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Three-dimensional bioprinting of mucoadhesive scaffolds for the treatment of oral mucosal lesions; an *in vitro* study

Maryam Koopaie^{1†}, Duha Hayder Mohammad Ali Nassar^{2*†} and Mahvash Shokrolahi³

Abstract

Background: Chronic oral lesions could be a part of some diseases, including mucocutaneous diseases, immunobullous diseases, gastrointestinal diseases, and graft versus host diseases. Systemic steroids are an effective treatment, but they cause unfavorable and even severe systemic side effects. Discontinuation of systemic corticosteroids or other immunosuppressive drugs leads to relapse, confirming the importance of long-term corticosteroid use. The present study aims to fabricate a mucoadhesive scaffold using three-dimensional (3D) bioprinting for sustained drug delivery in oral mucosal lesions to address the clinical need for alternative treatment, especially for those who do not respond to routine therapy.

Methods: 3D bioprinting method was used for the fabrication of the scaffolds. Scaffolds were fabricated in three layers; adhesive/drug-containing, backing, and middle layers. For evaluation of the release profile of the drug, artificial saliva was used as the release medium. Mucoadhesive scaffolds were analyzed using a scanning electron microscope (SEM) and SEM surface reconstruction. The pH of mucoadhesive scaffolds and swelling efficacy were measured using a pH meter and Enslin dipositive, respectively. A microprocessor force gauge was used for the measurement of tensile strength. For the evaluation of the cytotoxicity, oral keratinocyte cells' survival rate was evaluated by the MTT method. Folding endurance tests were performed using a stable microsystem texture analyzer and analytic probe mini tensile grips.

Results: All scaffolds had the same drug release trend; An initial rapid explosive release during the first 12 h, followed by a gradual release. The scaffolds showed sustained drug release and continued until the fourth day. The pH of the surface of the scaffolds was 5.3–6.3, and the rate of swelling after 5 h was $28 \pm 3.2\%$. The tensile strength of the scaffolds containing the drug was 7.8 ± 0.12 MPa. The scaffolds were non-irritant to the mucosa, and the folding endurance of the scaffolds was over three hundred times.

Conclusion: The scaffold fabricated using the 3D bioprinting method could be suitable for treating oral mucosal lesions.

Keywords: 3D bioprinting, Drug delivery systems, Controlled release, Mucoadhesive scaffold, Scaffold

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Background

Oral mucosal lesions are usually painful lesions associated with discomfort [1], leading to nutritional deficiencies in severe cases [2]. Oral ulcerative lesions can be divided into acute and chronic, depending on their manifestation and development [3]. Chronic oral lesions could manifest some diseases, including immunobullous



diseases such as pemphigus vulgaris and graft-versus-host disease (GVHD) [4, 5]. Wound healing involves a sequence of complex biological processes performed in all tissues with the same pattern to complete the healing process with minimal scarring [6, 7]. The current treatment for oral ulcerative lesions involves using medications such as mouthwashes, creams, or ointments that are less effective because the medication does not have enough time to contact the lesion. In addition, common types of buccal drug delivery systems do not allow the patient to consume food and drink simultaneously, or in some cases, patients will complain of difficulty speaking [6, 8]. Topical corticosteroids can be used as adhesive vehicles or mouth rinses [9]. However, their therapeutic benefits are not always evident, and side effects are caused by topical corticosteroids, including secondary candidiasis, nausea, refractory response, mucosal atrophy, oral dryness, sore throat, unpleasant taste, and delayed healing [9, 10]. Some creams currently contain steroids, ointments, or pastes specially made, tested, and suitable for the oral cavity with limited efficacy [11, 12]. Besides this, the attachment and adhesion of most creams, ointments, and pastes to the saliva covering the oral mucosal surfaces is unfavorable mouth and tongue movements also increase their removal after application, resulting in short drug delivery times and being less effective [13]. Systemic steroids are more effective but cause severe systemic side effects [14]. Discontinuation of systemic corticosteroids leads to relapse, confirming the importance of long-term corticosteroid use [15]. These problems intensify the clinical need for alternative treatments, especially for those who do not respond to routine therapy; alternative treatments should have the ability to deliver controlled amounts of the drug locally to the lesion site [10, 16]. Oral mucosa is considered an important route for drug delivery [17]. Oral mucosa has arteries that are very permeable and accessible, allowing for systemic absorption of the drug painlessly and with a stable rate, bypassing the stomach and initial metabolism in the liver, which leads to an increase in its biological activity [13, 18]. The permeability of the oral mucosa is estimated to be about 4000 times that of the epidermis, which helps the drug be absorbed rapidly [19, 20]. Mucoadhesion is valid for drug delivery, such as tablets, patches, and gels [21]. Benefits of mucoadhesive scaffolds include increased residence time at application sites, drug protection, increased drug penetration, and enhanced drug availability [22, 23]. Using a mucoadhesive membrane will reduce toxicity, maximize the drug dose to the lesion, and minimize dose [24]. Mucoadhesive improves the advantage of drug localization in the affected region [25]. There are various systems for drug delivery to the mucosal layer, including adhesive tablets, gels, and, more

recently, films, which have been developed. Buccal films are superior to sticky tablets in terms of flexibility and comfort. In addition, buccal films are suitable for protecting wound surfaces, reducing pain, and increasing the effectiveness of treatment [26, 27]. Although Nesic et al. reviewed three-dimensional (3D) printing techniques for tissue regeneration and oral vascular rehabilitation [28], limited studies have been performed on oral drug delivery to treat oral lesions through 3D printing [29]. Regarding limited data and studies about treating oral mucosal lesions with mucoadhesive scaffolds and the lack of standardization of these techniques, this study aims to fabricate a mucoadhesive scaffold by 3D bioprinting for drug delivery systems to treat oral mucosal lesions.

Methods

Ethical statement

This study was approved by the Tehran University of Medical Sciences Ethical Committee (ethical code: IR.TUMS.DENTISTRY.REC.1399.100). All methods were performed in accordance with the relevant guidelines and regulations.

Preparation of bio-ink

Single layered scaffold

The scaffolds in the first step were fabricated in one layer, and for the fabrication of the scaffolds and preparation of the biological ink, alginate was selected as the base material for preparing patches containing the drug. Regarding the alginate properties in mucosal adhesion and the ability to slow drug release [30, 31], alginate was selected as the scaffold polymer base, and calcium sulfate was used to cross-link alginate partially and improve its printability [32]. For this purpose, according to Table 1, solutions were prepared, and their printability was checked. Since the compounds proposed using CaSO₄ were not uniform enough and did not show good printability, the 3.0% gelatin and CaCl₂ as cross-linkers will improve the printability.

Table 1 Chemical composition of drug-containing inks

Solvent	Concentration		Crosslinker	BioInk: Crosslinker
	Alginate	Drug		
Deionized water	8.0%	0.8%	2.5% CaSO ₄	10:1 8:1
Deionized water	6.0%	0.8%	2.5% CaSO ₄	10:1 8:1
Deionized water	7.0%	0.8%	5.0% CaCl ₂	10:1 8:1

Table 2 Chemical composition of improved solutions as drug-containing inks

	Material	Concentration	Crosslinker (Type and concentration)	Solvent
Bottom layer	Ethylcellulose		5.0% CaCl ₂	Deionized water
Middle layer	Ethylcellulose & HPMC			
Top layer	Alginate	7.0%		
	Gelatin	3.0%		
	HPMC	0.8%		
	Drug	0.8%		

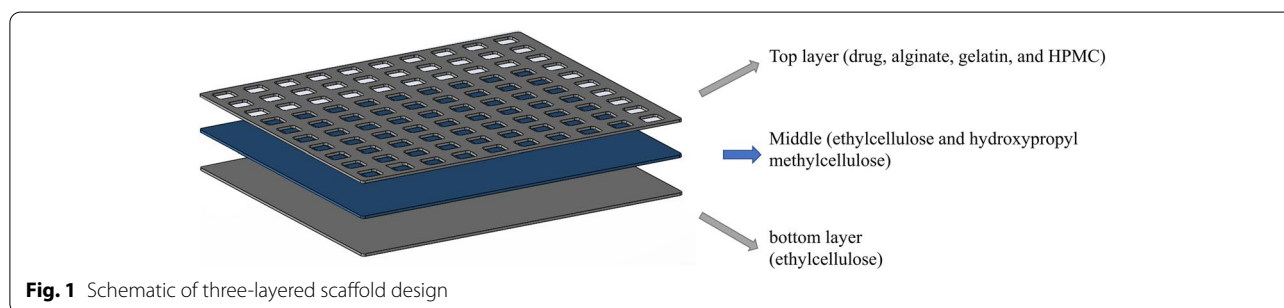


Fig. 1 Schematic of three-layered scaffold design

Three-layered scaffold

After fabricating a single-layered scaffold, we fabricated the scaffolds in three layers because of the need for a hydrophobic layer to protect the scaffold from the oral cavity. The three-layered scaffold is composed of the bottom layer, which is a hydrophobic layer of ethylcellulose that protects the scaffold in the oral cavity. The middle layer combines ethylcellulose and hydroxypropyl methylcellulose (HPMC) that holds the bottom layer and the top layer to be printed together. The top layer containing the drug is alginate, gelatin, and HPMC (Table 2).

