specific to collagen was preserved when mixed with up to 30% MC/HEC gel (Figure 5.1b).



a) b)

Fig. 5.1. a) Circular dichroism spectra of the formulated hydrogels; b) FT-IR spectra of the corresponding spongious matrices

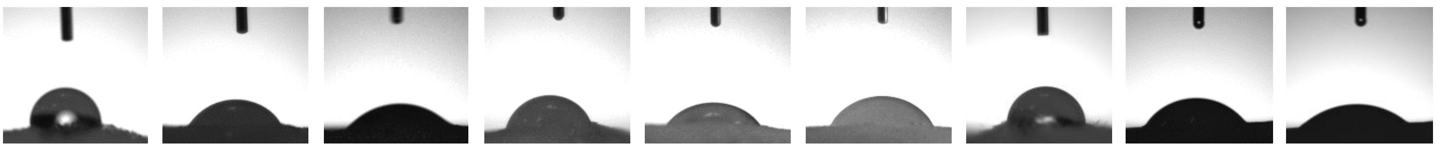
Scanning Electron Microscopy (SEM) analysis revealed that the spongious matrices exhibited a dense porous structure with a large number of micro- and macropores and a smooth surface for the samples containing up to 30% MC / HEC gel, whereas the matrices with a higher content (over 50%) of cellulose derivative gels displayed a dense architecture with long, parallel lamellar filaments, characteristic of cellulose (Figure 5.2).



Coll MC HEC Coll-MC1 Coll-MC Coll-MC3 Coll-HEC1 Coll-HEC2 Coll-HEC

Fig. 5.2. SEM images of the spongious matrices (200 x magnification, 500 µm scale bar)

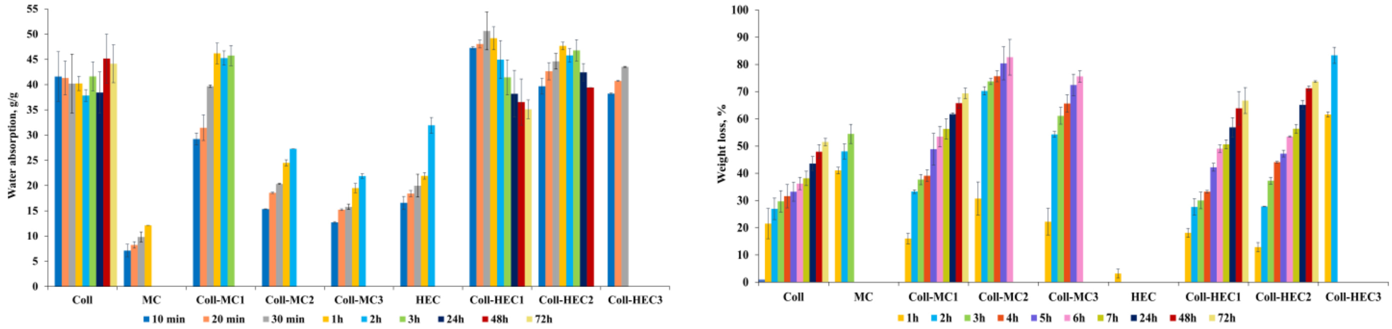
Figure 5.3 shows, as examples, the images of water droplets in contact with the surface of the spongious matrices, corresponding to the measured contact angles.



Coll	MC	HEC	Coll-MC1	Coll-MC2	Coll-MC3	Coll-HEC1	Coll-HEC2	Coll-HEC3
(85.69 ± 3.04°)	(44.98 ± 6.22°)	(44.07 ± 1.83°)	(62.96 ± 4.77°)	(50.67 ± 1.78°)	(56.49 ± 2.57°)	(57.09 ± 1.45°)	(51.43 ± 3.61°)	(38.73 ± 5.27)

