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ORIGINAL ARTICLE / ÖZGÜN MAKALE

DEVELOPMENT AND *IN-VITRO* EVALUATION OF MUCOADHESIVE BUCCAL NANOFIBER FORMULATIONS FOR USE IN ORAL ULCER TREATMENT

AĞIZ ÜLSERİ TEDAVİSİNDE KULLANIM İÇİN MUKOADEZİF BUKKAL NANOLİF FORMÜLASYONLARININ GELİŞTİRİLMESİ VE İN-VITRO DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: *Oral ulcers are common lesions that negatively affect patients' quality of life due to pain, inflammation, and discomfort. The aim of this study was to develop and evaluate mucoadhesive nanofiber formulations containing hydrocortisone (HC) for the local treatment of oral ulcers.*
Material and Method: *Different types of Eudragit (E100, S100, L100-55) and polyvinylpyrrolidone (PVP) were used as mucoadhesive polymers. Nanofibers were fabricated using electrospinning system. Nanofibers characterized by differential scanning calorimetry (DSC), mechanical testing, contact angle measurements, and ex vivo mucoadhesion studies for buccal application.*
Result and Discussion: *Nanofibers were successfully produced with electrospinning. Thermal analyses confirmed the HC within the nanofibers. Mechanical and wettability studies showed that the nanofibers had suitable physical properties for buccal application. In ex vivo mucoadhesion tests of the formulations, values were 0.20 ± 0.05, 0.12 ± 0.03, and 0.14 ± 0.01 mJ cm^{-2} for E2-HC, L2-HC, and S2-HC, respectively. Mucoadhesion tests demonstrated strong adhesion to the mucosal surface, indicating potential for extended residence time and effective local drug delivery. In vitro release profiles of formulations, HC released 40.26%, 47.87% and 47.03% at 1 h for E2-HC, L2-HC and S2-HC, respectively. The formulations demonstrated sustained release and favorable mucoadhesive strength, indicating potential clinical applicability. Hydrocortisone-loaded buccal electrospun nanofibers showed promise as a novel and effective drug delivery platform in the treatment of oral ulcers.*
Keywords: *Buccal nanofiber, electrospinning, hydrocortisone, oral ulcer*

ÖZ

Amaç: *Oral ülserler, ağrı, inflamasyon ve rahatsızlık nedeniyle hastaların yaşam kalitesini olumsuz etkileyen yaygın lezyonlardır. Bu çalışmanın amacı, oral ülserlerin lokal tedavisi için hidrokortizon (HC) içeren mukoadezif nanolif formülasyonları geliştirmek ve değerlendirmektir.*
Gereç ve Yöntem: *Eudragit'in farklı tipleri (E100, S100, L100-55) ve polivinilpirolidon (PVP) mukoadezif polimerler olarak kullanıldı. Nanolifler elektroçekim sistemi kullanılarak üretildi.*

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Bukkal uygulamaya yönelik olarak nanolifler; diferansiyel taramalı kalorimetri (DSC), mekanik testler, temas açısı ölçümleri ve ex vivo mukoadezyon çalışmaları ile karakterize edildi.
Sonuç ve Tartışma: *Nanolifler elektroçekim yöntemiyle başarıyla üretildi. Termal analizler, nanolifler içinde HC varlığını doğruladı. Mekanik ve ıslanabilirlik çalışmaları, nanoliflerin bukkal uygulama için uygun fiziksel özelliklere sahip olduğunu gösterdi. Formülasyonların ex vivo mukoadezyon testleri, E2-HC, L2-HC ve S2-HC için sırasıyla 0.20 ± 0.05, 0.12 ± 0.03 ve 0.14 ± 0.01 mJ cm⁻² değerlerine ulaştı. Mukoadezyon testleri mukozal yüzeye güçlü yapışma gösterdi ve bu da uzatılmış kalış süresi ve etkili lokal ilaç salımı potansiyeline işaret etti. Formülasyonların in vitro salım profillerinde, HC, E2-HC, L2-HC ve S2-HC için sırasıyla 1 saatte %40.26, %47.87 ve %47.03 oranında salındı. Formülasyon, sürekli salım ve uygun mukoadeziv güç göstererek potansiyel klinik uygulanabilirliğini gösterdi. Hidrokortizon yüklü bukkal elektro-eğrilmiş nanolifler, oral ülserlerin tedavisinde yeni ve etkili bir ilaç dağıtım platformu olarak umut vaat etmektedir.*
Anahtar Kelimeler: *Bukkal nanolif, elektroçekim, hidrokortizon, oral ülser*

INTRODUCTION

Oral health has been defined by the World Health Organization as one of the top ten standards for human health. Oral ulcers have high prevalence in the population also affect oral health and consequently, systemic health. They cause pain, impair nutrition and speech in patients and also reduce the quality of life [1,2]. The causes of oral ulcers are varied, complex and vary individually including stress, local trauma, lack of some vitamins, cytotoxic treatments, genetic factors and malignant transformations. Due to substantial interindividual variability among patients, there is no single rational treatment. However, symptomatic management remains the most common therapeutic strategy [1,3,4].