Three dimensional (3D) bioprinting of oral scaffolds

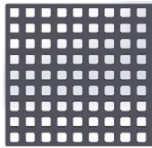
The 3D bioprinting method was used for the fabrication of the scaffolds. A mesh-like design was used for 3D bioprinting (20 × 20 × 2 mm dimensions) to print the oral scaffold. Printing geometry and characteristics of the single-layered and three-layered scaffolds (Fig. 1) are shown in Tables 3 and 4, respectively.

Evaluation of *in vitro* drug profile release

Betamethasone

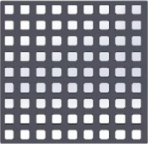
Artificial saliva was selected as the release medium to evaluate the drug release profile. For this purpose, first, specific concentrations of betamethasone as a drug were prepared in artificial saliva, and after reading their absorption rates at 190 to 500 nm NanoDrop (BOECO Micro UV-VIS, Germany), a graph of the absorption

Table 3 Printing geometry and characteristic of the single-layered scaffold

Print geometry	The diameter of the nozzle	0.5 mm
	The distance between two strings	2.2 mm
	Dimensions	20 × 20 × 2 mm
	Print design	
Print conditions	Temperature	25 °C
	Pressure	0.3 bar
	Print speed	15 mm/s
	Layer height	Each layer is 100 μm

rate was drawn according to the concentration. The printed scaffold was placed in 20 ml of artificial saliva in a 37 °C incubator shaker to simulate physiological conditions. At regular intervals, 1 ml of the release medium was removed, and the same amount of fresh saliva was added to the release medium. After absorption, samples were taken from the release medium and were read at a wavelength of 246 nm and calculated using the standard diagram and Eq. 1 [33]. The cumulative value of the drug released from the sample was calculated.

Table 4 Printing geometry and characteristics of the three-layered scaffold

Print geometry	The diameter of the nozzle	0.5 mm
	The distance between two strings	2.2 mm
	Dimensions	20 × 20 × 2 mm
	Print design of top layer	
Print conditions	Temperature	25 °C
	Pressure	0.3 bar
	Print speed	15 mm/s
	Layer height	Each layer is 50 μm

$$Er(\%) = \left(\frac{V_o \times C_n + V_r \times \sum 1^{n-1} C_i}{M_{total}} \right) \times 100 \quad (1)$$

M_{total} : The total amount of drug trapped in the sample

V_o : Volume of the release medium

V_r : Volume of the alternative medium

C_n : Drug concentration in the samples

Prednisolone

In the first step, before examining the ability to release prednisolone from multilayer scaffolds externally, its standard concentration diagram was drawn. For this purpose, artificial saliva solutions were considered as a receptor phase (release medium) in drug release studies. Artificial saliva solutions containing different amounts of the drug were prepared and after normalizing the device compared to the artificial saliva solution, the drug absorption at the maximum wavelength (243 nm) was determined by NanoDrop (BOECO Micro UV-VIS, Germany). This experiment was repeated for three days, and the standard graph of mean absorption was plotted as a function of their concentration in the saliva solution.

In order to evaluate the amount of drug released from the prepared scaffolds, specific dimensions (15 mm × 15 mm) of each scaffold with a specific weight were immersed in jars containing 4 ml of artificial saliva solution and then incubated at 37 °C; This operation was repeated three times for each scaffold. At specified times, the solution inside the jars was transferred to the NanoDrop machine tank and immediately replaced with a fresh saliva solution. After measuring the amount of absorption by the NanoDrop device, the amount of absorption was converted to concentration using a standard concentration chart, and the average amount of the drug was calculated by repeating the measurement of its

release. Then, the cumulative release percentage of the drug ($Er\%$) was calculated by Eq. 1, and the cumulative release diagram of the drug was plotted as a function of incubation time. The scaffolds were printed using BioFab X2 machine (3D BIO, Omidafarinan company, Iran) at 25°C during the printing. Printed scaffolds were cured using the UltraViolet-C (UV-C) lamp for 4 min.

Scanning electron microscopy (SEM) and 3D surface reconstruction

SEM images were taken using scanning electron microscopy (ZEISS EVO MA 25, Zeiss Evo 25, Germany). Images were prepared with a voltage of 5–10 kV. Samples were dehydrated before imaging. The specimens were connected to a 5 mm diameter stub pin using a 350 mm diameter pin. The samples were coated with gold using a sputtering method for 2 min at 15 Ma (JEOL Ltd., Tokyo, Japan) under an argon atmosphere, and images were taken at different resolutions. To evaluate the surface topography of scaffolds, 3D surface reconstruction (3DSM, Carl Zeiss scanning electron microscopy) and MountainsSEM® were used.

PH evaluation

The pH of mucoadhesive scaffolds was measured using a pH meter, and the sample with dimensions of 10 cm² was dissolved in 10 ml of water. This process was repeated three times for each sample.

Folding endurance test

A folding endurance test assesses the scaffold’s ability by folding and bonding the scaffold without breaking or cracking it. Folding endurance tests were performed using a stable microsystem texture analyzer and analytic probe mini tensile grips. A folding endurance test was performed by bending the scaffold several times, up to 300 times, until the scaffold was torn.

Swelling efficacy profile measurement

It was measured by using Enslin dipositive. The samples were exposed to a buffer solution at pH 6.8 for 5 h. The dimensions of the scaffold used were 1 cm². Our scaffold was placed on a sintering filter, and the volume of material absorbed by the samples was measured by pipetting after 5 h. The volume of fluid absorbed by the samples was recorded.

Evaluation of cytotoxicity

To evaluate the toxicity of the scaffold, oral keratinocyte cells cultured from DMEM medium were cultured in 6-well plates (3 × 10⁵ cells per well). The cells were incubated for 24 h; Then the cells were examined in 3 groups: 1- Cells in the vicinity of the drug-free scaffold, 2- Cells in

the vicinity of the drug-containing scaffold, and 3- Cells in the control group. 4.5 ml of culture medium was added to each group. After 24 h of cell incubation in the vicinity of the film, the rate of cell survival in 3 groups was evaluated by 4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) method. Each group was repeated three times, and the mean value (95% CI) was reported.

Measurement of tensile strength

A microprocessor force gauge and a scaffold evaluated the tensile strength using SANTAM-STM50 (SANTAM-Eng. Design Co. LTD) with the force–displacement (F–D) and stress–strain (σ – ϵ) diagram (ASTM D1708). The scaffold was mounted between the upper and lower clamps. The upper clamp was moved at 2 mm/min speed until the scaffold was torn. The drug-free and drug-containing scaffold tensile strength was recorded based on the number displayed in the force gauge.

Results

Printability

Single layered scaffold

By examining different formulations, it was observed that the combination of 7wt% alginate and 3wt% gelatin was found to have good printability after evaluating various formulas. As the number of layers increased and the ink warmed up, the material dispersed, and the layers collapsed. Macro image of a 3D printed scaffold is shown in Fig. 2.

Three-layered scaffold

By examining the different formulations that were described in the material section, it was observed that the combination of 7 wt% alginate, 3 wt% gelatin, 0.8 wt% HPMC, and drug (based on the weight of polymers) for the top layer has acceptable printability. Cross-section view of scaffolds with different magnifications are illustrated in Figs. 3 and 4.

As shown in Fig. 3, the thickness of the three layers is about 150 μ m, and Fig. 4 depicts that the thickness of each layer is 50 μ m, confirming the desired thickness of scaffolds.

SEM images of 3-layered scaffold surface are shown in Fig. 5 and Fig. 6.

SEM images of 3-layered scaffold surface with a magnification of 50 to 500 are shown in Fig. 7.

SEM images (Fig. 8-A), surface topography (Fig. 8-B), and line scan of surface roughness (Fig. 8-C) of scaffolds using 3D surface reconstruction (3DSM, Carl Zeiss scanning electron microscopy) and MountainsSEM[®] are shown in Fig. 8.