Fig. 5.3. Images of water droplets on the surface of the spongious matrices

The spongious formulations exhibited contact angle values ranging from 38.73 ± 5.27° to 85.69 ± 3.04° (Figure 5.3), indicating a hydrophilic surface. They also demonstrated an adequate swelling ratio, characteristics essential for a wound dressing intended to absorb wound exudate [26]. Increasing the content of cellulose derivative gels resulted in a decreased swelling ratio, due to their high solubility in aqueous media (Figure 5.4a). The matrices composed of 100% collagen gel and those containing lower amounts of MC / HEC gel (30-50%) maintained their structural integrity best in collagenase solution for up to 72 hours (Figure 5.4b).



a) b)

Fig. 5.4. a) Water absorption capacity of the biopolymeric matrices; b) Enzymatic degradation of the spongious matrices in collagenase solution at 37°C

Thermogravimetric analysis revealed that blending collagen gel with MC / HEC gels resulted in spongious matrices with superior thermal stability compared to the spongious matrix composed of 100% collagen gel.

As shown in Figure 5.5, none of the tested spongious matrices exhibited cytotoxic effects or inhibitory activity on the motility of human dermal fibroblasts, supporting the biocompatibility of the polymer blends.

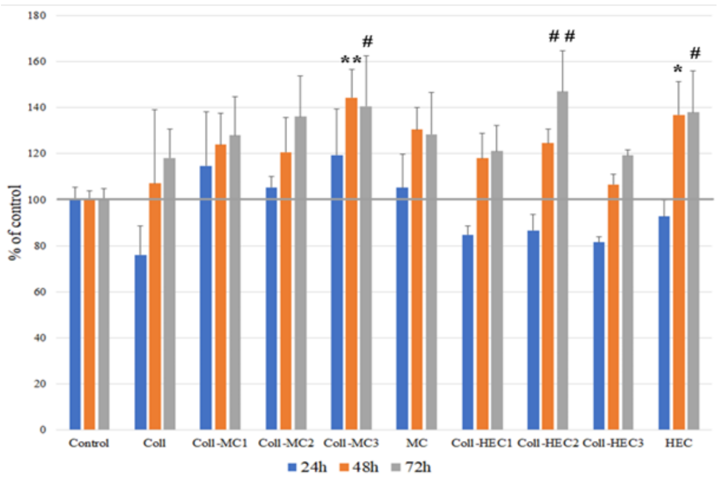


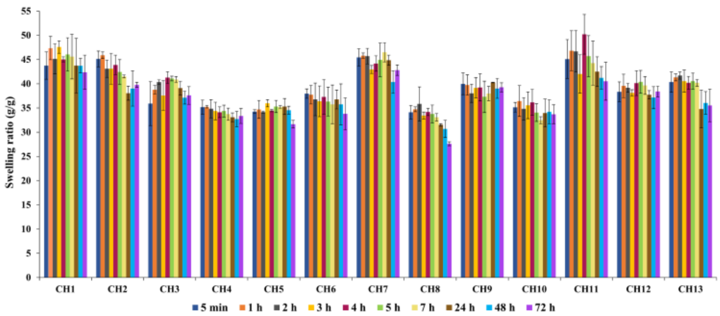
Fig. 5.5. XTT assay showing the viability of human adult dermal fibroblasts after 24, 48, and 72 h of incubation in the presence of porous matrices extracts (* $p < 0.05$ versus Control 48 h, ** $p < 0.01$ versus Control 48 h, # $p < 0.05$ versus Control 72 h, ## $p < 0.01$ versus Control 75 h)

The results indicate that spongious matrices containing up to 30% MC / HEC gel represent promising supports for the development of new modern dressings incorporating non-steroidal anti-inflammatory drugs intended for skin lesion regeneration.

