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Original article

Effect of a combination of mometasone furoate, levofloxacin, and retinyl palmitate with an in situ gel-forming nasal delivery system on nasal mucosa damage repair in an experimental rabbit model

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ABSTRACT

Background: In this study a combination of Mometasone Furoate (MF) + Levofloxacin hemihydrate (LH) + Retinyl palmitate (RP) with an in situ gel-forming delivery system was evaluated at different stages of nasal mucosal damage repair in a rabbit maxillary sinus model.

Methods: In this study, 28 rabbits were included and assigned randomly to four groups. In all rabbits, a standard ostium was opened in the medial wall of the maxillary sinus by using a drill. Two different subsequently prepared gels with an in situ gel-forming delivery system were used. Of these 14 nasal cavities, combination 1 (active combination) was applied daily to 5, combination 2 (placebo) to 5, while 4 did not receive any pharmaceutical treatment. The diameter of the ostium was measured. Histopathological assessment was performed.

Results: After 2, 3 and 4 weeks, the ostium diameter was significantly wider in the group where gel 1 had been applied compared to both the placebo group and control group. In the group treated with gel 1, after 2, 3 and 4 weeks the presence of superficial cilia was significantly greater, surface epithelium significantly less. In the 4th week, histologic scores for fibroblastic proliferation and vascular proliferation in the group treated with gel 1 were better than in either the control group or the placebo group. With gel 1, chronic inflammation parameters were also significantly lower than in the other groups.

Conclusion: The MF + LH + RP mixture with an in situ gel-forming nasal delivery system applied for wound healing after FESS prevents the formation of stenosis and is favorable for proper wound healing.

1. Introduction

For chronic sinusitis not responding to medical treatment, functional endoscopic sinus surgery (FESS) is frequently performed. Despite advanced surgical techniques and instruments, major complications such as postoperative scarring, ostium stenosis or adhesions between the medial concha and the lateral nasal wall can arise, causing severe problems by disrupting the mucociliary drainage of the sinuses [1–3]. To guarantee successful surgical interventions, maximum postoperative wound healing needs to be achieved. Nasal packings (tamponage inside the nose) and wound debridement applied to prevent these complications have been shown to have significant efficacy in the prevention of ostium stenoses and adhesions. Yet as foreign objects staying inside the body, they promote scar formation [4–6].

FESS allows the restoration of natural mucus clearance pathways with a minimally invasive intervention [7]. As this technique became popular, the importance given to mucosal protection increased, given that bone tissue exposed by mucosa ablation can develop chronic inflammation leading to osteitis [8]. In addition, abnormal mucosal healing in these regions can result in insufficient mucosal clearance [9]. However, an unchanging etiological source shows that disrupted mucociliary clearance and immobile sinonasal secretions are a principal pathophysiological outcome [10]. It also shows how important post-FESS wound healing is. Wound healing has been researched before in other tissues (gingiva, skin) [11]. Wound healing phases vary between tissues. Generally, they consist of clotting, inflammation, matrix deposition and remodeling, cell proliferation, and maturation [12]. This process is organized by a wide range of contributors, such as growth

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factors, cytokines, and proteases [13]. Local wound healing problems may also affect normal mucosal structure. Thus, the most important factor for the success of surgery is the quality of healing in the mucosa.

To improve nasal mucosal healing post-FESS, systemic antibiotic therapy, systemic corticosteroid, topical steroid sprays, and physiological saline sprays (hypotonic, isotonic, and hypertonic) are in use. These methods may not be sufficiently effective individually, and when used in combination can produce side effects and even then not lead to a complete recovery of the nasal mucosa. To this day, no drug application to minimize post-FESS formation of scar tissue and adhesions and to quickly restore normal sinus functions through improved wound healing has been found.

A drug to improve nasal mucosal healing needs to feature the following four important characteristics [14]:

1. The substance needs to be applicable in a suitable manner
2. In order to be absorbed in the sinus wound, it needs to remain there for a sufficient period
3. It needs to be biocompatible
4. Wound healing needs to be achieved without scarring

The aim of our study is to develop a product that will improve the healing of the newly formed ostium after FESS and improve the mucus healing. In this respect, we aim to increase patient satisfaction with surgical success. For this purpose, in our study we developed a new mucoadhesive nasal drug delivery system, which is liquid at room temperature but on the nasal mucosa converts into gel form, using fast gelating poloxamer 407 and mucoadhesive carbopol 974P NF polymers containing mometasone furoate (MF), levofloxacin hemihydrate (LH), and retinyl palmitate (RP) as active ingredients.

With this combination, we assessed the various stages of nasal mucosal healing in a rabbit maxillary sinus model.