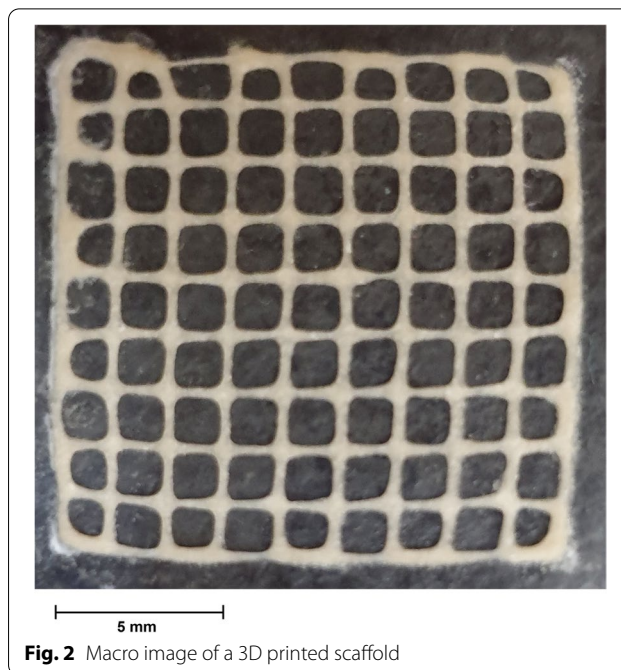


Fig. 2 Macro image of a 3D printed scaffold

pH and folding endurance measurement results

The pH of the scaffold surface was in the range of 5.3–6.3. This range is close to the normal pH of the oral cavity, and therefore it does not irritate the mucosa. The folding endurance of the scaffold is more than 300 times.

Swelling index and Tensile strength

The swelling index of the oral scaffold should ensure the patient's comfort. Excessive swelling leads to discomfort to the patients and results in removing the scaffold from the mucosa. Swelling further causes faster and uncontrollable drug release. Swelling further causes faster and uncontrollable drug release. The rate of swelling after 5 h was $28 \pm 3.2\%$. The tensile strength of the scaffold without the drug was 7.1 ± 0.34 MPa and 7.8 ± 0.12 MPa in the scaffold containing the drug.

Evaluation of Cytotoxicity

MTT results showed that after 1 h of incubation, drug-free scaffolds did not reduce cell viability compared to the control group, and therefore it can be considered non-irritant (Fig. 9).

Drug Release

Betamethasone

An absorption spectrum was first taken from the betamethasone drug between 190 and 800 nm to prepare the standard diagram and release profile (Fig. 10).

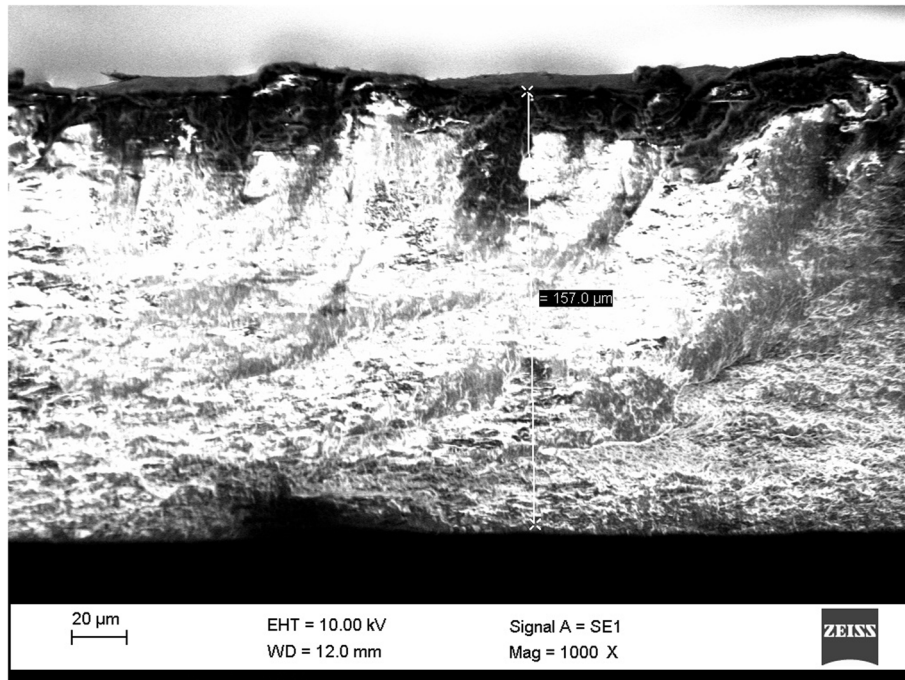


Fig. 3 Cross-section view of three-layered scaffold (Magnification = 1000 X)

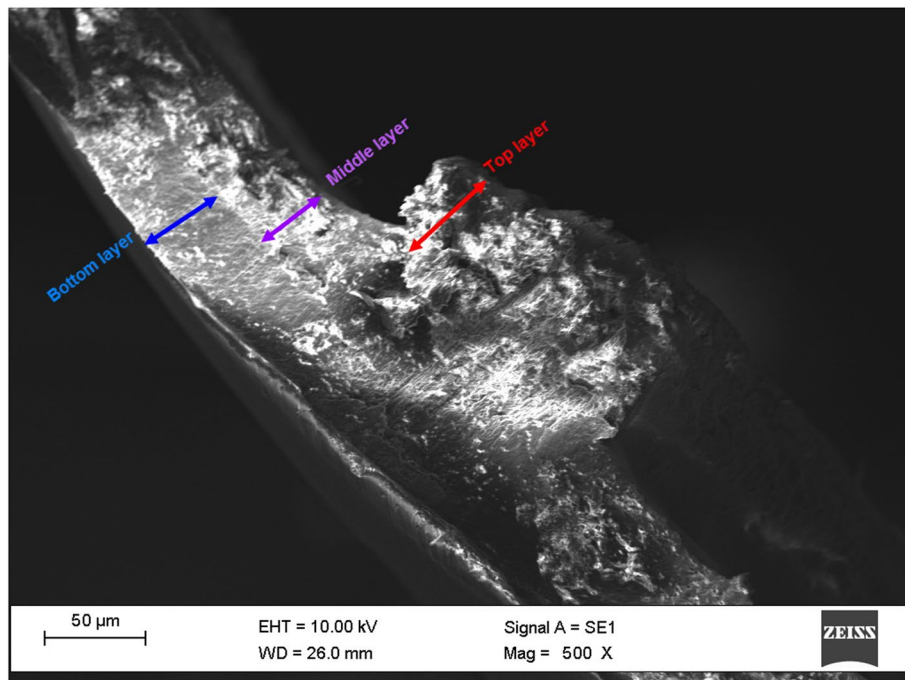


Fig. 4 Cross-section view of three layered scaffold (Magnification = 500 X)

In the absorption spectrum of betamethasone, it was observed that there was a peak at the wavelength of 246 nm. Therefore, this wavelength was chosen as an

identifier to draw the standard diagram and read the absorption of release samples. After the scaffold containing the drug was printed and placed in the release

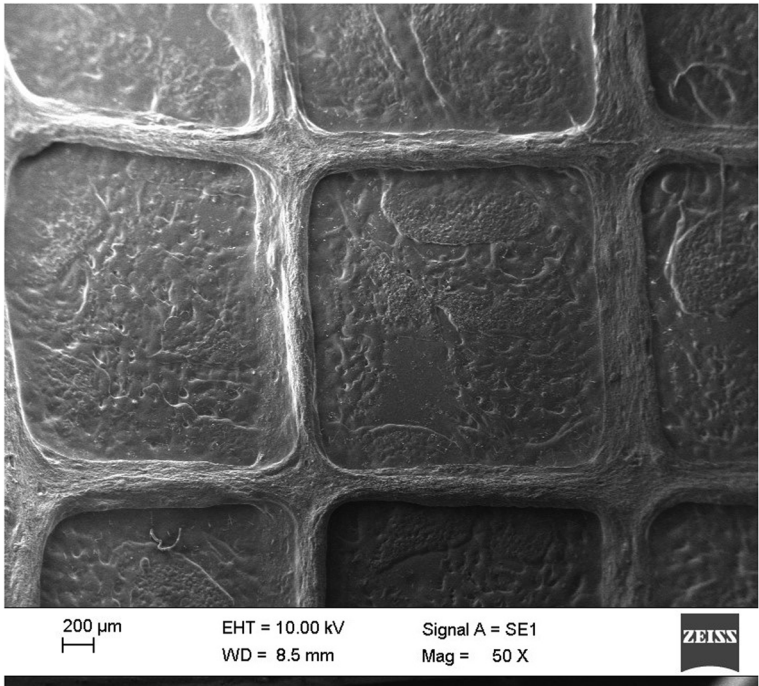


Fig. 5 SEM image of 3 layered scaffold (Magnification = 50 X)

