UNIVERSITY OF MEDICINE AND PHARMACY

"CAROL DAVILA", BUCHAREST

DOCTORAL SCHOOL

PHARMACY FIELD

Multifunctional polymeric systems for vaginal drug delivery

ABSTRACT OF THE DOCTORAL THESIS

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TABLE OF CONTENTS

INTRODUCTION

Drug administration via mucosal routes is often an effective alternative to the oral route. Among the mucosal sites used for drug delivery is the vaginal mucosa [1], which has a long history of use in medical practice [2,3]. However, conventional formulations are limited by short retention time and uneven distribution within the vaginal cavity, factors that can compromise therapeutic outcomes [4]. Polymers play a crucial role in the development of modern vaginal drug delivery systems due to properties such as mucoadhesion and biocompatibility [2,5]. The use of such therapeutic systems is particularly relevant for cervicovaginal conditions, including cervical cancer, where localized therapies can provide a promising alternative to standard treatments, reducing systemic side effects and minimizing impact on fertility [6].

The novelty of this doctoral thesis lies in the development of bilayered wafers based on collagen, hydroxypropylmethylcellulose (HPMC), and gellan gum (GG) for the co-administration of two model drugs with complementary mechanisms of action – 5-fluorouracil (5-FU) and sodium diclofenac (DNa) – via the vaginal route, intended for the localized treatment of precancerous and cancerous lesions of the cervix.

This doctoral thesis is structured into two main parts: (i) the theoretical section (*Chapters 1–2*), which provides the conceptual framework on vaginal drug administration and the role of polymers in the development of modern therapeutic systems, and (ii) the experimental section (*Chapters 4–6*), dedicated to the development, optimization, and evaluation of polymeric systems intended for vaginal administration, with potential application in the localized therapy of precancerous and cancerous cervical lesions. Initially, collagen-based spongious matrices incorporating mucoadhesive polymers (HPMC and Carbomer 940) were designed and characterized in terms of polymers compatibility, physicochemical and morphological properties of the obtained systems, and their biocompatibility. Subsequently, the systems were optimized through the integration of a stimuli-responsive polymer (gellan gum) to enhance functional performance under conditions simulating the intravaginal environment. In the final stage, the selected systems were loaded with two active substances with complementary mechanisms of action, evaluated in terms of physicochemical and functional properties, and, for the first time, integrated into a bilayered system with potential for the localized therapy of precancerous and cancerous cervical lesions.

I. GENERAL SECTION

1. VAGINAL DRUG ADMINISTRATION

In recent years, drug delivery via mucosal routes has attracted significant attention as an alternative to conventional routes (oral and parenteral), due to its noninvasive nature, ease of administration, and potential for developing targeted therapies, while simultaneously reducing systemic side effects [1].

Vaginal drug administration has proven to be a promising approach for achieving both local and systemic effects. It has numerous applications in medical practice, including the prevention and treatment of vaginal infections, contraception, labor induction, management of menopause-specific conditions, and treatment of cervical cancer [7].

Cervical cancer, predominantly associated with persistent infection by high-risk HPV strains, represents a significant global health issue. In addition to surgical or chemotherapeutic treatments, localized therapeutic approaches are increasingly being investigated. Administration of active substances directly to the cervix can achieve higher local concentrations while minimizing systemic effects, which is particularly relevant for precancerous lesions and early stages of the disease [8].

Numerous classes of active substances are currently used for vaginal administration, either for contraceptive purposes or for the management of gynecological conditions. These include antimicrobial agents, antifungals, spermicides, hormones, probiotics, anti-inflammatory drugs, and antiviral or antiproliferative agents [9]. In the localized treatment of cervical cancer, agents such as 5-fluorouracil, imiquimod, trans-retinoic acid, cidofovir, and interferon are being investigated for administration to the cervical mucosa, alongside non-pharmacological or plant-derived alternatives [10]. 5-Fluorouracil (5-FU) is a well-known antimetabolite chemotherapeutic agent widely used in the treatment of solid tumors [11] and investigated as a topical treatment for cervical intraepithelial neoplasia [12]. In clinical studies, 5-FU administered as a 5% cream has shown promising efficacy in lesion regression, with manageable local side effects [12]. Diclofenac, a nonsteroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic, and antipyretic properties, is being studied in the context of drug repurposing for its anticancer effects, due to COX-2 inhibition and reduction of chronic inflammation implicated in carcinogenesis [13,14]. In combination with 5-FU, diclofenac has demonstrated synergistic cytotoxic effects against several cancer cell lines [15], supporting its selection as a model drug in the present study.

The performance of intravaginal therapeutic systems depends on both the characteristics of the vaginal environment (pH, vaginal fluid, microbiota, etc.) and the properties of the active substance and pharmaceutical formulation, as both can influence drug release and absorption [7]. Although vaginal administration offers numerous advantages, the dynamic intravaginal environment and natural protective mechanisms can reduce treatment efficacy [16]. Conventional dosage forms present limitations such as short residence time at the administration site, local discomfort, and dose non-uniformity [9,17,18], which can be overcome through the use of polymers as multifunctional excipients to optimize drug delivery systems [19,20].

2. POLYMERS IN THE DESIGN OF VAGINAL DRUG DELIVERY SYSTEMS

The development of therapeutic systems for intravaginal administration needs to address the classic quality requirements (efficacy, safety, stability) as well as the specific characteristics of the vaginal environment. Formulations should be biocompatible, non-irritating, stable under acidic pH, mucoadhesive, and easy to use [9]. A key attribute of vaginal drug delivery supports is biocompatibility, since such systems must interact safely with the mucosal surface without triggering immune responses, irritation, or toxicity [21,22]. Another critical aspect is mucoadhesion, which increases residence time, bioavailability, and targeted drug delivery [23]. Combining the biocompatibility of natural polymers with mucoadhesive properties enables the development of effective systems for the treatment of infections, inflammation, and cervical lesions [24].

Polymers are essential for the development and optimization of vaginal drug delivery systems, contributing to enhanced stability, bioavailability, and controlled drug release [2,5]. Natural polymers are biocompatible and biodegradable, presenting minimal risk of immune reactions or toxicity [25,26]. Collagen, due to its ability to support cell adhesion, proliferation, and tissue regeneration [27], helps maintain the integrity of the vaginal mucosa and promotes epithelial repair in inflammatory or dystrophic conditions [28,29]. At the same time, mucoadhesive polymers – whether natural, derivative, or synthetic – prolong the residence time of formulations through interactions with mucus and allow sustained drug release [30]. Polyacrylic acid (carbomer) and hydroxypropylmethylcellulose (HPMC) are two widely used mucoadhesive polymers in topical and mucosal drug delivery systems,

owing to their favorable properties that can maximize the performance of various pharmaceutical formulations [31]. Stimuli-responsive polymers, such as gellan gum, respond to factors like ions in biological fluids, providing mucoadhesive properties and ensuring stable adhesion to the mucosa [32,33]. Gellan gum (GG) stands out for its biocompatibility, stability under acidic pH, and versatility, being used not only in vaginal formulations but also in regenerative medicine and wound healing [34-36].