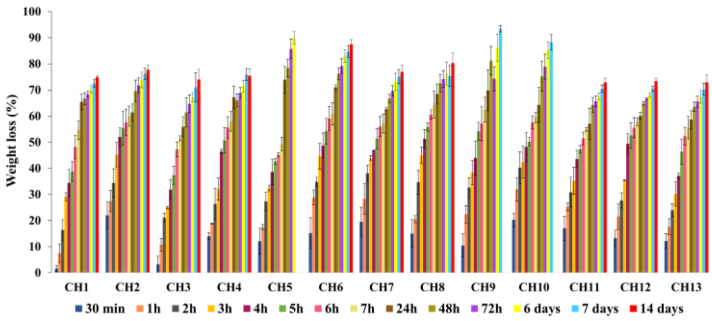
In the ***second stage of this study***, the focus was on the design, optimization, and *in vitro/in vivo* evaluation of naproxen-loaded spongious matrices, incorporating the biopolymeric support based on collagen and hydroxyethylcellulose selected during the first stage. The formulations were developed as drug delivery systems for post-burn wound treatment using a 3^3 fractional factorial Box–Behnken design. Three independent variables (X_i) were selected: the mixing ratio of 1.1% collagen gel and 3% HEC gel - collagen : HEC (X_1, g%:g%), NPX concentration (X_2, g%), and glutaraldehyde concentration (X_3, g%). Four dependent variables (Y_i) were evaluated: contact angle (Y_1, °), swelling ratio at 24 hours (Y_2, g/g), cumulative percentage of drug released at 24 hours (Y_3, %), and percentage of mass loss at 24 hours (Y_4, %). The designed spongious matrices were coded as CH1-CH13.

FT-IR analysis confirmed the preservation of the triple-helix structure specific to collagen, even in the presence of the drug. Morphological analysis using SEM revealed that the designed spongious matrices exhibited a porous network with interconnected pores and cellulose filaments typical of HEC at maximum gel concentrations (30%). SEM also indicated the presence of the drug within the matrices, localized both free at and near the surface and entrapped within the porous network of the spongious formulations.

The spongious matrices exhibited hydrophilic surfaces with contact angle values ranging from 26.58° to 90.36°, optimal swelling capacity (Figure 5.6a), and good structural stability in collagenase solution for more than seven days (Figure 5.6b).



a)



b)

Fig. 5.6. a) Swelling ratio of the spongious matrices immersed in pH 7.4 phosphate buffer; b) Enzymatic degradation of the spongious matrices in collagenase solution at 37°C

The *in vitro* release kinetics exhibited a biphasic profile: (i) during the first hour – a rapid release of NPX (burst release effect), beneficial for the immediate reduction of inflammation and pain associated with acute tissue trauma, followed by (ii) a controlled release over the next 24 hours, ensuring prolonged inflammation control corresponding to the inflammatory phase of the healing process. The total amount of NPX released after 24 hours ranged from 62.24% (CH7) to 96.12% (CH4), values considered appropriate for burn treatment, as the first 24-48 hours are critical in the healing of an acute skin lesion [13] (Figure 5.7). The Power Law model provided the best fit for the experimental data, suggesting a non-Fickian drug transport mechanism.

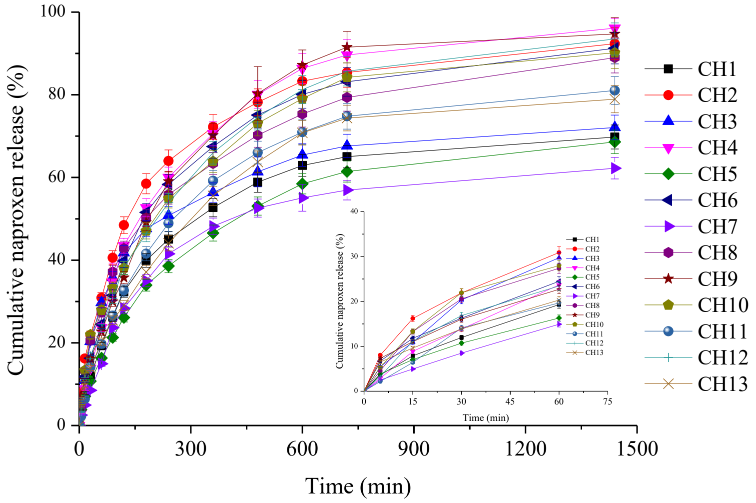


Fig. 5.7. Cumulative release of naproxen from the spongious matrices

The relationship between each dependent variable Y_i and the formulation variables X_i is illustrated in Figure 5.8a,b,c,d through response surface plots, which allow the three-dimensional visualization of the effects of the independent variables on each dependent variable.