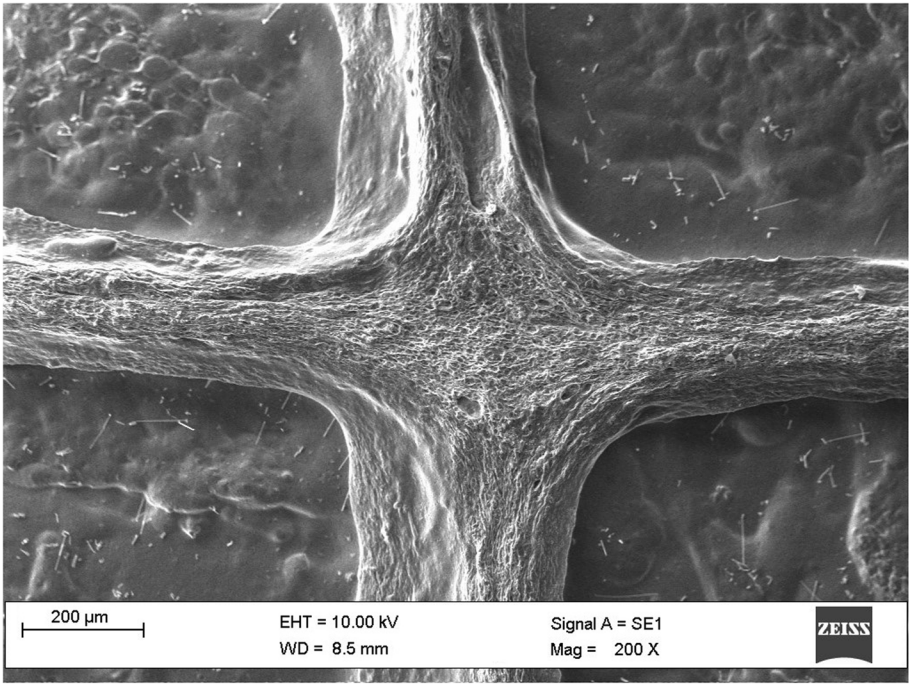


Fig. 6 SEM image of 3-layered scaffold (Magnification = 200 X)

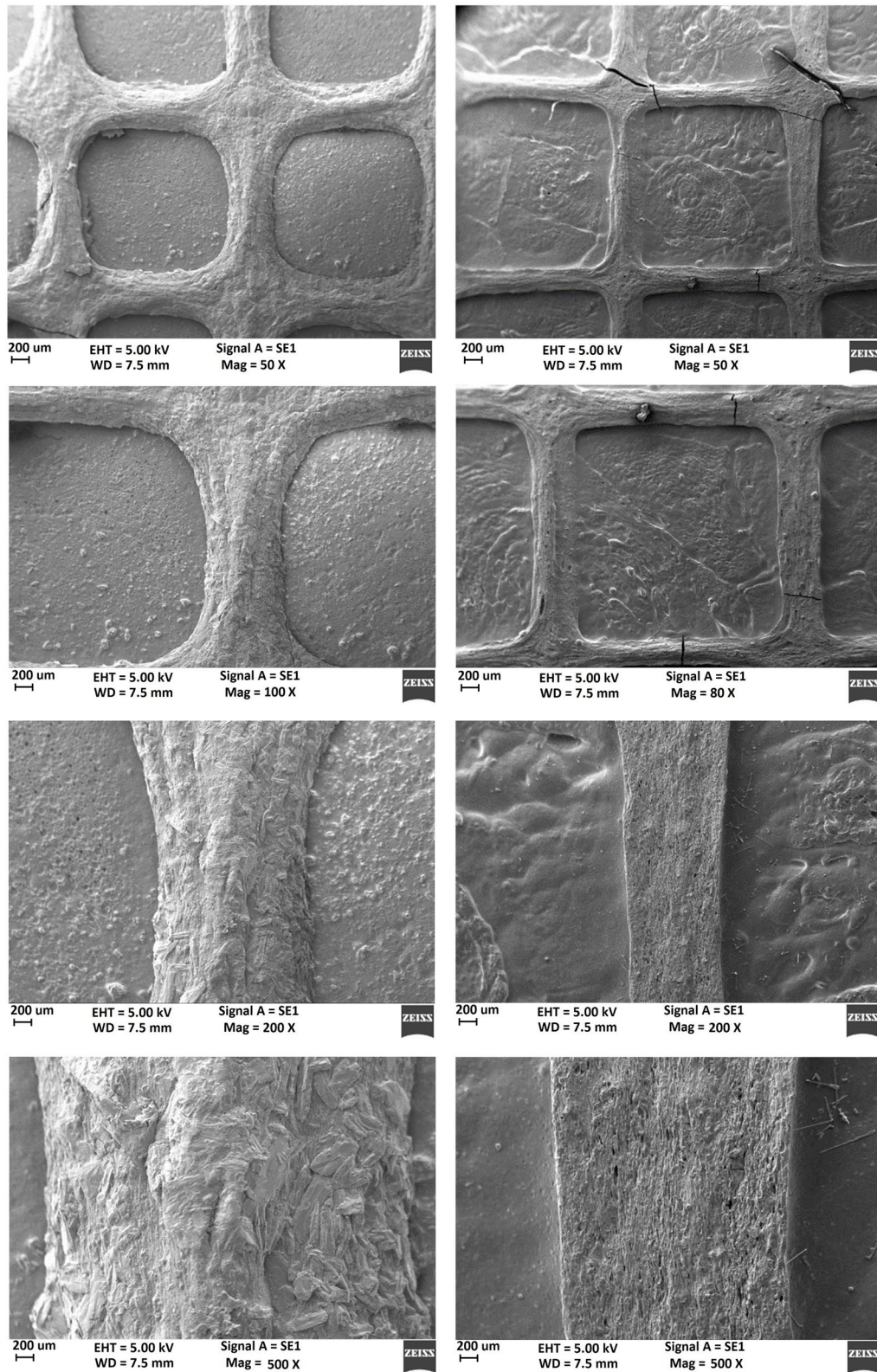
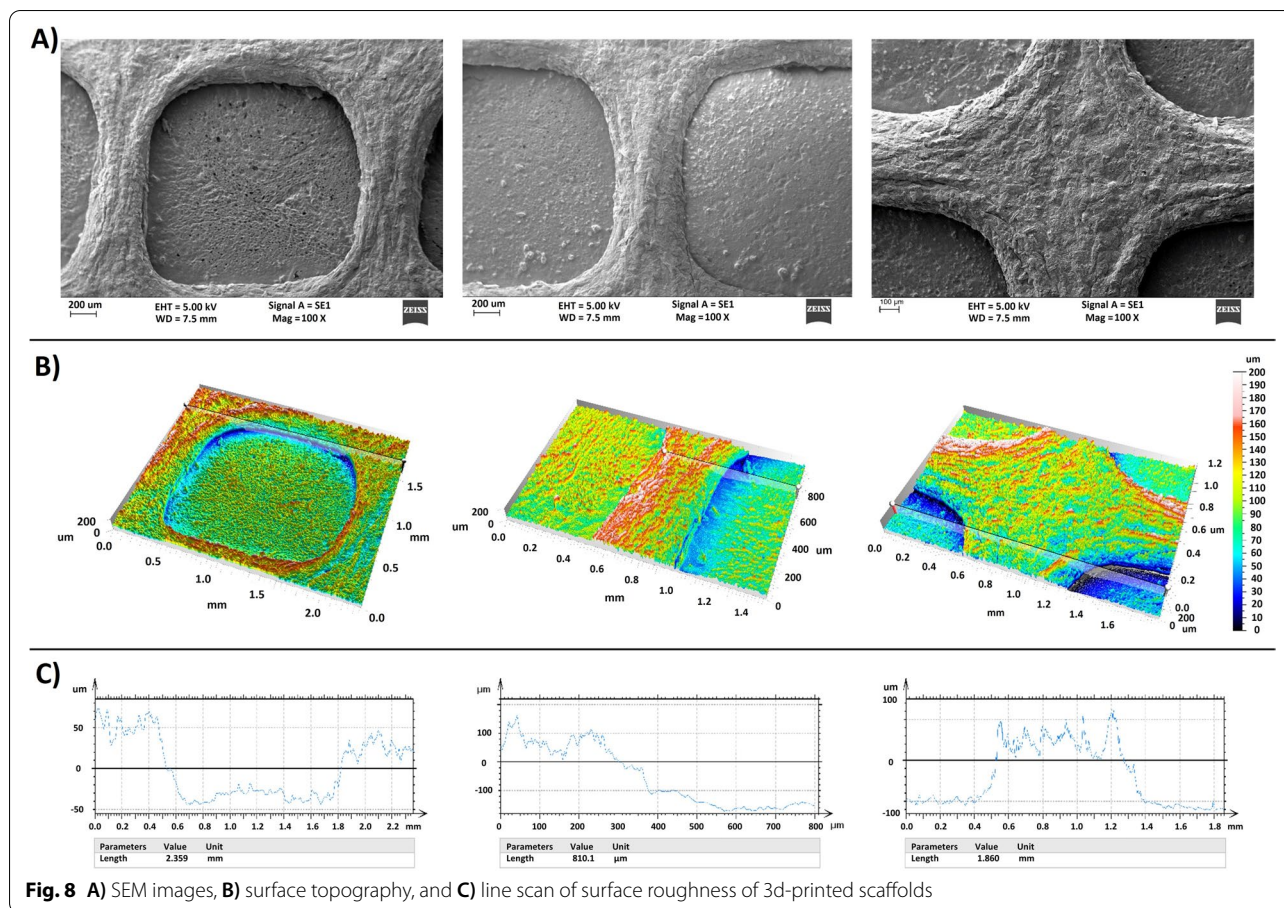
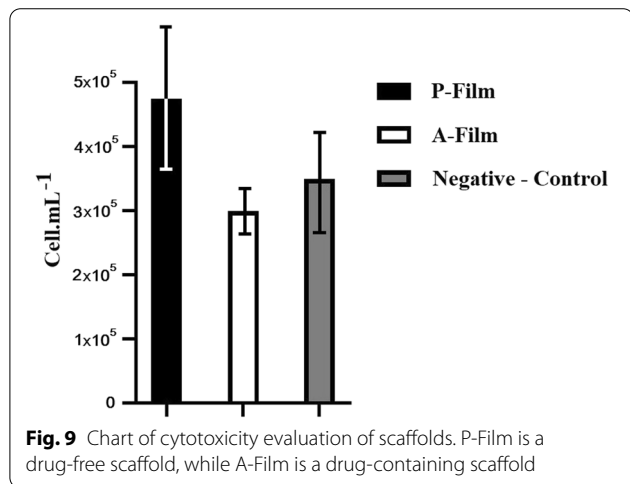