Thus, polymers are essential supports in the development of intravaginal therapeutic systems, playing important roles in controlling drug release and increasing contact time with the mucosa. Modern dosage forms include gels, films, tablets, and spongious matrices, while nanotechnology opens new perspectives in this field [2]. In the present study, attention was focused on spongious matrices, or wafers – porous structures capable of absorbing biological fluids and incorporating drugs, which are subsequently released via a complex mechanism involving diffusion, swelling, and erosion, offering the advantage of localized delivery and reduced systemic effects [37]. In addition, dual or bilayered systems, such as bigels [38] or bilayered tablets [39], allow controlled drug release while combining therapeutic efficacy with improved system stability.

Building on the potential advantages of bilayer or biphasic formulations, as well as the relevant properties of spongious matrices, the final objective of the research presented in this doctoral thesis was to develop, for the first time, some bilayered wafer systems. Moreover, despite the diversity of vaginal drug delivery systems, no formulation – whether reported in the published literature or available commercially – has been identified that integrates the specific combination of the three polymers (collagen, HPMC, gellan gum) proposed in the present study.

II. PERSONAL CONTRIBUTIONS

3. WORKING HYPOTHESIS AND OBJECTIVES

The main objective of this doctoral thesis was the development and characterization of vaginal drug delivery systems based on **collagen** – a natural and biocompatible polymer, and other polymers intended to improve residence time at the site of administration: mucoadhesive and stimuli-responsive polymers. These systems were designed to ensure a balance between functionality, efficacy, and safety, being adapted to the intended purpose: targeted therapy of precancerous and cancerous lesions of the cervix.

The achievement of the main objective was carried out through the following **specific objectives**: (1) development and selection of polymeric spongious matrices based on collagen and mucoadhesive polymers (**HPMC, Carbomer 940**) intended for mucosal administration; evaluation of polymer compatibility and characterization of the obtained systems in terms of physicochemical properties and biocompatibility; (2) optimization of the selected systems through the introduction of a smart polymer, **gellan gum**, and development of hydrogels and spongious matrices with functional properties suitable for intravaginal administration; evaluation of polymer compatibility and the influence of gellan gum on the characteristics of the designed systems, with selection of the optimal matrices; (3) incorporation of two model drugs (**5-FU** and **DNa**), at different concentrations, into the selected spongious matrices and testing of developed single-layer single-drug wafers in terms of morphology, swelling capacity, hydrophilicity, resistance to enzymatic degradation, mucoadhesion, and *in vitro* release kinetics, with selection of the optimal formulations; (4) development of **bilayered systems** based on the selected single-layer wafers for the co-administration of the two model drugs; (5) evaluation of **the synergistic antiproliferative effec**t *in vitro* on the cervical cancer cell line CaSki as a method to validate the functionality of the developed systems in the proposed applications; (6) dissemination of the results through publication of scientific articles and presentation of the results as oral communications or posters at scientific events.

4. INFLUENCE OF MUCOADHESIVE POLYMERS ON THE PHYSICOCHEMICAL PROPERTIES AND BIOCOMPATIBILITY OF COLLAGEN SPONGIOUS MATRICES

The main objective of this study was the development and characterization of collagen-based spongious matrices with two mucoadhesive polymers, namely hydroxypropylmethylcellulose (HPMC) and Carbomer 940 (CBM), intended for mucosal administration. At the same time, the study aimed to evaluate the compatibility between the mucoadhesive polymers and collagen, as well as the properties of the developed polymeric supports (physicochemical and biological), with the purpose of selecting the optimal compositions that formed the basis for subsequent development stages [40].

Individual hydrogels of fibrillar type I collagen (of bovine origin, obtained at the Collagen Department, ICPI - INCDTP, Bucharest), HPMC, and Carbomer 940 were

prepared and combined in different proportions to obtain mixed hydrogels (COL-HPMC and COL-CBM). Both the simple and mixed hydrogels were lyophilized to obtain spongious matrices. The characterization of the systems focused on (i) polymer compatibility (circular dichroism, FT-IR spectroscopy, thermogravimetric analysis) and (ii) evaluation of the matrices in terms of morphology (SEM), swelling capacity, hydrophilicity (contact angle), and biocompatibility with human fibroblasts.

Circular dichroism analysis (CD) confirmed the preservation of the collagen triple helical structure in the presence of the two mucoadhesive polymers; however, more pronounced changes were observed upon the addition of Carbomer 940 (Figure 4.1).

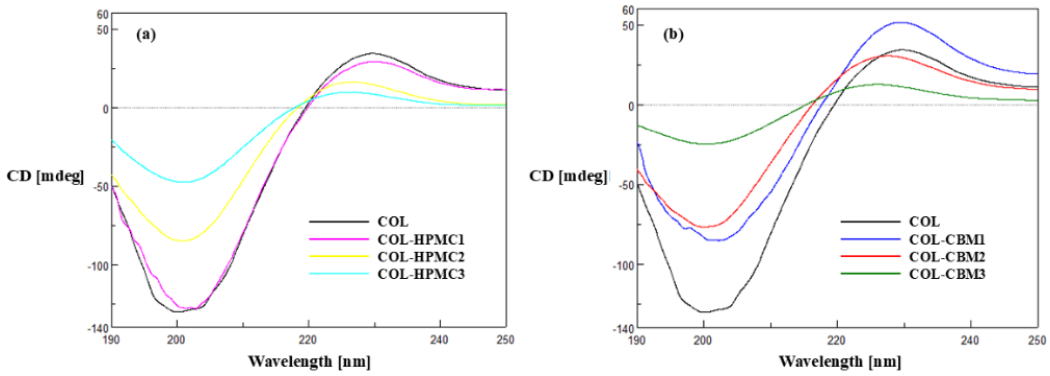
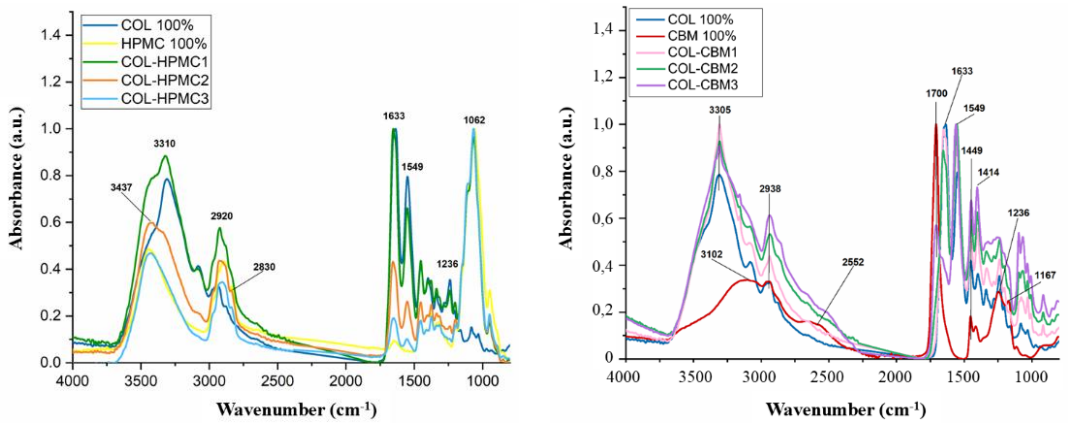


Fig. 4.1. CD spectra of hydrogels from a) COL-HPMC and b) COL-CBM series

The FT-IR spectra (Figure 4.2) highlighted the characteristic peaks of all incorporated polymers, with position or intensity variations depending on the composition of the matrices.