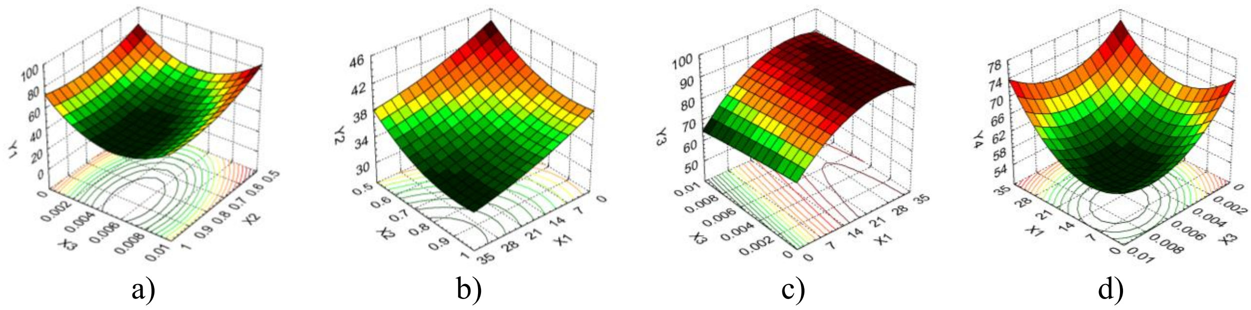


Fig. 5.8. 3D response surface and contour plots illustrating the effect of different formulation factors on the system responses: a) X_2 and X_3 on the contact angle (Y_1); b) X_1 and X_2 on the swelling ratio (Y_2); c) X_1 and X_3 on the cumulative percentage of NPX released (Y_3); d) X_1 and X_3 on the percentage mass loss (Y_4)

As the final stage of the optimization process, the Taguchi technique was employed, allowing the selection of four optimal spongious matrices (one, CH4, belonging to the experimental design, and three, CH14-CH16, developed outside of it). These matrices were robust and stable, with the system responses (Y_i) being least affected by noise factors. Their application to post-burn lesions induced in experimental animals accelerated the wound healing process, achieving complete closure after 17 days (Figure 5.9), compared with the control, the commercial dressing, and the collagen matrix without drug. This effect was achieved through the reduction of inflammation due to naproxen and the stimulation of tissue regeneration by the collagen-hydroxyethylcellulose biopolymeric support.

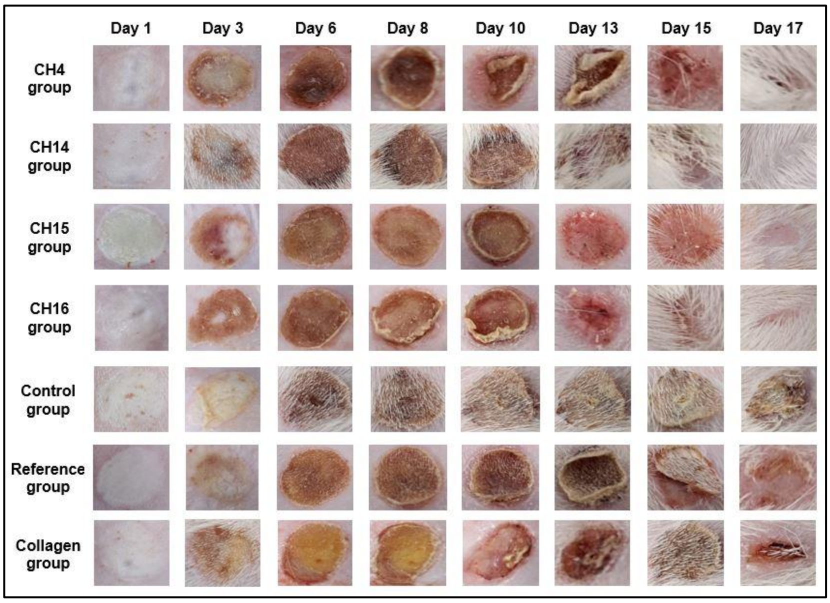
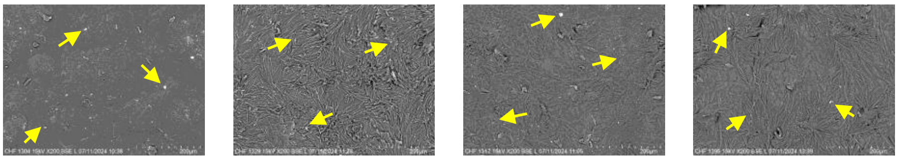