Anesthetics, corticosteroids, and antimicrobials are used as topical drugs for buccal application [1,5]. Topical corticosteroids are suggested for painful oral ulcers [6,7]. Hydrocortisone (HC), considered a first-choice corticosteroid for ora ulcer, is a Biopharmaceutics Classification System (BCS) class II drug characterized by low solubility and high permeability [5, 8-10]. Various studies in the literature have investigated the use of HC for oral ulcers treatment. Adinaraya et al. developed various *in situ* gels containing 1% HC and they found proper for local treatment of oral ulcers [11]. Ceschel et al produced buccal adhesive tablets containing hydrocortisone acetate could found to use in ulcerative and inflammatory oral diseases treatment [12].

Several forms of currently available treatments for oral ulcers are used on the market, including gargle, gel, cream and tablet. These dosage forms need to be frequently administered because they tend to mix with saliva in the mouth. However, the buccal patch is more acceptable to apply on the mouth ulcers. Buccal patch has the advantage of adhering to the ulcer site and delivering the drug locally and protecting the ulcer wound. Therefore, it extends the action of the drug, reduces frequency of doses and weakens pain [1]. Buccal patches can be produced by different methods such as film, nanofiber or 3D printing [13-15].

Electrospinning is a novel method for fabricating buccal patches with mucoadhesive polymers. Electrospinning produces nanofibers with simple one-step processing, low cost and enhanced solubility for poorly water-soluble drugs [16]. Nanofibers have several advantages, including adjustable diameter and structure, high porosity, high permeability, high surface area, mechanical flexibility, functionalization capability, ease of use, high encapsulation capacity and the potential to encapsulate various active substances [17,18].

Mucoadhesive polymers strongly interact with buccal mucosa and overcome the buccal drugs application challenge and preferred over conventional dosage forms [19]. Polymethacrylate derivatives, commercially known as Eudragit, are copolymers derived from acrylic and methacrylic acid esters that exhibit various physicochemical properties due to their functional groups. Eudragit L, S, FS, and E polymers exhibit strong adhesive properties. PVP is a hydrophilic, stable, biodegradable, bio-compatible polymer with low cytotoxicity, high thermal resistance [20]. These polymers will increase retention time and the solubility of poorly soluble active ingredients in the oral cavity.

This study aims to develop buccal nanofiber formulations with PVP and different types of

Eudragit for local treatment of oral ulcers. Buccal mucoadhesive nanofibers provide significant advantages for the topical treatment of oral ulcers. There is no nanofiber formulation containing HC for the treatment of oral ulcers that has been developed using the electrospinning technique according to the current literature.

MATERIAL AND METHOD

Materials

Hydrocortisone was a pleasant gift from Gen İlaç, Turkiye. Eudragit E100, S100 and L 100-55 (Evonik Röhm GmbH, Germany) and PVP (M.W. 40,000, BASF, Germany) were used in nanofiber formulations. Ethanol: N, N-Dimethylformamide (DMF) (7:3) was used as a solvent system in electrospinning process. NaCl, KH2PO4, NaHPO4, HCl and distilled water were used for pH 6.8 simulated saliva fluid. All chemicals were of analytical grade.

Preparation of the Polymer Mixture for Electrospinning

Different ratios of Eudragit derivatives and/or PVP were used in formulation production. Eudragit S100 (ES100), Eudragit L 100-55(EL-100-55) and Eudragit E100 (EE100) were coded as ES, EL, and EE, respectively. For the preparation of formulations, Eudragit was dissolved in DMF and ethanol at room temperature stirring at 500 rpm. Subsequently, the required amount of PVP was gradually added and mixed until a homogeneous mixture was obtained. Blank formulations were produced, and those exhibiting the most favorable mechanical strength and mucoadhesive performance were selected for further development. During this evaluation, the polymer solutions were also characterized in terms of viscosity, conductivity, and electrospinnability to ensure stable nanofiber formation. In contrast, some formulations such as E1 and L3 demonstrated less favorable characterization results, which hindered the production of uniform nanofiber mats and buccal use. Therefore, only the E2-HC, L2-HC, and S2-HC formulations were selected for subsequent studies as they provided the most suitable balance of physicochemical and functional properties.