2. Material and methods

2.1. Study design

Ethics approval was obtained from the local experimental animal ethics committee (Number 14/138). Our work is a prospective, randomized, placebo-controlled, double-blind experimental study. For the experiments, 28 white, male New Zealand rabbits weighing between 2 and 4 kg were used. It is accepted that New Zealand rabbits are a suitable animal model for the assessment of nasal mucosal healing in experimental sinusitis models [15–18]. Despite the known physiological differences between humans and rabbits, no more suitable alternatives have been found to date [19].

The animals were divided into 4 groups, conforming with the numbers and times specified in the literature [20,21]. Each group was divided into three subgroups. The groups were sacrificed sequentially at a distance of one week in order to assess the width of the ostium and the recovery of the wound. Thus, within each group placebo and control (negative control) were assessed. For this purpose, in each group the right nasal cavity of five rabbits was used as the study group and the left cavity as placebo group. In two more rabbits, both nasal cavities served as control group (Fig. 1).

3. Composition of mometasone furoate (MF) + levofloxacin hemihydrate (LP) + retinyl palmitate (RP) mixture with an in situ gel-forming delivery system

3.1. A. Materials used in the preparation of the combination

Mometasone furoate (Sigma Aldrich, Germany), Levofloxacin hemihydrate (Sigma Aldrich, Germany), Carbopol 974P NF (Lubrizol, Belgium), Dexpanthenol (Fluka, Germany), Benzalkonium chloride (Fluka, Germany), Pluronic® F-127 (BASF, Germany), Retinyl palmitate (Sigma Aldrich, Germany), Polyethylene glycol 400 (Tekkim, Turkey). All other chemicals used were of analytical grade.

For the study, two combinations were prepared. Combination 1 contained active ingredients plus additional substances (components to achieve in situ gelation). Combination 2 (placebo) contained additional substances only (Table 1).

3.1.1. Combination 1 (active)

Mometasone furoate (MF), Levofloxacin hemihydrate (LH), Retinyl palmitate (RP) + ***Combination 2*** (Carbopol 974P NF, PF127, PEG 400, Dexpanthenol (as a humidifier), Sodium chloride, Benzalkonium chloride, Citric acid solution (50% m/v), distilled water)

3.1.2. Combination 2(placebo)

Carbopol 974P NF, PF127, PEG 400, Dexpanthenol, Sodium chloride, Benzalkonium chloride, Citric acid solution (50% m/v), distilled water

3.2. B. Preparation and optimization of heat-gelating PF 127 gels

PF 127 gels without and with active ingredients were prepared according to the cold preparation method described by Schmolka [22]. Initially, in order to optimize the formulation, gels without drugs were prepared with various concentrations of PF 127 and the gelation temperatures were assessed. The series with an optimized PF 127 concentration was used in further studies to research the effect of the mucoadhesive polymer on the gelation temperature. The mucoadhesive polymer Carbopol 974P NF was prepared in two different concentrations (0.25% and 0.5%).

According to the chosen cold preparation method, the drug-laden formulations were prepared stirring MF, LH, RP, PEG 400, Carbopol 974P NF and the other ingredients at room temperature into the appropriate quantity of distilled water. After cooling the dispersion in a refrigerator to 4 °C, PF 127 was slowly added to the dispersion with continuous stirring with a magnetic stirrer. The final dispersion was kept in the refrigerator until it became a clear solution [22]. Finally, the formulations were calibrated to the required volume using distilled water. The transparency, pH, gelation temperature and viscosity of the final formulations were assessed.

3.3. C. Assessment of the Formulations

3.3.1. Transparency

Transparency of the formulations was assessed visually in front of a black and white background, using the categories turbid (+), clear (++), very clear (glass-clear) (+++) [23].

3.3.2. pH

To determine the pH values of the formulations, 1 ml from each formulation was transferred into a 10 ml beaker and diluted with distilled water to 10 ml. The pH values of the resulting solutions were measured with a pH meter (WTW inolab pH 720) [23].

3.3.3. Determination of the solution-gel-transition temperature (T sol-gel)

Gelation temperature of the aqueous solution containing PF 127 was determined in the following way: 10 ml solution was transferred into a 20 ml transparent beaker with a magnetic stirring bar. At a stirring speed of 100 rpm, the temperature on the heated stirrer was increased by 1 °C/min. The temperature at which the stirring bar stopped turning was determined as the gelation temperature [23].