Fig. 5.9. Wound healing evolution in untreated rats (Control) versus those treated with the optimal spongious matrices, the reference dressing, and the collagen matrix without drug, at different time points

6. DEVELOPMENT OF INTELLIGENT pH-SENSITIVE BIOPOLYMERIC FILMS FOR RELEASE OF AN ANTI-INFLAMMATORY DRUG FOR POST-BURN SKIN WOUND HEALING

The local pH value of a wound is a critical factor in wound management, as it not only reflects but also directly influences the physiological and biochemical processes involved in tissue regeneration [7]. The aim of this study was to develop multifunctional pH-sensitive film systems, based on the optimal composition previously determined. These advanced systems incorporated phenol red as a pH indicator, serving a dual role in post-burn wound therapy: (1) *diagnostic* - enabling real-time monitoring of wound pH through the indicator's color changes, shifting from fuchsia in alkaline environments, to orange in neutral media, and to yellow in acidic conditions [27]; and (2) *therapeutic* - providing controlled local release of the anti-inflammatory drug to reduce inflammation and pain [28].

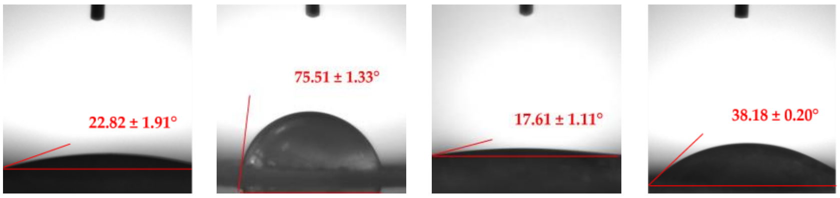
The resulting films, coded F1-F4, exhibited suitable physical and functional properties for application on burn injuries, such as flexibility, transparency, and ease of peelability from casting molds. The films possessed an appropriate thickness for dressing applications, ranging from 0.12 to 0.19 mm, and mechanical properties favorable for post-burn wound treatment. SEM imaging revealed that the films had relatively smooth, compact, nonporous, and continuous surfaces, suggesting a uniform molecular network, with the drug present on the film surface (visible as white spots), highlighted by yellow arrows in the images shown in Figure 6.1.



F1 F2 F3 F4

Fig. 6.1. SEM images of the obtained films (200× magnification, 200 µm scale bar).