The polymer concentration of formulations prepared with Eudragit E100 was adjusted to be higher than those prepared with Eudragit S100 and L100-55. This adjustment was necessary because solutions containing Eudragit E100 exhibited lower viscosity and weaker chain entanglement at the same concentrations, which caused unstable jet formation and bead formation during electrospinning. To achieve stable electrospinning and obtain uniform nanofibers, the concentration of Eudragit E100 had to be increased. In contrast, formulations with Eudragit S100 and L100-55 already showed sufficient viscosity and electrospinnability at lower concentrations. Formulations containing HC were mixed until homogeneously dissolved. Codes and content of formulations were shown in Table 1.

Table 1. Content and codes of nanofiber formulations

Formulation code	HC (%)	EE100 (%)	EL-100-55 (%)	ES100 (%)	PVP (%)
E1		40			
E2		30			10
E3		20			20
E2-HC	1	30			10
L1			15		
L2			10		5
L3			7.5		7.5
L2-HC	1		10		5
S1				15	
S2				10	5
S3				7.5	7.5
S2-HC	1			10	5

Characterization of Electrospinning Solutions

Conductivity, viscosity and surface tension of the polymer mixtures were characterized for electrospinnability. The conductivity meter was used for conductivity measurements at room temperature (Seven2Go Cond meter S3, Mettler Toledo, UK). Conductivity values of the solutions were measured and evaluated as μs/cm [21]. Approximately 10 ml of each polymer solution was transferred into a clean, dry beaker, ensuring that the probe was fully immersed into the polymer solution. Conductivity values were measured in triplicate and expressed in μS/cm. Between measurements; the probe was rinsed with distilled water and gently dried to prevent contamination between samples.

Viscosity of polymer solutions were measured using a cone-plate viscometer equipped with spindle type CPE-52 (Brookfield, DV-III Rheometer, USA) [22]. Viscosity measurements were carried out with 0.5 ml of polymer solution at room temperature. The viscosity measurements were obtained at a constant shear rate of 40 s^{-1}. Each sample was analyzed in triplicate to ensure reproducibility.

Surface tension of polymer solutions was measured using an optical tensiometer (Attension-Theta Lite, Biolin Scientific, Finland). Surface tension was calculated by the device software using the Young-Laplace equation [23]. A drop of the polymer solution (5–10 μl) was formed at the tip of a syringe. The device's software automatically calculated the surface tension values based on the Young–Laplace equation by fitting the droplet shape. All measurements were conducted at room temperature. The surface tension values were reported as mean ± SD from three measurements.

Production of Nanofibers by Electrospinning Method

Single nozzle equipment electrospinning (Inovenso Ltd, NE300, Turkey) was used for the produce of nanofiber formulations. Solutions were kept in ultrasonic bath to remove air bubbles before electrospinning process. Following the preparation of the formulations, each solution was loaded into 10-ml syringes for electrospinning process. The syringe was mounted on the electrospinning apparatus, and nanofibers were collected on a flat stationary aluminum foil-covered collector. To achieve produce nanofibers, critical process parameters including solution flow rate (0.5–6 ml/h), applied voltage (5–20 kV), and the distance between the needle tip and the collector (10–20 cm) were optimized as detailed in Table 2.

Table 2. Process parameters of nanofibers produced using the electrospinning method

Formulation code	Feed rate (ml/h)	Voltage (kV)	Distance (mm)
E1	2	15	130
E2	5	14.5	150
E3	4.5	15	140
E2-HC	1,5	14.5	145
L1	6	14	150
L2	5.2	13	160
L3	1.4	16.5	150
L2-HC	1.75	14.3	122
S1	1.5	16	150
S2	5	14	150
S3	4.2	14	150
S2-HC	1	14.5	122

The electrospinning parameters such as feed rate, applied voltage, and tip-to-collector distance were optimized individually for each formulation, since the solution properties such as viscosity, conductivity, and surface tension varied depending on the type and ratio of Eudragit polymer used and the presence of hydrocortisone. These physicochemical differences directly affect jet stability and fiber formation. Therefore, formulation-specific adjustments were required to achieve stable Taylor

cone formation and continuous jet flow. All electrospinning procedures were conducted under ambient room temperature conditions. The collected nanofiber mats were carefully removed from the collector and stored at room temperature until further characterization.