Fig. 7 SEM image of 3-layered scaffold surface (Magnification = 50 to 500 X)



medium and the incubator, the sample was taken at different hours. Finally, the absorbance of the samples at 246 nm was read. The concentration of drugs released into the release medium was calculated by placing the absorption numbers in the formula obtained in the



standard diagram. To calculate the cumulative percentage of the drug, the weight of the printed scaffold was first measured. By establishing the ratio between the weight of the scaffold and the weight of one milliliter of unprinted ink, the volume of ink printed with a drug concentration of 0.8%, the resulting amount of drug in the scaffold was obtained (Fig. 11).

According to calculations, 148.304 micrograms of the drug were placed in the release environment in the printed scaffold. The following chart was drawn by placing the concentrations obtained from the standard chart in the cumulative percentage formula (Table 5).

As seen in the cumulative release chart, after nine days, about 21% of the drug in the printed scaffold was released into the environment (Fig. 12).

Repeated release test

The ink containing the drug was prepared by combining 5wt% gelatin, 7wt% by weight alginate, and 1.2 mg/ml betamethasone to repeat the test. With drug-free ink and drug-containing ink, all three scaffolds were printed with the design mentioned in the previous test and placed in 5wt% calcium chloride for 15 min

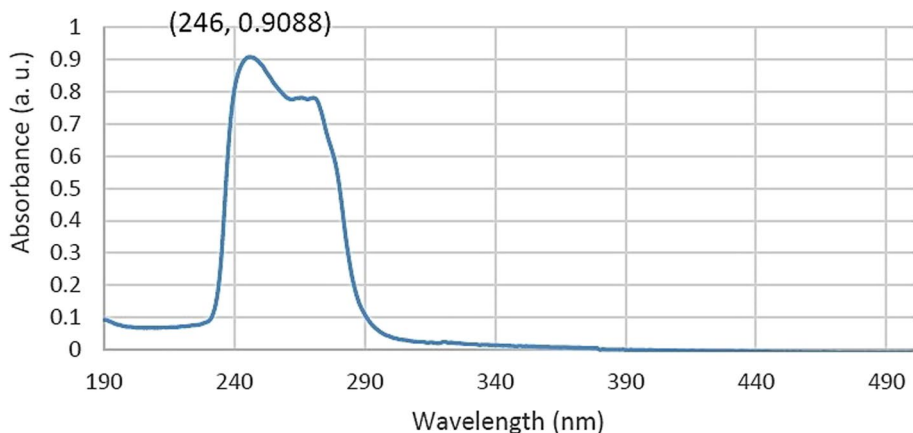


Fig. 10 Absorption spectrum of different concentrations of betamethasone in artificial saliva

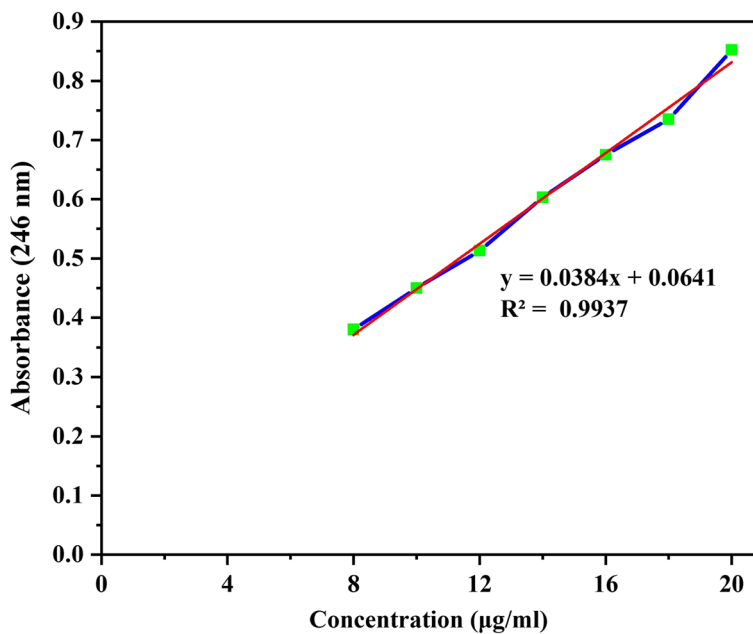


Fig. 11 Standard chart of betamethasone in artificial saliva

to cross-link. The scaffolds were then placed in a Falcon containing 20 ml of artificial saliva and transferred to a sugar incubator at 37 °C. After the scaffolds were placed in the release medium, and inside the incubator, samples were taken at different hours, and finally, the absorption of the samples was read at 246 nm. At each hour, a sample is taken from the environment where the scaffold was without medication; the device was first reset to zero. The concentration of drug released into the release medium was calculated by placing the adsorption numbers into the formula obtained in the

standard diagram. To calculate the cumulative percentage of the drug, the weight of the printed scaffold was first measured by establishing the ratio between the weight of the scaffold and the weight of one milliliter of unprinted ink, the volume of ink that was printed. Then the amount of drug present in the scaffold was obtained. According to calculations, there was an average of 0.115 mg of drug per scaffold. By placing the concentrations obtained from the standard graph in the cumulative percentage Eq. 2, and cumulative drug release was calculated (Fig. 13).

Table 5 Concentration and cumulative percentage of drug released into the release medium at different hours

Time (hour)	ΣC (ug/ml)	Cumulative release (%)
1	0.017	2.304
2	0.018	2.674
3	0.019	2.930
4	0.019	3.058
5	0.021	3.471
24	0.057	8.749
72	0.059	9.415
144	0.064	10.450
216	0.136	21.087

$$Er(\%) = \left(\frac{V_o \times C_n + V_r \times \Sigma 1^{n-1} C_i}{M_{total}} \right) \times 100 \quad (2)$$

After nine days, when we used Betamethasone as a drug, only about 21% of the drug in the printed scaffold was released into the environment. We wanted to reach an optimum concentration in the release test we took, and Betamethasone had a low concentration; therefore, we changed the drug to Prednisolone 50 mg.

Prednisolone

Figure 14 shows the cumulative release and the explosive release of the three-layered scaffolds. All scaffolds had the same drug release curve: an initial rapid explosive release during the first 12 h, followed by a gradual release. The three scaffolds (0, 1, and 2) were released respectively 66.27%, 75.34%, and 81.88% of their loaded drugs during the first 12 h. Scaffold 2 had a smaller diameter than the other two, and as the diameter decreases due to the increase in the sample’s surface area, more drug molecules are in contact with the release medium, causing a higher drug to be released during the first hours. After 24 h, the entire loaded drug was released. Scaffold 0 had a slower release rate, and the initial slope of its release diagram was lower than the other two scaffolds, and the release of the drug continued until the fourth day (Fig. 14).

Scaffold containing Prednisolone could release all the drugs, and even the scaffold was disintegrated. We could reach to high drug concentration release that we could not reach with Betamethasone.