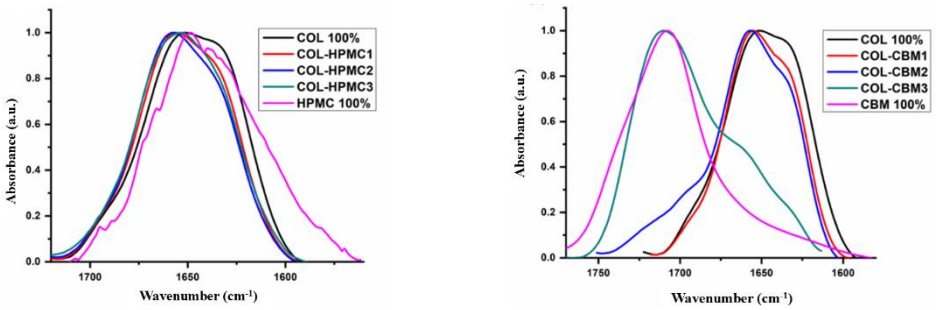


a) b)

Fig. 4.2. FT-IR spectra of samples from a) COL-HPMC and b) COL-CBM series

Deconvolution of Amide I spectral region allowed the identification of the contributions of different types of secondary structures, with the α-helix structural type being

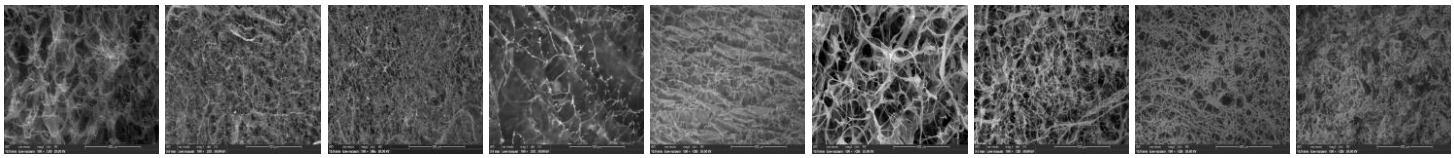
predominant in matrices with high collagen content and partially replaced by other structural types (random coil) as the proportion of mucoadhesive polymers increased. For both series, a slight shift of the absorption bands (Amide I) toward higher wavenumbers was observed, being more pronounced for the COL-CBM3 sample (Figure 4.3).



a) b)

Fig. 4.3. Overlay of the FT-IR spectra analyzed in the 1770–1580 cm^{-1} range (Amide I) for samples a) COL-HPMC and b) COL-CBM

The SEM images (Figure 4.4) revealed a microporous structure for collagen, with large pores interconnected by collagen fibers, while Carbomer exhibited a similar network, but more compact and with smaller pores. HPMC displayed a multilayered, lamellar morphology, and the biocomposite matrices combined the characteristics of the constituent polymers.



COL **COL-HPMC1** **COL-HPMC2** **COL-HPMC3** **HPMC** **COL-CBM1** **COL-CBM2** **COL-CBM3** **CBM**

Fig. 4.4. SEM images obtained for the COL-HPMC and COL-CBM matrices

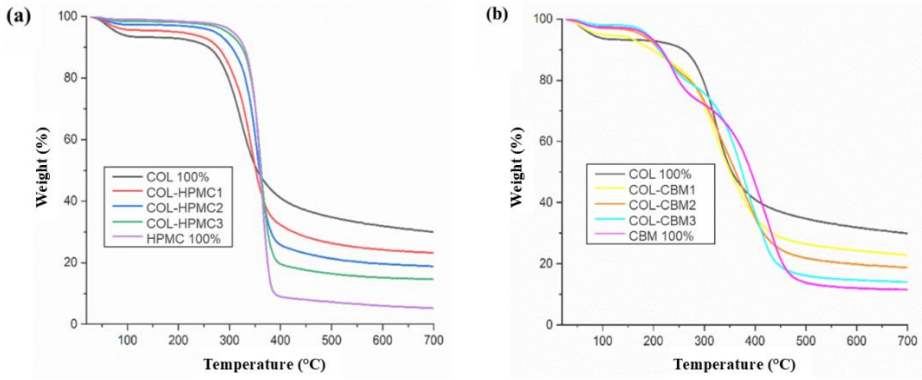


Fig. 4.5. TGA thermograms of samples a) COL-HPMC and b) COL-CBM

Thermogravimetric analysis (TGA) highlighted an improvement in the thermal stability of the collagen matrices correlated with the addition of HPMC, whereas the addition of Carbomer led to more pronounced thermal degradation, involving multiple degradation steps (Figure 4.5).

Goniometric analysis (Figure 4.6) revealed the hydrophilic nature of the spongious matrices, with contact angle values < 90° obtained for all analyzed samples.

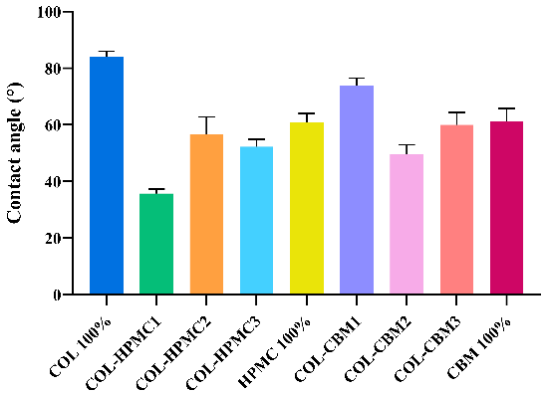
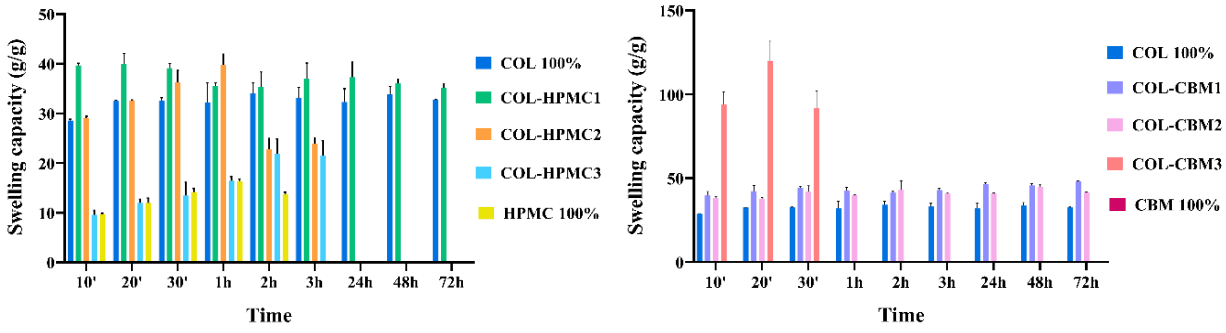


Fig. 4.6. Comparative representation of the mean contact angle values obtained for COL-HPMC and COL-CBM samples

Swelling capacity analysis (Figure 4.7) highlighted an adequate water absorption capacity for COL 100%, COL-HPMC1, COL-CBM1, and COL-CBM2 matrices, which maintained their structural integrity throughout the duration of the experiment (72 h).



a) b)

Fig. 4.7. Swelling capacity (g/g) of a) COL-HPMC and b) COL-CBM samples over the 72-hour testing period

With the exception of CBM 100%, all samples demonstrated good biocompatibility, maintaining cell viability (Figure 4.8) and cell migration capacity (Figure 4.9).