3.3.4. Determination of the formulations' viscosity

The static rheological characteristics of the preparations were determined using a rotational viscometer (Brookfield DV-II viscometer, Brookfield Engineering Laboratories Inc., MA, USA). The viscosity measures were taken at room temperature and at 34 °C, using a

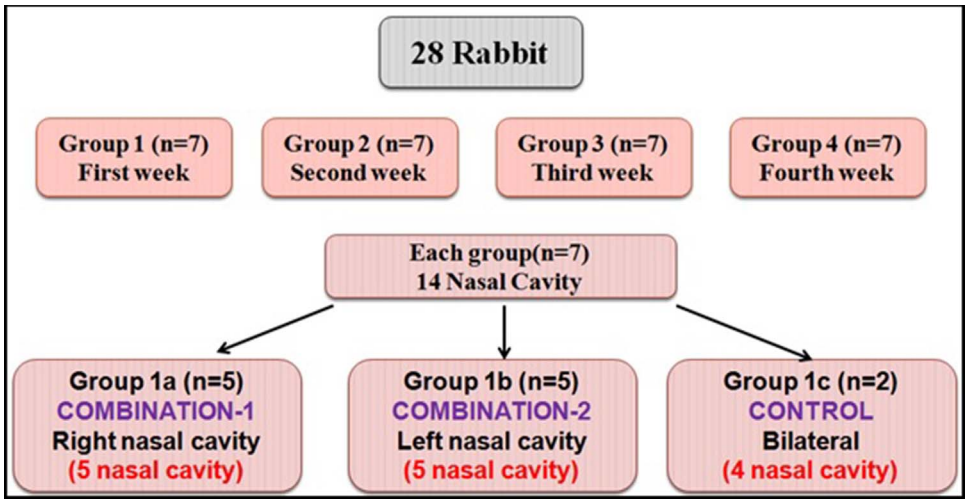


Fig. 1. Division of study groups.

Table 1
Composition of Mucoadhesive Thermoreversible In Situ Gels.

Conc (w/v)	Formulations	
	Placebo	Active
Mometasone furoate	–	0.05
Levofloxacin hemihydrate	–	0.5
Retinyl palmitate	–	0.05
Dexpanthenol	0.2	0.2
Pluronic® F-127	18	18
Carbopol 974P NF	0.25	0.25
Polyethylene glycol 400	8	8
Sodium chloride	1	1
Benzalkonium chloride	0.02	0.02
Citric acid solution	q.s.	q.s.
Distilled water	q.s.	q.s.

Helipath Stand and a T-bar spindle, increasing the rotational speed from 5 rpm to 150 rpm [24].

3.4. D. Surgical procedure

The rabbits were anesthetized with ketamine (35 mg/kg) and xylazine (5 mg/kg) IM. As an antiemetic, acepromazine (0.75 mg/kg) (SC) was given. On the nasal dorsum, an area of 2 × 3 cm was shaved and cleaned with povidone-iodine. Local anesthetic (3 ml 1% lidocaine with 1:100,000 epinephrine) was infiltrated SC. Under sterile conditions, a 2.5 cm vertical midline incision was made and the soft tissue above the maxillary sinus and the periosteum was elevated and removed (Fig. 2a). The maxillary sinus anterior wall was removed by use of a drill (Fig. 2b). Subsequently, in the maxillary sinus medial wall a 6 mm ostium, conforming with the literature, was created with an otologic drill (4 mm) [19], using a 2.5 size loop (Fig. 2c and d) [19].

The subcutaneous incision was closed with a rapidly absorbable suture, the skin incision with a non-absorbable suture. Transdermal fentanyl was applied for postoperative pain. One, 2, 3 and 4 weeks after surgery, respectively, one of the four groups was sacrificed. After scarification, the nasal dorsum was opened and the maxillary sinus and nasal mucosa were exposed and excised.

3.5. E. Assessment

3.5.1. E.1. Width of the ostium

After carefully opening the nasal dorsum, the ostia opened in the maxillary sinus medial wall were measured with a micrometer gauge. The observer did not know what treatment had been used.

3.5.2. E.2. Histological analysis

After measuring the ostium diameters, the bilateral medial maxillary walls and the nasal septum were resected. The samples were stained with hematoxylin eosin and assessed through an optical microscope by a pathologist, blinded to the treatment, using a 5-point scale (0–4).

In each sample, PNL infiltration, lymphocyte infiltration, fibroblastic proliferation, surface epithelium loss, and vascular proliferation were assessed [25,26]. To determine the amount of newly formed cilia, a semi-quantitative ranking system previously established in the literature [27] was used (0 = no cilia, 1 = < 30% cilia, 2 = 31–60% cilia, and 3 = > 60% cilia).