Discussion

The present study aimed to design a mucosal scaffold by 3D bioprinting and gradual release of the drug into the oral environment. The 3D bioprinting method is an accurate and practical method for making bio-scaffolds. In the present study, a mucoadhesive scaffold consisting of three layers was fabricated, which contained a

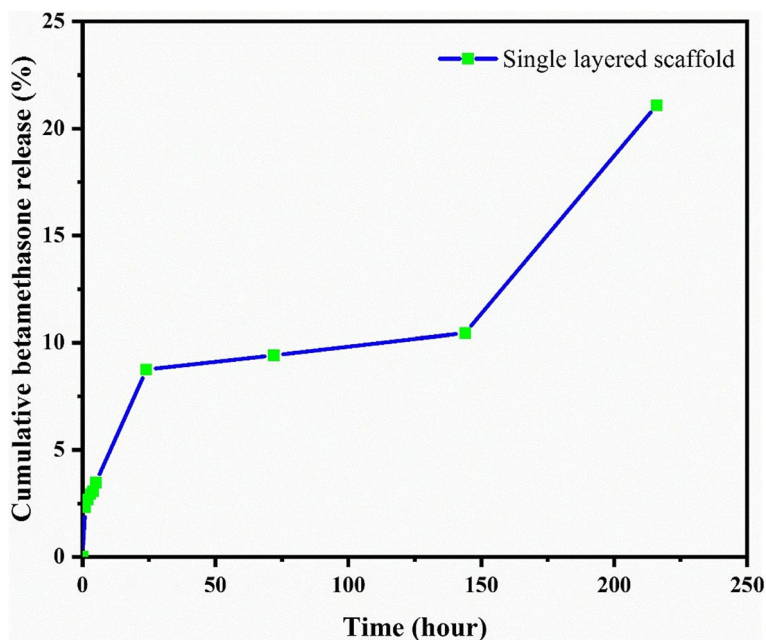
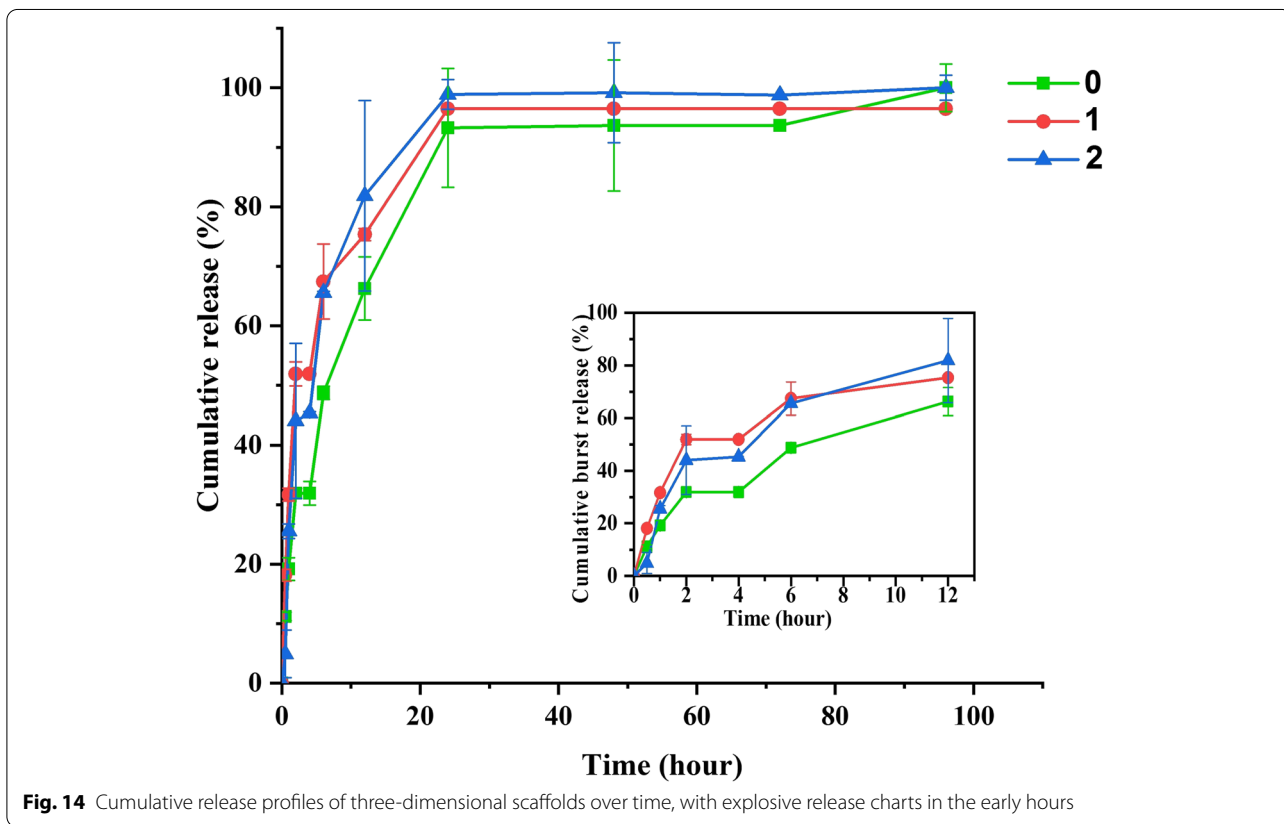
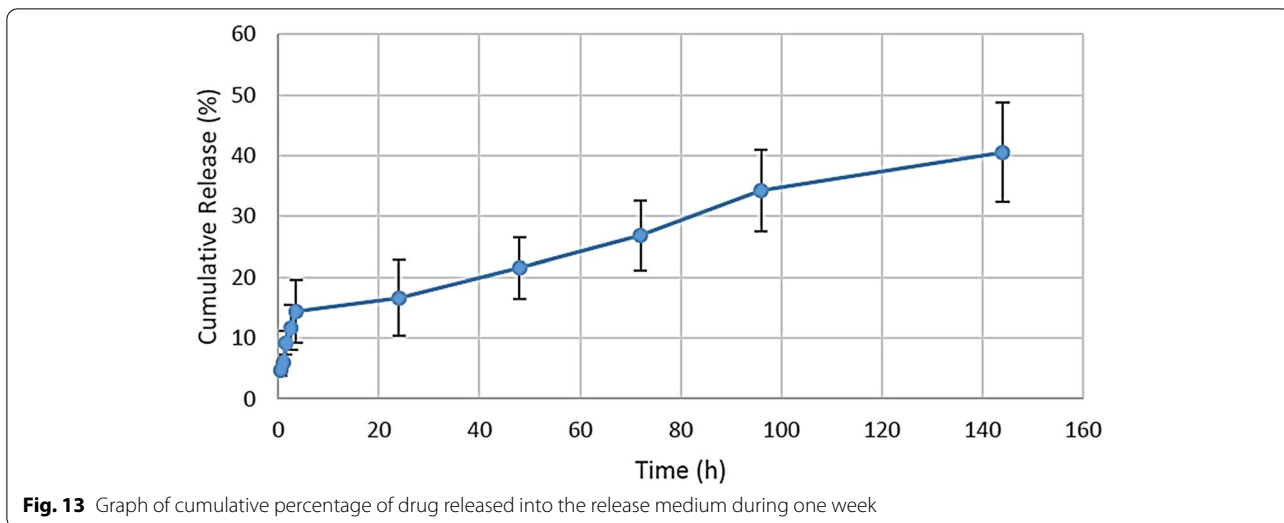


Fig. 12 Diagram of the cumulative release of betamethasone in artificial saliva



bioadhesive layer and a hydrophobic protective backing layer. These scaffolds showed acceptable mechanical and biological properties. The pH of the fabricated scaffolds was 5.3–6.3, similar to the saliva pH (5.6–7.9) [34, 35]. This pH does not cause irritation or tissue damage. Successful bio-adhesion depends on the rapid hydration

of scaffolds [36]. The scaffolds in the present study show rapid swelling, which is an acceptable feature for scaffolds. Another feature of the fabricated scaffold in the present study was sustained drug release, while most similar studies did not have this feature [37–40].

Santocildes-Romero et al. fabricated mucoadhesive membranes by electrospinning to treat mucosal lesions such as oral lichen planus lesions [41]. They used a double-layered (adhesive/drug-containing layer and a backing layer) membrane, while ours, a three-layered scaffold (protective layer/middle layer/drug-containing layer) was fabricated. They attached the layers using the melting method, while we used CaCl_2 cross-linker to attach the layers that other studies showed that CaCl_2 cross-linked could be used for prolonging drug release [42]. The pH of membranes in the study by Santocildes-Romero et al. was 8.2, which is slightly more alkali than normal saliva [35], but the pH of the scaffold surface of our study was in the desired range: (5.3–6.3) and close to the normal pH. Therefore, it does not irritate the mucosa and any changes in saliva could lead to oral microbiome imbalance and diseases [43, 44]. All their patches increased in weight to approximately 70% of their weight within 60 min, but the swelling rate in our study was only $28 \pm 3.2\%$ after 5 h. Excessive swelling leads to discomfort in the patient and results in the patch's removal from the mucosa. Swelling further causes faster and uncontrollable drug release [45]. Their results showed rapid drug release [41] while ours showed sustained drug release, which is advantageous to have increased drug interpenetration and longer time of drug contact to the lesions. In our study, the release of the drug continued until day four, but in this study, the drug release continued only for 6 h, which is a fast drug delivery time [33, 36].