Drug Content of Formulations

To determine the drug content, nanofiber mats were cut into circular pieces with a diameter of 1 cm and immersed in simulated saliva solution (SSS). Samples were placed on a shaker and stirred at 100 rpm for complete dissolution and release of hydrocortisone (HC) into the medium. The saliva solutions were filtered through a 0.45 µm PTFE syringe filter to remove any undissolved polymer or debris prior to analysis. The amount of hydrocortisone (HC) in the nanofibers was quantified using a UV-Visible spectrophotometer at a wavelength of 248 nm. Calibration curves were constructed using standard HC solutions (1-25 µg/ml) in simulated saliva medium over an appropriate concentration range ($R^2 > 0.999$). Drug content was calculated as the percentage of theoretical drug loading. All measurements were carried out in triplicate and results were expressed as mean ± SD.

Thermal Analysis of Nanofiber Formulations

Thermal analysis of Eudragit S100, Eudragit L 100-55, Eudragit E100, PVP and nanofiber formulations was performed using a differential scanning calorimeter (Shimadzu, DSC-60, Japan). Approximately 2.0 mg of each sample was weighed and placed into standard aluminum DSC pans. Samples were weighed for DSC analysis. DSC processes were conducted a heating rate of 10°C/min under a nitrogen atmosphere to 300°C.

Mechanical Properties of Nanofibers

Texture analyzer (TA.XTPlus Texture Analyzer, Stable Micro Systems, UK) was used to determine tensile strength and elongation at break values of the nanofiber formulations [24]. Nanofibers were cut into 3 cm x 1 cm and attached to the tensile grip apparatus. Tensile strength (MPa) and elongation at break (%) were determined from the stress–strain curves. The values on the stress-strain graphs at the point where the elongation is maximum and the rupture occurs were used calculating for elongation at break and tensile strength. Elongation at break corresponds to the value on the x-axis, whereas tensile strength corresponds to the value on the y-axis. Measurements were conducted in triplicate for each formulation.

Contact Angle Measurements

Contact angle measurements were used to examine the wettability of nanofibers [25]. An optical tensiometer was used to measure the contact angles nanofibers (Attension, Theta Lite, Finland). Nanofiber mats were cut into square samples and carefully placed on a flat, convex sample holder to ensure a smooth measurement surface. A 5 µl droplet of distilled water was dispensed onto the nanofiber surface using a precision syringe. Contact angles were determined using the device software by analyzing the shape of the drop formed on the nanofiber surface.

***In vitro* Drug Release Studies of Nanofibers**

Franz diffusion cells equipped with a dialysis membrane (12 kDa, Sigma®, USA) were used for the *in vitro* release of hydrocortisone (HC) from the buccal nanofibers. Prior to the experiment, the membranes were soaked in SSS to ensure complete hydration. Nanofibers were placed in the donor compartment and SSS was filled into the 2,5 ml receptor compartment of Franz diffusion cell. 2.5 ml samples were taken from the receptor phase at definite time intervals (0.25, 0.5, 1, 2, 4, 6, 8 and 24 h). An equal volume of fresh pH 6.8 phosphate buffer was added to maintain sink conditions. The release studies were extended to 24 h in order to obtain a comprehensive release profile and comparative data between different formulations. The release experiments were performed in triplicate, and results were expressed as cumulative drug release percentage (mean ± SD) versus time. Ultraviolet (UV) spectrometer (Cary 60 UVvis, Agilent Technologies, US) was conducted to define amount of HC at 248 nm. The analytical method was validated according to ICH guidelines in terms of linearity, precision, accuracy, limit of quantification (LOQ) and limit of detection (LOD).

Ex-Vivo Mucoadhesion Studies

Mucoadhesion studies were conducted by using the TA-XT Plus Texture Analyzer with cow buccal mucosa for mucoadhesion studies. In this study, probe force of 0,2 N, probe speed of 1 mm/s and contact time of 150 s with the mucosa were studied [26]. The buccal mucosa was fixed to the lower platform of the instrument and nanofiber samples were attached to the upper probe using double-sided adhesive tape. Each formulation was tested in triplicate The area under the force-distance curve was used to calculate the work of mucoadhesion. These values per cm^2 was calculated according to the formula:

$$(mJ/cm^2) = AUC/(\pi r^2)$$

and was compared [27].