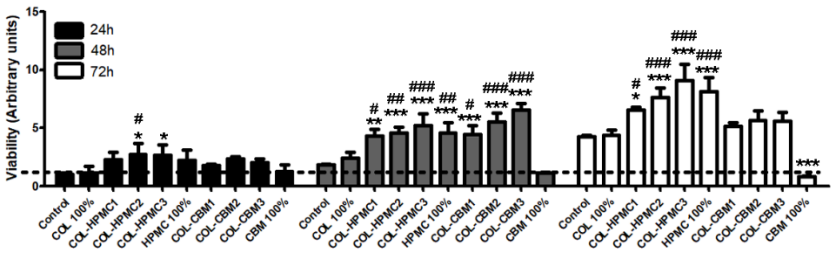


Fig. 4.8. XTT assay showing the viability of human fibroblasts after 24, 48, and 72 hours of incubation in the presence of spongious matrices extracts (* $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. Control at each time point, # $p<0.05$, ## $p<0.01$, ### $p<0.001$ vs. COL at each time point)

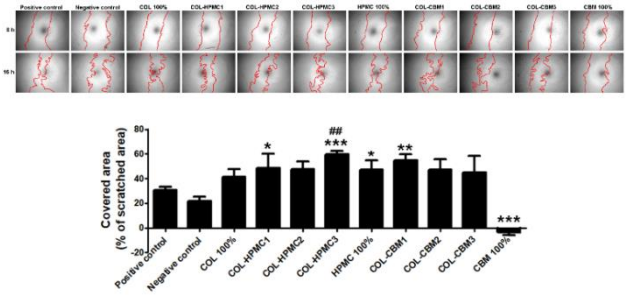


Fig. 4.9. *In vitro* scratch test: upper panel - phase-contrast microscopy showing the scratched area at 0 and 16 h later; lower panel - quantification of covered area as % of the initial scratched area where positive and negative controls are serum and serum-free medium (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. the positive control; $p < 0.01$ vs COL)

The data obtained for the evaluation of cell migration capacity in the presence of the tested biomaterials were validated by assessing cell morphology through fluorescence microscopy (Figure 4.10).

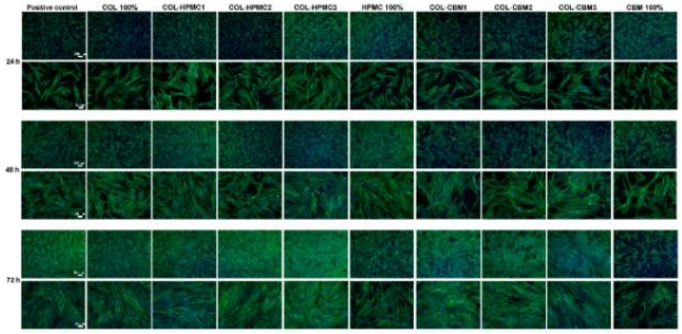


Fig. 4.10. Images of fluorescence-based staining of the actin cytoskeleton (green) and nucleus (blue): upper panels – evaluation of cell density (bar = 200 μm); lower panels - cytoskeleton organization and F-actin stress fibers (bar = 50 μm)

The addition of HPMC in the samples induced an increase in cell proliferation, with the best results observed for the COL-HPMC3 sample, which further demonstrated a certain chemoattractive effect on human fibroblasts (Figure 4.11).

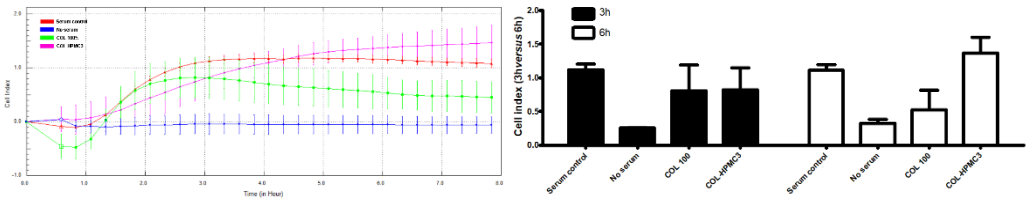


Fig. 4.11. Time-dependent assessment of chemotactic migration of human adult fibroblasts. The lower panel illustrates the migration index at 3 h versus 6 h.

Considering all the obtained results, the COL-HPMC samples exhibited superior performance throughout the analyses compared to the COL-CBM samples, representing a starting point for the subsequent stages of this study.

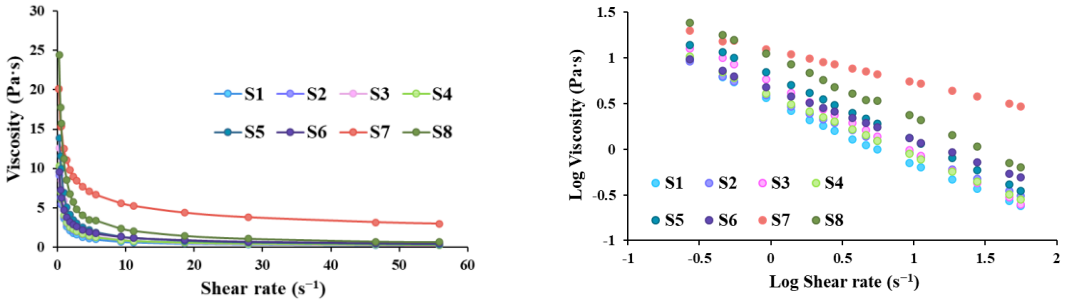
5. EFFECT OF GELLAN GUM ON THE PROPERTIES OF COLLAGEN-HPMC SPONGIOUS MATRICES DESIGNED FOR VAGINAL ADMINISTRATION

In this stage of the study, the aim was to optimize the previously developed collagen- and HPMC-based systems by integrating gellan gum, a stimuli-responsive polymer with good swelling capacity and stability in the acidic environment specific to the vagina.

Thus, the objective of the study was the development and evaluation of hydrogels and corresponding spongious matrices based on collagen, HPMC, and gellan gum, as safe, effective, and biocompatible polymeric systems for vaginal administration [41]. Fibrillar type I collagen, HPMC, and gellan gum hydrogels were prepared and combined in different proportions to obtain mixed hydrogels, which served as the basis for the spongious matrices subsequently obtained by lyophilization. Both the hydrogels and the spongious matrices were coded S1–S8.