Tonglairoum et al. produced a one-layer scaffold of clotrimazole-microemulsion-containing nanofibers to treat oral candidiasis by electrospinning method [38]. Although their scaffolds showed low toxicity, *in vitro* drug release showed rapid drug release and lasted for only 24 h, but our *in vitro* results showed sustained drug release, and the release continued until the fourth day, which shows longer drug delivery to the lesions.

Tonglairoum et al. used a hybrid polyvinylpyrrolidone/hydroxypropyl β -cyclodextrin (PVP / HP β CD) nanofiber mat composed of clotrimazole (CZ) fabricated a one-layer with low toxicity to treat oral candidiasis [39]. This study showed rapid drug dissolution and release, while our results showed a gradual and sustained drug release, resulting in a longer drug delivery time. In another study, Tonglairoum et al. produced sandwich nanofibers composed of CZ using electrospinning. This drug showed rapid drug release, which differs from our goal, which was sustained drug release [40].

Alves et al. make patches to treat oral mucosal infections, especially targeted drugs for mucositis. They fabricated a bi-layered patch containing lidocaine, and the results showed rapid drug release. The mucoadhesive films fabricated in this study were biologically safe [37].

Hajikhani et al. manufactured composite nanofiber encapsulated collagen and cefazolin dressing scaffold using a coaxial electrospinning method to release the encapsulated composite [46]. The release rate of Cefazolin from their scaffold was such that it could be used for several days. Their scaffold had no non-polar backing layer to create a unilateral drug release to prevent Cefazolin's migration to the other side of the scaffold, but our scaffolds contained a backing layer for unilateral drug release and protection from the oral cavity. A fibrous membrane using electrospinning from an ethanol/water mixture was fabricated by Edmansa et al. The resulting fibrous membrane released the protein at a clinically desirable rate, reaching a cumulative release of $90 \pm 13\%$ after 2 h. An additional protective poly (caprolactone) backing layer was introduced to facilitate unidirectional transfer without loss of enzymatic activity [47].

Davoudi et al., a chitosan/gelatin/keratin composite containing sodium hydrocortisone succinate was formed by casting as a buccal mucosal adhesive patch to treat desquamative gingival inflammation was developed. Ethylcellulose was used as a backing layer to control the release rate. *In vitro* drug release showed a burst effect occurring in the first 3–4 h, which releases about 80% of the drug concentration. Based on the interactions between keratin and drug particles, some drugs were trapped in the system even after 120 h [48]. Alopaus et al. prepared a double-layered buccal film using the solvent casting method. This study showed rapid drug release, different from our study results [49]. Szabo et al. manufactured nanofiber buccal film for antifungal agents *in vitro* and *in vivo* study [50]. Buccal films were fabricated by the electrospinning technique. The results show that the dissolution process takes place quickly and thoroughly, resulting in rapid drug delivery, which differs from the results of our study, which showed sustained drug release.

The scaffolds used in this research demonstrate fast swelling and sustained drug release, both of which were difficult to achieve in previous investigations [37–40]. Oral mucosal diseases such as lichen planus, pemphigus, and pemphigoid are chronic diseases requiring long-term corticosteroid treatment [51]. Long-term use of high doses of topical corticosteroids can lead to adrenal and hypothalamic–pituitary–adrenal axis suppression [52, 53].

Several limitations to this study need to be acknowledged. Although it has been tried to simulate the physiological conditions of the oral cavity, the scaffolds are immersed in different solutions, so our test conditions do not accurately reflect the physiological conditions of the oral cavity. A significant challenge and limitation were to study the combination of biological inks and drugs so

that the properties of printing and drug release can be provided together.

In comparison, the gradual and continuous release of the drug from the patch reduces the final cumulative dose of the drug and reduces the likelihood of adrenal suppression. The lesions could be associated with a pain sensation; applying mucoadhesives may protect the lesions from external stimuli.

Conclusion

This study designed and fabricated a three-layer mucoadhesive scaffold comprising a hydrophilic layer. The scaffolds showed sustained drug release into the environment. A hydrophobic protective backing layer protects the mucosa, and a middle bioadhesive layer is attached to the two layers. Our results showed that the 3D bioprinting method could be used to fabricate an oral mucoadhesive scaffold with acceptable mechanical properties for oral drug delivery of oral diseases. The presence of enzymes, temperature changes, and exposure to foods affect the rate of degradation and release of the drug into the oral cavity. For this reason, it is necessary to evaluate the scaffolds containing the drug *in vivo* as the next step.

Abbreviations

3D: Three-dimensional; CZ: Clotriazole; GVHD: Graft-versus-host disease; HPMC: Hydroxypropyl methylcellulose; MTT: 4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; PVP/HPBCD: Hybrid polyvinylpyrrolidone/hydroxypropyl β -cyclodextrin; SEM: Scanning electron microscope; UV-C: UltraViolet-C.

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Authors' contributions

MK and DHMAN conceived the study idea, created the study protocol, and wrote the original draft. MK, DHMAN, MSH contributed to data analysis / interpretation and preparation of the manuscript. MK and MSH led the review and editing. MK and DHMAN interpreted the results. All authors read and approved the final manuscript.

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Availability of data and materials

Any data or results regarding this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by Tehran University of Medical Science Ethical Committee (ethical code: IR.TUMS.DENTISTRY.REC.1399.100). After describing the study objectives, written informed consent was obtained from a parent or guardian for participants under 16 years old.

Competing interests

The authors declare that they have no competing interests.

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References

1. Abdalla-Aslan R, Benoliel R, Sharav Y, Czerninski R. Characterization of pain originating from oral mucosal lesions. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2016;121(3):255–61.
2. Elad S, Zadik Y, Caton JG, Epstein JB. Oral mucosal changes associated with primary diseases in other body systems. *Periodontol* 2000. 2019;80(1):28–48.
3. Fitzpatrick SG, Cohen DM, Clark AN. Ulcerated lesions of the oral mucosa: clinical and histologic review. *Head Neck Pathol.* 2019;13(1):91–102.
4. Carrozzo M, Porter S, Mercadante V, Fedele S. Oral lichen planus: A disease or a spectrum of tissue reactions? Types, causes, diagnostic algorithms, prognosis, management strategies. *Periodontol* 2000. 2019;80(1):105–25.
5. Porter S, Mercadante V, Fedele S. Oral manifestations of systemic disease. *Br Dent J.* 2017;223(9):683–91.
6. Politis C, Schoenaers J, Jacobs R, Agbaje JO. Wound healing problems in the mouth. *Front Physiol.* 2016;7:507.
7. Valero C, Javierre E, García-Aznar J, Menzel A, Gomez-Benito M. Challenges in the modeling of wound healing mechanisms in soft biological tissues. *Ann Biomed Eng.* 2015;43(7):1654–65.
8. Morales JO, McConville JT. Manufacture and characterization of mucoadhesive buccal films. *Eur J Pharm Biopharm.* 2011;77(2):187–99.
9. Alrashdan MS, Cirillo N, McCullough M. Oral lichen planus: a literature review and update. *Arch Dermatol Res.* 2016;308(8):539–51.
10. Yang H, Wu Y, Ma H, Jiang L, Zeng X, Dan H, et al. Possible alternative therapies for oral lichen planus cases refractory to steroid therapies. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2016;121(5):496–509.
11. Rudralingam M, Randall C, Mighell A. The use of topical steroid preparations in oral medicine in the UK. *Br Dent J.* 2017;223(9):633–8.
12. Arroyo GP, Lofters A, Clarkson E. Pharmacological Management of Common Soft Tissue Lesions of the Oral Cavity. *Oral Maxillofac Surg Clin.* 2022;34(1):99–114.
13. Teelavath M, Patnaik KR. Review on buccal adhesive drug delivery system: a promising strategy for poorly soluble drugs. *J Drug Deliv Ther.* 2019;9(3-s):778–92.
14. Rice JB, White AG, Scarpati LM, Wan G, Nelson WW. Long-term systemic corticosteroid exposure: a systematic literature review. *Clin Ther.* 2017;39(11):2216–29.
15. Lattanzi S, Cagnetti C, Danni M, Provinciali L, Silvestrini M. Oral and intravenous steroids for multiple sclerosis relapse: a systematic review and meta-analysis. *J Neurol.* 2017;264(8):1697–704.
16. Santocildes-Romero ME, Hadley L, Clitherow KH, Hansen J, Murdoch C, Colley HE, et al. Fabrication of Electrospun Mucoadhesive Membranes for Therapeutic Applications in Oral Medicine. *ACS Appl Mater Interfaces.* 2017;9(13):11557–67.
17. Homayun B, Lin X, Choi H-J. Challenges and recent progress in oral drug delivery systems for biopharmaceuticals. *Pharmaceutics.* 2019;11(3):129.
18. Kurćubić I, Vajić U-J, Cvijić S, Crevar-Sakač M, Bogavac-Stanojević N, Miloradović Z, et al. Mucoadhesive buccal tablets with propranolol hydrochloride: Formulation development and *in vivo* performances in experimental essential hypertension. *Int J Pharm.* 2021;610:121266.
19. Gupta P. An overview of applications of mucoadhesive buccal film in Oral Medicine. *J Orofac Res.* 2020;2(9):14–9.
20. El-Say KM, Ahmed TA. Buccal Route of Drug Delivery. The ADME Encyclopedia: A Comprehensive Guide on Biopharmacy and Pharmacokinetics. 2021:1–10.
21. Koopaie M. 22 - Nanoparticulate systems for dental drug delivery. In: *Nanoengineered Biomaterials for Advanced Drug Delivery*. edn. Edited by Mozafari M: Elsevier; 2020:525–559.