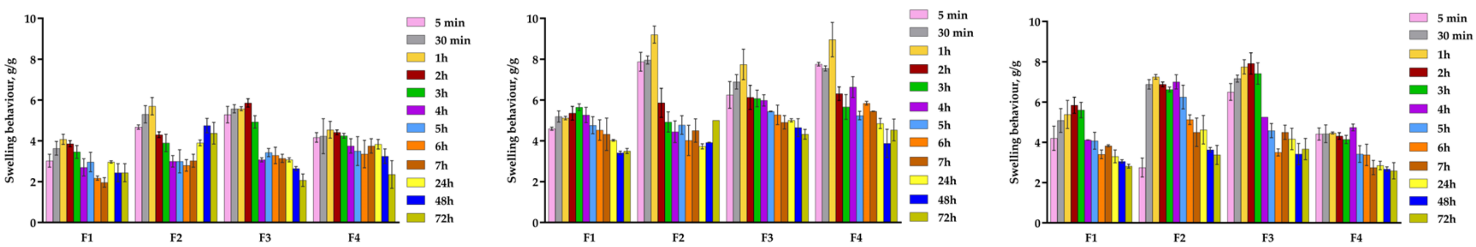
All films exhibited hydrophilic behaviour, with contact angle values ranging from 17.61 ± 1.11° to 75.51 ± 1.33° (Figure 6.2).



F1 F2 F3 F4

Fig. 6.2. Images of droplet shapes and corresponding contact angles recorded on the surface of the pH-sensitive films

The films exhibited a high swelling ratio in all three media with different pH values: acidic (pH 5.5) – typical of healthy skin, neutral (pH 7.4) – corresponding to the initial wound phase, and alkaline (pH 8.5) – characteristic of post-burn lesions [29] (Figure 6.3).

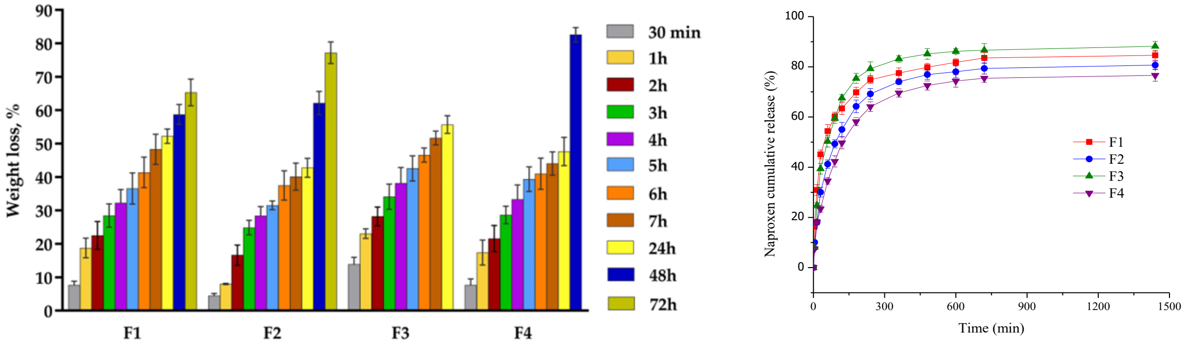


a) pH 5,5 b) pH 7,4 b) pH 8,5

Fig. 6.3. Swelling ratio of the pH-sensitive films in media with different pH values

The higher swelling ratios observed at pH 7.4 and 8.5 compared to pH 5.5 support the use of the designed formulations as effective dressings for post-burn wounds.

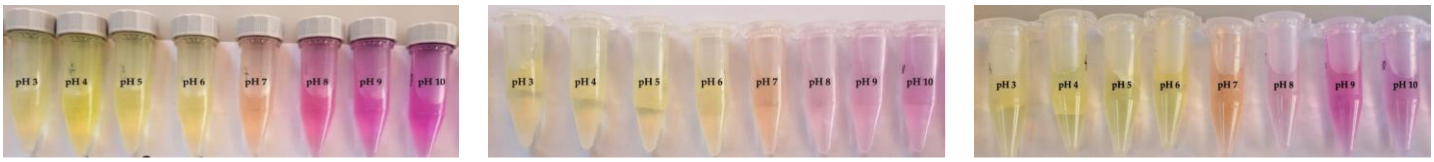
Moreover, the pH-sensitive films maintained their structural integrity for more than 24 hours, a favorable attribute in reducing the frequency of dressing changes for patients (Figure 6.4a). Their ability to absorb exudate facilitates efficient drug release, exhibiting a biphasic profile: an initial rapid release within the first hour, followed by a sustained release over 24 hours, providing effective control of inflammation and pain (Figure 6.4b).