Statistical Analysis

GraphPad Prism version 7.0 (GraphPad Software Inc., San Diego, CA, USA) was used for all statistical analysis. $p < 0.05$ was considered statistically significant.

RESULT AND DISCUSSION

Characterization Result of Electrospinning Solutions

The electrospinning performance of polymer solutions is strongly influenced by their physicochemical properties, particularly conductivity, viscosity, and surface tension, which collectively determine fiber formation and morphology [28]. All characterization results of the electrospinning solutions are shown in Table 3.

Conductivity increases the charge carrying capacity, providing the polymer jet with stretch and high bending instability [29]. Formulations containing hydrocortisone generally exhibited higher conductivity. This effect may be due to the ionic contribution of HC or interactions between HC and the polymer. A statistically significant difference in conductivity values was observed among the HC-free formulations (E1, L1, and S1) ($p < 0.05$). This variation can be explained by the distinct chemical structures and the presence or absence of ionizable functional groups in the Eudragit® derivatives. Specifically, Eudragit® S100 and L100-55 contain free carboxyl groups that can partially ionize in the ethanol/DMF solvent system, thereby contributing to higher conductivity. In contrast, Eudragit® E100 lacks such ionizable groups, which results in comparatively lower conductivity values. Studies have shown that increasing the electrical conductivity of the solution can produce fibers with smaller diameters and fewer beads [30]. However, excessively high conductivity may cause bead formation due to jet instability. If the solution has high conductivity, the radius of curvature of the Taylor cone will be very small, making the initiation of bending instability more challenging. Therefore, electrical conductivity within an appropriate range will lead to the formation of thinner fibers.

Solution viscosity also represents chain entanglement, which is directly affected by the molecular weight or polymer concentration [31]. If a polymer solution exhibits insufficient viscosity, the lack of chain entanglement will result in jet break up, because the droplets have lower surface tension and consequently less resistance to the electric field. This gives rise to a phenomenon known as Rayleigh instability, which describes the interaction between surface tension and applied forces. Rayleigh instability causes jet breakage and bead formation in fibers formed at low concentrations or low molecular weights. The viscosity of the polymer solutions was strongly dependent on both the polymer concentration and the type of Eudragit derivative used. The L2-HC formulation contained a lower concentration of Eudragit L100-55 compared to other formulations, which reduced chain entanglement and resulted in the lowest viscosity values. Additionally, the incorporation of hydrocortisone (HC) further influenced viscosity. HC, being a small and poorly water-soluble molecule, may interact with the polymer chains and partially disrupt intermolecular interactions, thereby decreasing overall viscosity. This effect was particularly evident in L2-HC, where the lower polymer concentration combined with drug incorporation produced the lowest viscosity among the

studied formulations. In a study by Zhang et al., increasing polymer concentration altered solution viscosity [32]. At lower polymer concentrations, beads appeared in the fiber structure, and the average fiber diameter was 87 ± 14 nm. With increasing concentration, the morphology changed from beaded fibers to a homogeneous fiber structure, and the fiber diameter gradually increased to 246 ± 50 nm. Tort and colleagues developed doxycycline-loaded nanofibers. Increasing the polymer solution concentration resulted in increased viscosity, which in turn increased the average fiber diameter [33]. This is because increased viscosity leads to increased chain entanglement. These concentration/viscosity effects observed in our study are consistent with literature reports.

Surface tension is more dependent on the solvent composition than the polymer concentration [34]. According to Renker and Doshi, the formation of bead-free fibers is facilitated by lowering the surface tension of the polymer solution [35]. The polymer solutions' surface tension values were found to be similar in our study because the same solvent system was used for all formulations. Overall, the interplay between polymer type, concentration, and hydrocortisone incorporation significantly influenced solution properties, which in turn affected fiber morphology and uniformity in the electrospinning process.

Table 3. Viscosity, surface tension, and conductivity of the polymer solutions (n = 3, mean ± SD)

Formulation code	Conductivity (μS.cm^{-1})	Viscosity (cP)	Surface tension (mN.m^{-1})
E1	6.5±0.04	195.7±6.24	25.64±0.62
E2	8.04±0.02	225±8.50	27.29±0.20
E3	8.96±0.03	239±8.17	29.55±0.35
E2-HC	13.39±0.01	225±8.45	27.29±0.20
L1	13.27±0.10	70±4.08	25.74±0.12
L2	17.24±0.07	2.08±0.08	27.76±0.11
L3	13.05±0.02	1.73±0.03	30.18±0.14
L2-HC	36.84±0.13	1.26±0.02	27.76±0.11
S1	14.62±0.39	523.7±13.12	32.48±0.93
S2	13.84±0.05	825.10±10.80	33.17±0.57
S3	15.32±0.33	773±9.43	28.36±0.11
S2-HC	27.96±0.06	825±10.8	33.17±0.57