22. Parhi R. Drug delivery applications of chitin and chitosan: a review. *Environ Chem Lett.* 2020;18(3):577–94.
23. Asati S, Jain S, Choubey A. Bioadhesive or mucoadhesive drug delivery system: a potential alternative to conventional therapy. *J Drug Deliv Ther.* 2019;9(4-A):858–67.
24. Tentor F, Siccardi G, Sacco P, Demarchi D, Marsich E, Almdal K, et al. Long lasting mucoadhesive membrane based on alginate and chitosan for intravaginal drug delivery. *J Mater Sci - Mater Med.* 2020;31(3):1–12.
25. Dahl DK, Whitesell AN, Sharma-Huynh P, Maturavongsadit P, Januszewicz R, Fox RJ, et al. A mucoadhesive biodissolvable thin film for localized and rapid delivery of lidocaine for the treatment of vestibulodynia. *Int J Pharm.* 2021;121288.
26. Bandi SP, Bhatnagar S, Venuganti VVK. Advanced materials for drug delivery across mucosal barriers. *Acta Biomater.* 2021;119:13–29.
27. Zierden HC, Josyula A, Shapiro RL, Hsueh HT, Hanes J, Ensign LM. Avoiding a sticky situation: bypassing the mucus barrier for improved local drug delivery. *Trends Mol Med.* 2021;27:436–50.
28. Nestic D, Durual S, Marger L, Mekki M, Sailer I, Scherrer SS. Could 3D printing be the future for oral soft tissue regeneration? *Bioprinting.* 2020;20:e00100.
29. Sahranavard M, Zamanian A, Ghorbani F, Shahrezaee MH. A critical review on three dimensional-printed chitosan hydrogels for development of tissue engineering. *Bioprinting.* 2020;17:e00063.
30. Shtenberg Y, Goldfeder M, Prinz H, Shainsky J, Ghantous Y, El-Naaj IA, et al. Mucoadhesive alginate pastes with embedded liposomes for local oral drug delivery. *Int J Biol Macromol.* 2018;111:62–9.
31. Laffleur F, Küppers P. Adhesive alginate for buccal delivery in aphthous stomatitis. *Carbohydr Res.* 2019;477:51–7.
32. Amr M, Counts M, Kernan J, Mallah A, Mendenhall J, Van Wie B, et al. 3D printed, mechanically tunable, composite sodium alginate, gelatin and Gum Arabic (SA-GEL-GA) scaffolds. *Bioprinting.* 2021;22:e00133.
33. Edmans JG, Clitherow KH, Murdoch C, Hatton PV, Spain SG, Colley HE. Mucoadhesive electrospun fibre-based technologies for oral medicine. *Pharmaceutics.* 2020;12(6):504.
34. Baliga S, Muglikar S, Kale R. Salivary pH: a diagnostic biomarker. *J Indian Soc Periodontol.* 2013;17(4):461.
35. Nimma V, Talla H, Poosa M, Gopaladas M, Meesala D, Jayanth L. Influence of hypertension on pH of saliva and flow rate in elder adults correlating with oral health status. *J Clin Diagn Res.* 2016;10(11):34.
36. Colley H, Said Z, Santocildes-Romero M, Baker S, D'Apice K, Hansen J, et al. Pre-clinical evaluation of novel mucoadhesive bilayer patches for local delivery of clobetasol-17-propionate to the oral mucosa. *Biomaterials.* 2018;178:134–46.
37. Alves TF, Rios AC, da Silva PK, Portella DL, Aranha N, Severino P, et al. Bilayer mucoadhesive buccal film for mucosal ulcers treatment: development, characterization, and single study case. *Pharmaceutics.* 2020;12(7):657.
38. Tonglairoum P, Ngawhirunpat T, Rojanarata T, Kaomongkolgit R, Opanasopit P. Fabrication of a novel scaffold of clotrimazole-microemulsion-containing nanofibers using an electrospinning process for oral candidiasis applications. *Colloids Surf, B.* 2015;126:18–25.
39. Tonglairoum P, Ngawhirunpat T, Rojanarata T, Kaomongkolgit R, Opanasopit P. Fabrication and evaluation of nanostructured herbal oil/hydroxypropyl- β -cyclodextrin/polyvinylpyrrolidone mats for denture stomatitis prevention and treatment. *AAPS PharmSciTech.* 2016;17(6):1441–9.
40. Tonglairoum P, Ngawhirunpat T, Rojanarata T, Panomsuk S, Kaomongkolgit R, Opanasopit P. Fabrication of mucoadhesive chitosan coated polyvinylpyrrolidone/cyclodextrin/clotrimazole sandwich patches for oral candidiasis. *Carbohydr Polym.* 2015;132:173–9.
41. Santocildes-Romero ME, Hadley L, Clitherow KH, Hansen J, Murdoch C, Colley HE, et al. Fabrication of electrospun mucoadhesive membranes for therapeutic applications in oral medicine. *ACS Appl Mater Interfaces.* 2017;9(13):11557–67.
42. Szekalska M, Sosnowska K, Czajkowska-Kośnik A, Winnicka K. Calcium chloride modified alginate microparticles formulated by the spray drying process: A strategy to prolong the release of freely soluble drugs. *Materials.* 2018;11(9):1522.
43. Pathak JL, Yan Y, Zhang Q, Wang L, Ge L. The role of oral microbiome in respiratory health and diseases. *Respir Med.* 2021;185:106475.
44. Zhang Y, Wang X, Li H, Ni C, Du Z, Yan F. Human oral microbiota and its modulation for oral health. *Biomed Pharmacother.* 2018;99:883–93.
45. Shakya P, Madhav NS, Shakya AK, Singh K. Palatal mucosa as a route for systemic drug delivery: a review. *J Control Release.* 2011;151(1):2–9.
46. Hajikhani M, Emam-Djomeh Z, Askari G. Fabrication and characterization of mucoadhesive bioplastic patch via coaxial polylactic acid (PLA) based electrospun nanofibers with antimicrobial and wound healing application. *Int J Biol Macromol.* 2021;172:143–53.
47. Edmans JG, Murdoch C, Santocildes-Romero ME, Hatton PV, Colley HE, Spain SG. Incorporation of lysozyme into a mucoadhesive electrospun patch for rapid protein delivery to the oral mucosa. *Mater Sci Eng, C.* 2020;112:110917.
48. Davoudi Z, Rabiee M, Houshmand B, Eslahi N, Khoshroo K, Rasoulian-boroujeni M, et al. Development of chitosan/gelatin/keratin composite containing hydrocortisone sodium succinate as a buccal mucoadhesive patch to treat desquamative gingivitis. *Drug Dev Ind Pharm.* 2018;44(1):40–55.
49. Alopaeus JF, Hellfritzsch M, Gutowski T, Scherließ R, Almeida A, Sarmiento B, et al. Mucoadhesive buccal films based on a graft co-polymer–A mucin-retentive hydrogel scaffold. *Eur J Pharm Sci.* 2020;142:105142.
50. Szabó P, Daróczy TB, Tóth G, Zekó R. *In vitro* and *in silico* investigation of electrospun terbinafine hydrochloride-loaded buccal nanofibrous sheets. *J Pharm Biomed Anal.* 2016;131:156–9.
51. Buonavoglia A, Leone P, Dammacco R, Di Lernia G, Petruzzi M, Bonamonte D, et al. Pemphigus and mucous membrane pemphigoid: an update from diagnosis to therapy. *Autoimmun Rev.* 2019;18(4):349–58.
52. Ahmet A, Brienza V, Tran A, Lemieux J, Aglipay M, Barrowman N, et al. Frequency and duration of adrenal suppression following glucocorticoid therapy in children with rheumatic diseases. *Arthritis Care Res.* 2017;69(8):1224–30.
53. Sampieri G, Namavarian A, Lee JJW, Hamour AF, Lee JM. Hypothalamic-pituitary-adrenal axis suppression and intranasal corticosteroid use: A systematic review and meta-analysis. *Int Forum Allergy Rhinol.* 2022;12(1):11–27.

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