The rheograms obtained from the rheological analysis of the collagen-, HPMC-, and GG-based hydrogels are shown in Figure 5.1. Analysis of the rheograms indicates that all hydrogels exhibited pseudoplastic behavior, facilitating administration and local distribution. The experimental data fit the Power Law model, and the analysis of the rheological parameters *m* (consistency index) and *n* (flow index) indicated that gellan gum

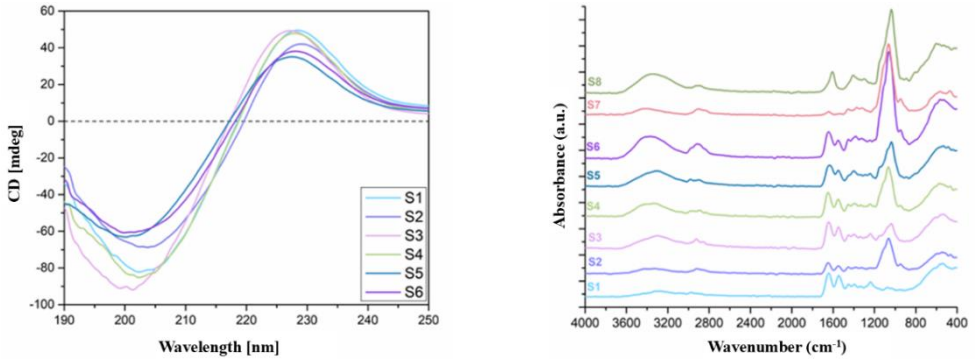
primarily influenced the consistency, while HPMC mainly affected the pseudoplasticity of the hydrogels, both properties being essential for spreading and retention of the formulations on the mucosa.



a) b)

Fig. 5.1. Rheograms of a) viscosity versus shear rate and b) log-log viscosity versus shear rate for hydrogels based on collagen, HPMC, and GG

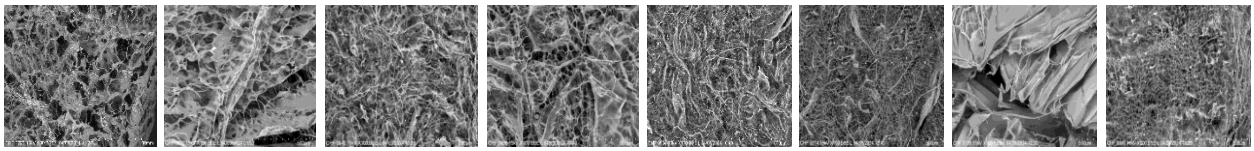
Circular dichroism and FT-IR spectroscopy analyses (Figure 5.2) confirmed the preservation of the collagen triple helical structure in all formulations – important for maintaining the mechanical and biological properties of collagen.



a) b)

Fig. 5.2. a) CD and b) FT-IR spectra of S1–S8 spongious matrices

SEM images (Figure 5.3) revealed the microporous structure specific to collagen matrices (S1), while the HPMC-based matrix (S7) exhibited a more compact, multilayered structure. The gellan gum-based matrix (S8) displayed a microstructure intermediate between the porous one, with a spider-web-like polymer network, and the compact one, similar to cellulose derivative-based samples. The mixed samples (S2–S6) exhibited a combined morphology, dependent on the constituent polymers.



S1 S2 S3 S4 S5 S6 S7 S8

Fig. 5.3. SEM images obtained for S1–S8 samples

All developed matrices exhibited contact angle values (Figure 5.4) with simulated vaginal fluid (SVF) < 90°, while gellan gum led to an increase in hydrophilicity, particularly observed in the collagen and GG-based samples (S3, S5).

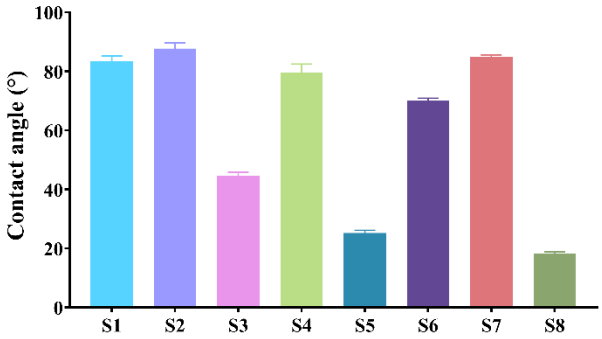
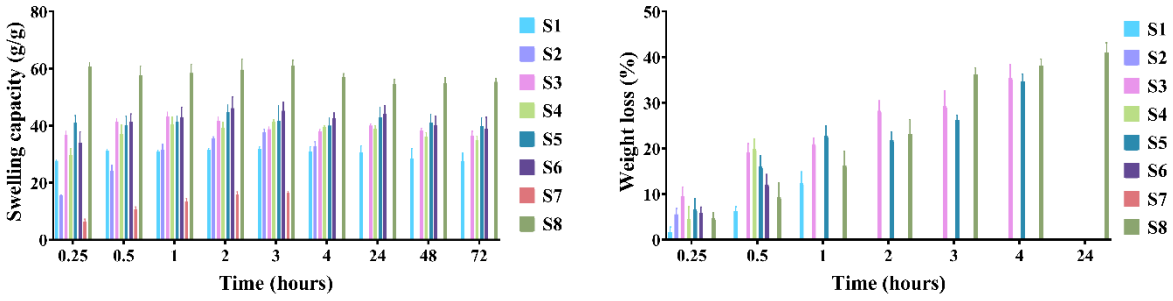


Fig. 5.4. Contact angle values for S1-S8 sampls

Combined samples exhibited a balanced hydration profile, supporting adequate comfort upon administration. Additionally, gellan gum promoted the maintenance of the structural integrity of the matrices in SVF and provided superior resistance to enzymatic degradation (Figure 5.5) [42].



a) b)

Fig. 5.5. a) Swelling capacity; b) Weight loss of the spongious matrices S1–S8

Regarding cytotoxicity (Figure 5.6), the S2–S8 samples did not exhibit cytotoxic effects on human fibroblasts and allowed cell proliferation without inducing changes in F-actin stress fibers (Figure 5.7).

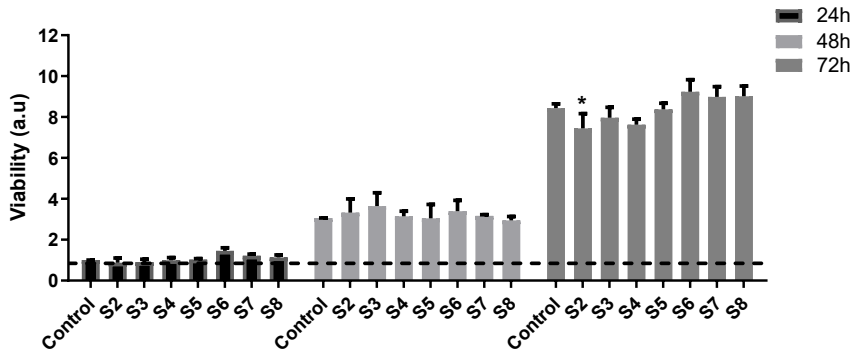


Fig. 5.6. Cell viability (XTT assay) of human fibroblasts after 24, 48, and 72 hours of incubation with spongious matrices extracts (*p < 0.005 vs. control at 72 hours).