Drug Content of Nanofiber Formulations

The HC content was found to be 116.74 ± 2.20% for E2-HC, 99.34 ± 1.15% for L2-HC, and 100.96 ± 1.27% for S2-HC. All formulations exhibited drug content values within or close to the acceptable pharmacopeial limits for content uniformity. The electrospinning process provided efficient drug loading into the formulations. These findings confirm that the ethanol–DMF solvent system and the selected polymer compositions enabled complete incorporation of hydrocortisone into the nanofiber matrix, without degradation or loss during processing. Drug loading is critical for dose accuracy in buccal drug delivery systems. Reda et al., developed ketoprofen-loaded nanofiber formulations for the treatment of oral mucositis [36]. High entrapment efficiency is expected due to the nanofibers' high surface area and the passive drug-loading technique, which involves incorporating the drug into a polymeric solution that is subsequently spun and solidified during electrospinning. Because "the drug remains entrapped within the polymer fiber mat, the risk of drug loss during this process is low. The resulting high entrapment efficiency allows the production of significantly smaller nanofibers suitable for buccal administration, thereby facilitating use.

DSC Analysis of Nanofiber Formulations

DSC is a technique that allows observation of melting points and displays crystal structures [37]. The thermogram of HC displayed a sharp endothermic melting peak at 220°C, consistent with literature [11]. In contrast, PVP and the Eudragit® polymers showed no melting peaks over the scanned range. The endothermic peak of HC was not observed in any of the HC-loaded nanofiber

mats. The loss of the crystalline HC peak suggests that HC obtained by electrospinning was dispersed within the polymer matrices. The hydrocortisone melting endotherm was not observed in any of the HC-loaded nanofiber mats. The amorphous polymer baselines and loss of the crystalline HC peak suggests that HC was dispersed within the polymer matrices (Figure 1).

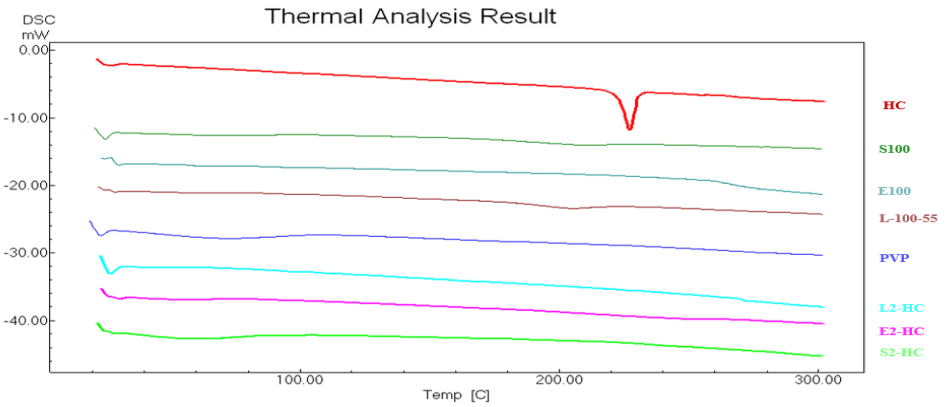

Figure 1. DSC thermograms HC, Eudragit polymers and HC-loaded nanofiber formulations

Mechanical Properties of Nanofibers

The tensile strength and elongation at break values of the nanofiber formulations are summarized in Table 4. Tensile strength values changed according to polymer type and composition. Formulations containing Eudragit L100-55 at higher concentrations generally exhibited superior tensile strength compared to Eudragit E100 and S100-based nanofibers. Formulations with higher elongation values are considered advantageous for buccal application, as they can better adapt to the dynamic movements of the oral cavity while resisting fracture. Drug incorporation generally reduced tensile strength in Eudragit S100-based fibers, as HC molecules interfering with polymer–polymer interactions. Buccal drug delivery systems require a balance between tensile strength (to maintain integrity during application) and flexibility (to prevent brittleness and ensure patient comfort). In this study, L100-55-based fibers, particularly L2-HC, provided an optimal combination of tensile strength and elongation, making them promising candidates for clinical use. Özcan et al. developed a sertraline-loaded nanofiber formulation for buccal application [38]. Tensile strength decreased from 4.301 ± 0.546 in blank nanofibers to 0.449 ± 0.284 in the sertraline-loaded formulation. Elongation at break values decreased from 35.9 ± 7.46% in the blank nanofibers to 22.6 ± 3.66% in the sertraline-loaded formulation. This reduction indicates that the fibers' elasticity was compromised, leading them to break more quickly under load. Both the elongation at break (%) and tensile strength values were reduced after the addition of sertraline. Similarly, according to our results, the addition of HC to the formulations caused a decrease in their mechanical properties.