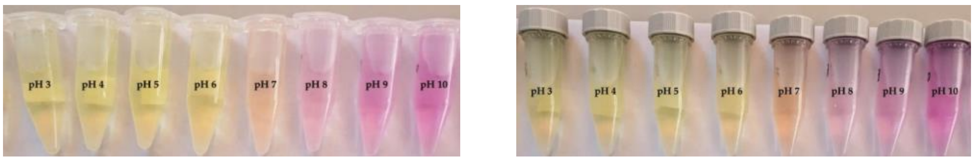
a) b)

Fig. 6.4. a) *In vitro* enzymatic degradation of the pH-sensitive films; b) Naproxen release profiles from the pH-sensitive films.

As shown in Figure 6.5, with the pH variation from 3 to 10, the color of the developed films changed from yellow (pH below 6.8) to orange (pH 6.8-7.5), and finally to fuchsia (pH above 7.5), with these changes being clearly visible to the naked eye.



Phenol red F1 F2



F3 F4

Fig. 6.5. Images of the pH-sensitive films in phosphate buffer solutions with pH 3-10

These chromatic transitions of the films, when applied to a wound, serve as an excellent visual indicator of the wound status, guiding medical personnel in selecting the appropriate treatment for rapid and effective healing.

7. CONCLUSIONS AND PERSONAL CONTRIBUTIONS

The primary objective of this doctoral thesis was the development of modern biopolymeric systems for topical application in the treatment and monitoring of acute skin lesions of traumatic origin (soft tissue injuries) or thermal origin (burns). The designed supports, based on one natural biopolymer (collagen) and two semi-synthetic biopolymers (methylcellulose and hydroxyethylcellulose), aimed to stimulate tissue regeneration, while the incorporation of an anti-inflammatory agent, naproxen, contributed to the reduction of inflammation and pain through its controlled release.

The experimental studies, structured in Chapters 4-6, were conducted in three main stages. Chapter 4 focused on the preliminary selection of suitable grades and concentrations (3-3.5%) of methylcellulose and hydroxyethylcellulose, based on the rheological characterization of hydrogels. Subsequently, hydroxyethylcellulose hydrogels containing various penetration enhancers (Transcutol® P, PEG 200, ethanol, and isopropanol) and naproxen were formulated, exhibiting pseudoplastic and thixotropic rheological properties that favored uniform cutaneous application and ensured adequate delivery of the anti-inflammatory drug to the injured skin area, as demonstrated by two *in vivo* acute inflammation models.

Chapter 5 presented the development of mixed collagen-MC / HEC hydrogels and their lyophilized forms (spongious matrices). Analyses confirmed the preservation of the native collagen structure for formulations containing up to 30% cellulose derivative gels, with physicochemical characteristics suitable for use as dressings. All formulations were biocompatible, supporting the viability of human dermal fibroblasts. Based on these findings, collagen-HEC spongious matrices loaded with naproxen were designed and optimized using Box–Behnken factorial design, response surface methodology, and the Taguchi technique. The matrices exhibited biphasic drug release: an initial burst release followed by controlled release over 24 hours, attributed to the formation of a host-guest complex. When applied to burn-induced lesions in experimental animals, the optimized spongy matrices facilitated wound healing by reducing inflammation and pain, while promoting skin tissue regeneration.

In Chapter 6, pH-sensitive films incorporating collagen, HEC, naproxen, and phenol red were developed. These films demonstrated favorable mechanical, wetting, swelling, and enzymatic degradation properties, biphasic naproxen release, and a visual pH indicator function (yellow-orange-fuchsia, pH 3-10), thus fulfilling both a diagnostic role (real-time visual monitoring of post-burn wound status) and a therapeutic role (supporting the tissue regeneration process).

Therefore, the personal experimental contributions of this thesis led to the development of innovative biopolymeric supports capable of providing efficient management of acute skin lesions of various etiologies.

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