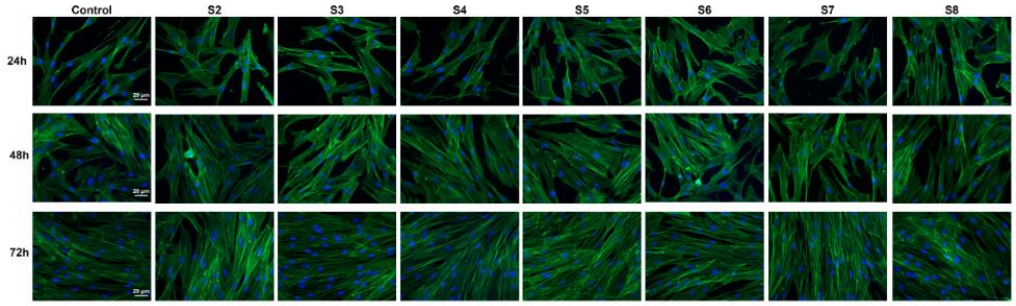


Fig. 5.7. Fluorescence microscopy images illustrating the distribution of actin fibers (green) in dermal fibroblasts and the nucleus (blue)

The developed biopolymeric formulations proved promising as platforms for vaginal drug delivery, providing the foundation for the subsequent development of safe and effective therapeutic systems with potential applications in the localized treatment of gynecological conditions.

6. DEVELOPMENT OF BILAYER BIOPOLYMER WAFERS WITH 5-FLUOROURACIL AND DICLOFENAC SODIUM FOR THE LOCALIZED TREATMENT OF CERVICAL CANCER

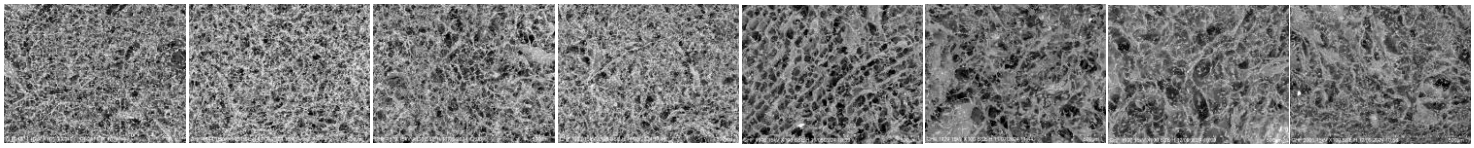
Cervical cancer remains a major public health challenge, particularly in regions with limited access to adequate screening and treatment [43,44]. Localized treatment strategies can help limit disease progression and reduce systemic adverse effects. In this context, biopolymeric systems represent promising platforms for localized drug delivery due to their biocompatibility, prolonged residence time, and the regenerative properties of the polymers [45,46]. Moreover, a multimodal therapeutic approach, based on complementary

mechanisms of action, could enhance the cytotoxic effect on precancerous or cancerous lesions of the cervix [47].

In this context, the final objective of this research was to develop, for the first time, some bilayered wafers based on collagen, HPMC, and gellan gum, intended for the simultaneous administration of 5-fluorouracil and diclofenac sodium, aiming to increase local efficacy and reduce systemic adverse effects in the treatment of precancerous or cancerous cervical lesions.

Starting from the formulations developed and tested previously (*Chapter 5*), spongious matrices based on collagen-GG were selected for the incorporation of 5-FU, due to GG's potential to enhance swelling capacity and surface hydrophilicity of the systems – features that may contribute to increased mucoadhesive properties of the matrices, promoting intimate and prolonged contact with the mucosa. Regarding the incorporation of DNa, matrices containing HPMC were chosen to achieve prolonged drug release, aiming to maintain the anti-inflammatory effect over an extended period and, consequently, ensure sustained control of local inflammation. Thus, single-layer polymeric systems were obtained by mixing collagen (1%), HPMC (2%), and GG (1.2%) gels in different proportions, incorporating one of the two model drugs – 5-fluorouracil or diclofenac sodium – crosslinking with glutaraldehyde, and lyophilization. The single-layer wafers were characterized in terms of morphology, swelling capacity, hydrophilicity, enzymatic degradation, mucoadhesion, and drug release kinetics.

SEM images (Figure 6.1) showed that matrices with higher collagen content exhibited microporous structures, while the addition of gellan gum or HPMC resulted in more compact networks and smoother surfaces. The distribution of both drugs was uniform, with superficial accumulation at higher concentrations, possibly responsible for the initial burst release effect observed during drug release from the spongious matrices.

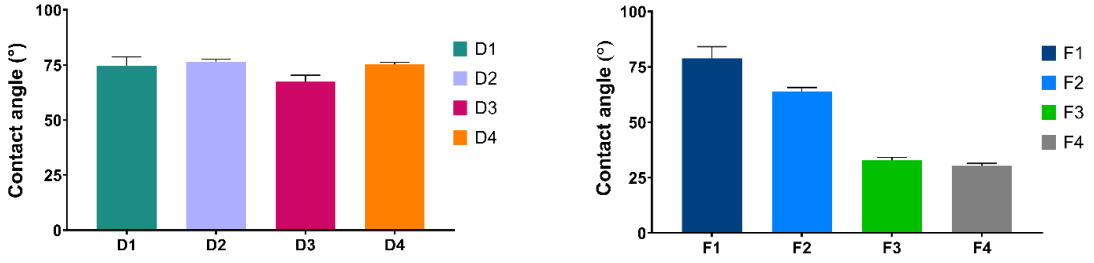


D1 **D2** **D3** **D4** **F1** **F2** **F3** **F4**

Fig. 6.1. SEM images for D1-D4 and F1-F4 wafers

Contact angle analysis (Figure 6.2) confirmed the hydrophilic nature of all matrices (angle < 90°), favorable for wetting and mucoadhesion [48,49]. Hydrophilicity increased with the proportion of gellan gum, being more pronounced in the 5-FU series, while the

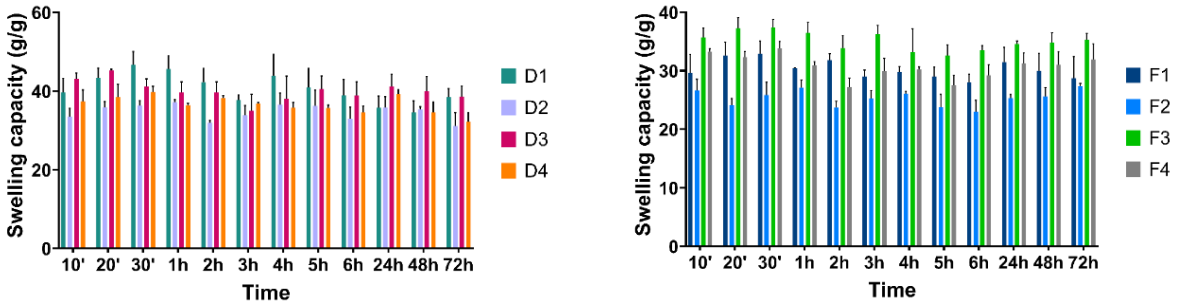
DNa-containing matrices exhibited higher contact angle values, possibly associated with their higher collagen and HPMC content.