Contact Angle of Nanofibers

Lower contact angle values (<90°) indicated higher surface hydrophilicity, while higher values (>90°) reflected more hydrophobic behavior, which is critical in predicting mucoadhesion and hydration behavior in the buccal environment. The water contact angle measurements of the nanofiber formulations ranged from 83.29 ± 3.66° to 123.60 ± 1.72°, indicating notable differences in surface wettability depending on polymer type and composition (Table 4). According to the statistical evaluation of water contact angle measurements, no significant differences were observed between E2-HC vs. L1, E2-HC vs. L3, E2-HC vs. S2, and L1 vs. L3 ($p > 0.05$). However, all other comparisons showed statistically significant differences ($p < 0.05$). These results indicate that although certain formulations displayed comparable wettability, the majority exhibited distinct surface properties, which can be attributed to differences in polymer composition and hydrophobic/hydrophilic balance.

Such variations are particularly relevant for buccal application, as they may influence the degree of hydration, swelling, and ultimately the mucoadhesive performance of the nanofiber mats. Surface wettability affects mucosal adhesion strength. The binding affinity between the formulation and the mucosal surface is due to intermolecular interactions and surface tension between the liquid and the substrate surface [39]. Hydrophobic surfaces limit polymer-mucus interactions, while excessively hydrophilic surfaces may shorten the retention time due to premature erosion [40].

Release Studies of Nanofibers

The release profiles of HC from the buccal nanofiber formulations demonstrated a dependence on polymer composition. E2-HC exhibited with approximately 40.26 ± 19.56% of HC released within the first hour (Figure 2). In contrast, L2-HC and S2-HC showed more sustained release patterns, with 47.87 ± 4.61% and 47.03 ± 11.08% release at 1 h and extending up to 24 h for HC release. The initial burst release observed within the first 30 minutes in all formulations is due to the presence of HC on the surface. The extent of this burst release varied depending on the formulation. L2-HC and S2-HC could be advantageous for acute symptom relief due to its rapid onset, while E2-HC may be more suitable for sustained therapeutic effects, reducing the need for frequent reapplication in buccal ulcer therapy. The relatively slower release of E2-HC compared to what was initially described can be attributed to the physicochemical properties of Eudragit E100, which is soluble at acidic pH values but shows limited solubility under the near-neutral buccal pH (6.8) used in the *in vitro* release studies. This pH-dependent solubility reduced the dissolution rate of the polymer matrix, thereby delaying the complete release of hydrocortisone. Vasantha et al. developed mucoadhesive buccal patches based on Eudragit and loaded with salbutamol sulfate [41]. All formulations released more than 80% of the drug within 90 minutes. No correlation was found between the drug release profile and the polymer composition. Mann et al developed buccal patch formulations using various polymers using solvent casting and electrospinning techniques [14]. Drug release from the formulations ranged from 84.80% to 89.90%. The formulations exhibited controlled release, reaching a maximum within 6 hours, and therefore, they stated that no penetration enhancers were required. Among these, L2-HC emerges as the most promising sustained-release candidate with favorable mechanical strength and mucoadhesive performance.

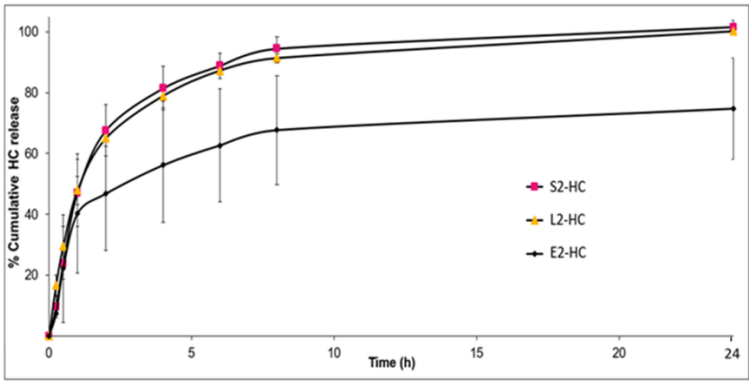

Figure 2. *In vitro* release study with Franz Diffusion cells over 24 hours (n=3, Mean± SD)