a) b)

Fig. 6.2. Contact angle values for a) DNa and b) 5-FU wafers

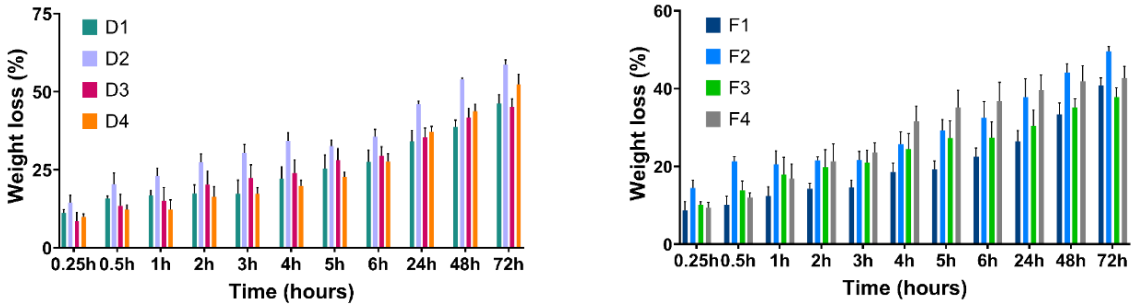
All developed single-layer wafers exhibited a moderate and stable swelling capacity (Figure 6.3), advantageous for ensuring strong mucoadhesion without compromising structural integrity or adhesion duration [48]. The DNa-containing matrices showed higher swelling capacity values, likely due to the higher crosslinking degree of the 5-FU samples. Regarding the influence of the two model drugs, the results of the present experiment revealed a decrease in swelling capacity correlated with increasing drug concentration for all samples.



a) b)

Fig. 6.3. Swelling capacity of a) DNa and b) 5-FU samples in the presence of SVF

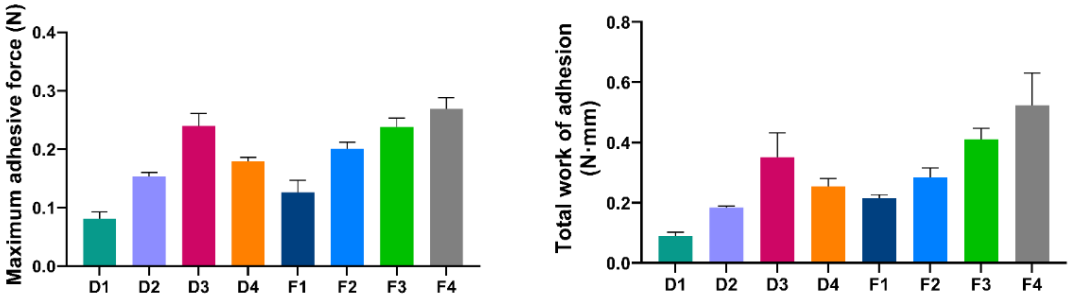
Evaluation of enzymatic degradation in the presence of collagenase (Figure 6.4) revealed a gradual degradation of the matrices, relevant for estimating residence time and drug release in the vaginal environment. Increasing drug concentration reduced resistance to degradation. Complete degradation of the systems occurred within 24 hours from the end of the experiment, likely due to the combined action of surface erosion and bulk matrix degradation, triggered by matrix rehydration and enzymatic breakdown [50].



a) b)

Fig. 6.4. Weight loss of a) DNa and b) 5-FU wafers in the presence of collagenase

Evaluation of wafers mucoadhesion (Figure 6.5), under conditions simulating the intravaginal environment, revealed superior mucoadhesive properties for the 5-FU-containing matrices compared to those with DNa.



a) b)

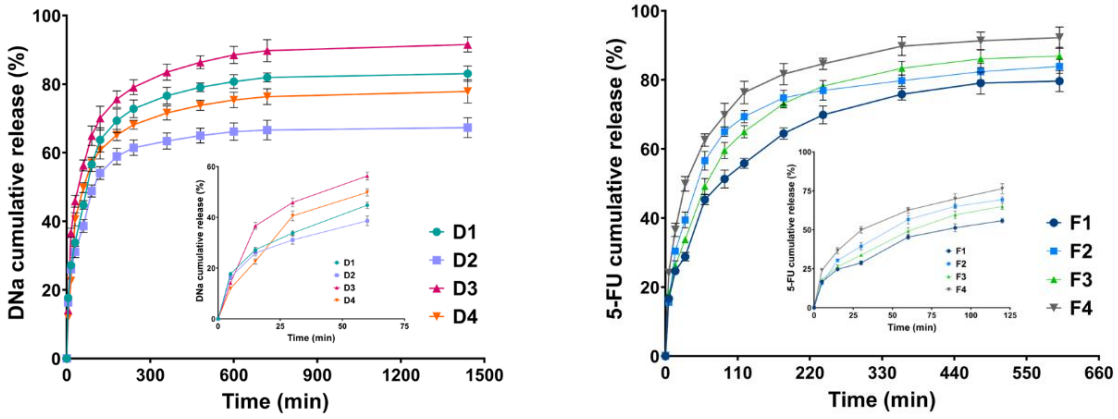
Fig. 6.5. a) Maximum adhesive force and b) Total work of adhesion for DNa (D1-D4) and 5-FU (F1-F4) wafers

The incorporation of gellan gum significantly contributed to increasing the maximum adhesive force and the total work of adhesion, with formulations containing higher GG content (F3 and F4) exhibiting the best potential for vaginal retention.

The release profiles (Figure 6.6) of the two model drugs from the single-layer matrices revealed a biphasic release pattern, with 5-fluorouracil released over approximately 10 hours and diclofenac sodium over 24 hours.

The Power Law model most accurately described the kinetic behavior under the experimental conditions, and analysis of the obtained release exponent values allowed the conclusion that drug release from the spongious matrices occurred via a complex mechanism, governed by multiple physicochemical processes: (i) rapid wetting of the matrix upon contact with vaginal secretions, facilitating interaction with the mucosa, (ii) initial drug release through desorption from the surface, (iii) absorption of vaginal fluid and swelling of

the spongious matrix, with relaxation of the polymer network and reinforcement of mucoadhesion through interpenetration of polymer chains with mucus, simultaneously with (iv) diffusion of the drug retained within the polymeric structure, and eventual (v) gradual erosion of the matrix. .



a) b)

Fig. 6.6. *In vitro* drug release kinetic profiles of (a) DNa and (b) 5-FU from the single-layer wafers

Based on the results obtained from the evaluation of morphological, structural, and functional characteristics, two formulations for each drug (D1, D3, F3, F4) were selected and combined to obtain **bilayered wafers** for the co-administration of both therapeutic agents.

To validate the functionality of the developed systems, the cytotoxic effect of the bilayered wafers was evaluated both by the XTT assay (Figure 6.7) and by morphological examination of CaSki cells using phase-contrast microscopy (Figure 6.8).

Phase-contrast microscopy showed that treatment with extracts from the spongious matrices containing 5-FU and DNa (Figure 6.8a) induced morphological changes in CaSki cells, as well as a concentration-dependent decrease in cell population. In the absence of DNa (Figure 6.8b), cells treated with 5-FU-containing samples did not exhibit morphological changes or significant differences in proliferation compared to untreated control cells.

The biological evaluation of the bilayered systems demonstrated a synergistic antiproliferative effect on CaSki cervical cancer cells, confirming the potential of this systems for localized therapy of precancerous or cancerous cervical lesions.

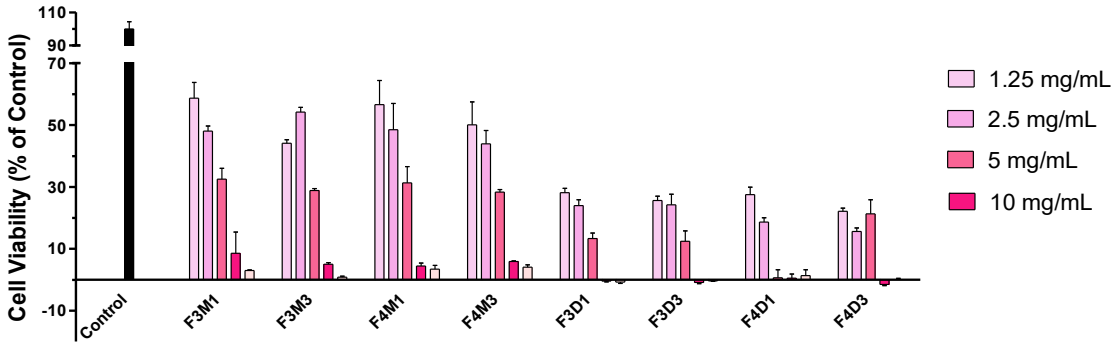
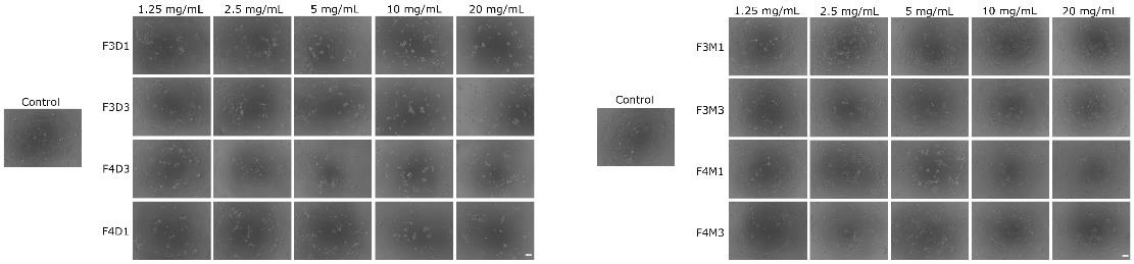


Fig. 6.7. 24-hour cytotoxicity graphs showing the antiproliferative effect of spongious matrices extracts containing 5-FU (F3M1, F3M3, F4M1, F4M3) and 5-FU combined with DNa (F3D1, F3D3, F4D1, F4D3) against the CaSki cell line.
Values are expressed as mean ± SD.



a) b)

Fig. 6.8. Effects of 5-FU and DNa on the morphology and proliferation of cervical cancer CaSki cells. Morphological changes were observed using phase-contrast microscopy after treatment with 0 (Control) and 1.25, 2.5, 5, 10, and 20 mg/mL of extracts obtained from the spongious matrices: a) containing both drugs (samples F3D1, F3D3, F4D1, and F4D3) and b) 5-FU only, without DNa (samples F3M1, F3M3, F4M1, and F4M3) for 24 hours.

7. CONCLUSIONS AND PERSPECTIVES

The main objective of this doctoral thesis was the development of biocompatible and bioresorbable polymer-based drug delivery systems, intended for vaginal drug delivery, capable of ensuring an appropriate balance between polymer compatibility, the physicochemical and biological properties of the obtained systems, and their biocompatibility. The experimental part, representing the personal contributions of this thesis, was carried out in three consecutive stages, each focusing on the characterization and optimization of different polymeric systems.

In the first stage of the research, spongious matrices based on collagen and mucoadhesive polymers (HPMC and Carbomer 940) were obtained and characterized for mucosal administration, highlighting the influence of composition on collagen structure, thermal stability, morphology, and biocompatibility of the systems. Based on the integrated evaluation of the results, the collagen–HPMC combination was selected as the starting point for the subsequent development stages.

The second stage of the research aimed to optimize the previously selected system by introducing gellan gum, an intelligent polymer sensitive to ions in the vaginal environment, leading to the development of hydrogels and spongious matrices with increased hydrophilicity, good swelling capacity, and improved enzymatic degradation stability, while maintaining collagen structural integrity and material biocompatibility. Studies conducted under conditions partially simulating the intravaginal environment allowed the functional validation of the collagen, HPMC, and GG-based systems and provided relevant information for the selection of formulations with optimal profiles, suitable for the development of advanced intravaginal drug delivery systems.

In the final stage, starting from the previously selected formulations, single-layer biopolymeric wafers based on collagen, HPMC, and GG were developed and characterized, each incorporating one of the two model drugs (5-FU or DNa). These matrices exhibited favorable properties regarding hydrophilicity, swelling capacity, mucoadhesion, enzymatic degradation profiles, and drug release, demonstrating their potential for localized vaginal drug administration. Based on the optimal formulations, bilayered spongious matrices were obtained, for the first time, for the co-delivery of two therapeutic agents with complementary mechanisms of action. Biological evaluation of these systems revealed a synergistic antiproliferative effect on CaSki cervical cancer cells, confirming the potential of the developed systems for localized combination therapy of precancerous and cancerous cervical lesions.

In conclusion, the personal contributions of this doctoral thesis highlight the potential of biopolymers in the development of multifunctional therapeutic systems for vaginal drug delivery, intended for the localized treatment of cervical cancer, as a possible alternative to existing therapeutic strategies.

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LIST OF PUBLISHED SCIENTIFIC PAPERS

I. Articles Published in ISI Web of Science Clarivate-Indexed Journals, as First Author

1. Luca I, Albu Kaya MG, Titorencu I, Dinu-Pîrvu CE, Marin MM, Popa L, Roșca AM, Antoniac A, Anuța V, Prisada RM, Kaya DA, Ghica MV, *Influence of mucoadhesive polymers on physicochemical features and biocompatibility of collagen wafers*, ACS Polymers Au, 2025, 5(3), 282-297, **FI – 6,9/2024, Q1,** chapter 4, pp. 33-61.
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II. Studies Published as Abstracts in the Proceedings of Scientific Events

1. Luca I, Ghica MV, Albu Kaya MG, Dinu-Pîrvu CE, Popa L, Anuța V, Prisada RM, *Development and evaluation of some biopolymeric systems designed for vaginal administration of an anti-inflammatory drug*, e-poster presentation (ID 982) presented at the 13th Congress of the "Carol Davila" University of Medicine and Pharmacy, Bucharest, October 23–25, 2025.
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