***Ex Vivo* Mucoadhesion Properties of Nanofibers**

Mucoadhesion is a fundamental prerequisite for effective buccal application; poor adhesion can lead to the film being spit out or swallowed [42]. Therefore, mucoadhesive properties are a critical performance parameter in formulation development, and mucoadhesive force measurements quantify the ability of the polymer to adhere to the buccal mucosa. The mucoadhesion data was used to compare the retention potential of different polymer combinations in the buccal environment. Among the HC-loaded formulations, E2-HC exhibited the highest mucoadhesion value, indicating a

synergistic contribution of polymer composition and fiber morphology to adhesion strength. This enhanced adhesion is associated with increased surface roughness and wettability, which facilitates intimate contact between the fiber mat and the mucosal surface, thus improving interfacial interactions. However, mucoadhesion was not considered as the sole criterion for buccal application. Since the formulation is expected to remain at the application site for a certain period, mechanical flexibility and balance between adhesion and elasticity were also taken into account. In addition, contact angle values were evaluated, as it directly influences hydration and polymer–mucosa interactions, which are critical for sustained adhesion and patient comfort. Tort et al. developed rapidly dissolving ornidazole-loaded PVP fibers for gingivitis [43]. They reported that mucoadhesion values ranging from 0.085 ± 0.035 to 0.117 ± 0.064 mJ/cm². The mucoadhesion results obtained 0.10 ± 0.01 to 0.26 ± 0.01 mJ/cm² in our study. These results demonstrate that the developed HC-loaded nanofiber formulations showed mucoadhesive performance comparable previous study. Reda et al reported that ketoprofen-loaded Eudragit electrospun nanofibers were develop for the treatment of oral mucositis [36]. They stated that the mucoadhesive properties of the nanofibers could be due to the large number of COOH groups in the polyanionic structures of the Eudragit polymers used, which could form hydrogen bonds with mucosal tissue, and the large surface area of the nanofibers that allows them to interact with biosurfaces.

Table 4. Characterization results of nanofiber formulations (n = 3, mean ± SD)

Formulation code	Contact angle (°)	Tensile strength (MPa)	Elongation at break values (%)	Work of mucoadhesion (mJ/cm^{-2})
E1	86.35±4.08	0.17±0.01	1.24±1.20	0.10±0.01
E2	86.57±9.73	0.17±0.04	13.35±6.33	0.12±0.01
E3	85.67±12.51	0.48±0.07	11.62±1.26	0.10±0.02
E2-HC	83.29±3.66	0.28±0.09	9.26±2.01	0.20±0.05
L1	123.6±1.72	5.29±1.10	6.69±1.56	0.11±0.02
L2	104.42±0.26	1.48±0.65	2.69±0.25	0.15±0.03
L3	109.15±0.91	0.87±0.57	1.057±0.37	0.18±0.07
L2-HC	99.52±5.84	0.93±0.24	13.42±6.44	0.12±0.03
S1	94.15±3.94	1.42±0.19	13.09±2.35	0.11±0.01
S2	93.72±2.15	1.38±0.65	5.72±1.57	0.12±0.04
S3	109.19±2.06	0.83±0.22	2.49±0.56	0.26±0.01
S2-HC	88.10±6.63	0.25±0.13	3.52±1.42	0.14±0.01

Conclusion

Hydrocortisone-loaded buccal nanofibers produced via the electrospinning technique demonstrate considerable potential as an innovative drug delivery platform for the treatment of oral ulcers, offering prolonged residence time, targeted drug release, and improved patient compliance compared to conventional dosage forms. Physicochemical characterization demonstrated that polymer composition and HC incorporation significantly influenced solution properties, fiber morphology, mechanical performance, wettability, and mucoadhesion. Among the tested formulations, S2-HC exhibited the highest mucoadhesion, while L2-HC achieved the most favorable balance between tensile strength, elongation at break, and adhesion performance. Tensile strength and elongation at break are parameters of critical importance for buccal application. If these parameters are considered the L2-HC formulation can be regarded as one of the most promising candidates, offering a favorable balance between mechanical and adhesion performance. Overall, the nanofiber formulations demonstrated the capability to provide effective HC delivery to the buccal mucosa, with formulations offering mechanical robustness, adequate adhesion, and controlled drug release. Future *in vivo* studies are required to confirm the clinical efficacy, retention behavior, and patient acceptability of these buccal nanofiber patches.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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