4. Statistical analysis

Statistical analysis was carried out using the Statistical Package for the Social Sciences version 13.0 software for Windows (SPSS Inc, Chicago, Illinois, USA). All quantitative variables were estimated using measures of central location (i.e. mean and median) and measures of dispersion (i.e. standard deviation, SD). Data normality was checked using the Kolmogorov-Smirnov tests of normality.

To compare quantitative data, Kruskal-Wallis variance analysis was used. To find the group causing difference, Mann-Whitney *U* test was used. To compare qualitative data, Chi-square test was used. Significance level was set at $p < 0.05$.

5. Results

5.1. Ostium width

In group 1 (1st week), there was no significant difference between the subgroups (group 1a: 4.4 ± 0.7, group 1b: 3.4 ± 0.7, group 1c: 3.9 ± 1.1) regarding ostium diameter ($p = 0.172$) (Fig. 3a).

In group 2 (2nd week), ostium width in group 2a (3.6 ± 0.6) was significantly greater than in group 2b (2.4 ± 0.5) and group 2c (2.5 ± 0.5) (in the order $p = 0.020$, $p = 0.036$) (Fig. 3b) (Fig. 4). There was no significant difference between groups 2b and 2c ($p = 0.775$) (Fig. 3b).

In group 3 (3rd week), ostium width in group 3a (3.2 ± 0.3) was significantly greater than in group 3b (1.3 ± 0.2) and group 3c (0.7 ± 0.1) (in the order $p = 0.008$, $p = 0.012$) (Fig. 3b) (Fig. 5). There was no significant difference between groups 3b and 3c ($p = 0.211$) (Fig. 3c).

In group 4 (4th week), ostium width in group 4a (3.3 ± 0.3) was significantly greater than in group 4b (1.1 ± 0.3) and group 4c (0.6 ± 0.2) (in the order $p = 0.009$, $p = 0.014$) (Fig. 3d) (Fig. 6).

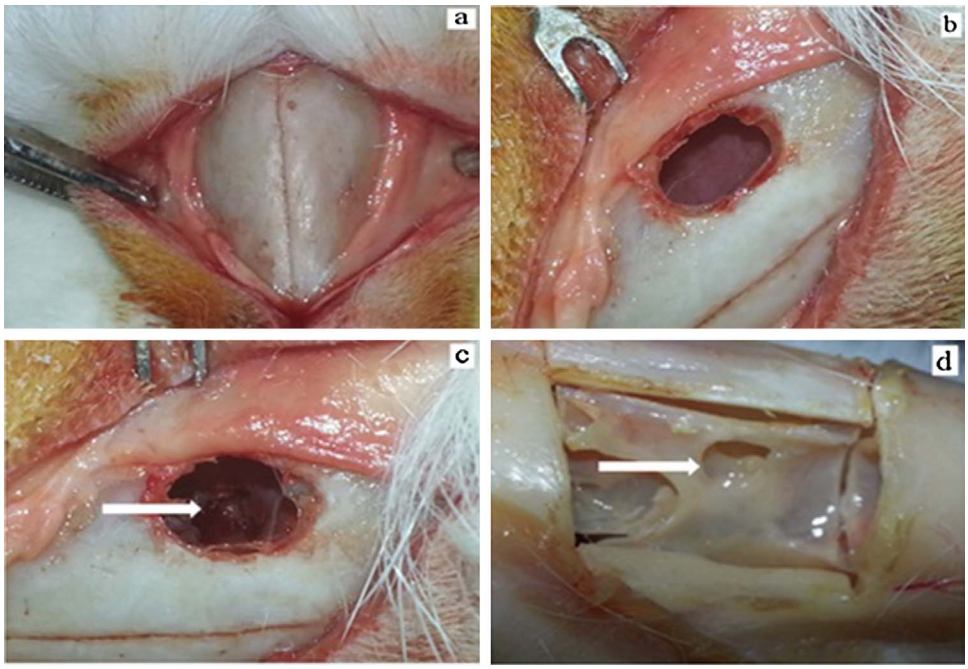


Fig. 2. Surgical procedure: Opening an ostium in the maxillary sinus medial wall.

There was no significant difference between groups 4b and 4c (p = 0.185) (Fig. 3d).

Between the subgroups receiving active drug (1a, 2a, 3a, 4a), no significant difference in the diameter of the ostium was found (p = 0.109).

In the subgroups receiving placebo, between 1b and 2b as well as between 2b and 3b there was a significant difference (in the order 0 = 0.031, p = 0.044). Between 3b and 4b, no significant difference was found (p = 0.371).

In the subgroups receiving only surgery, between 1c and 2c as well as between 2c and 3c there was a significant difference (in the order p = 0.027, p = 0.047). Between 3c and 4c, no significant difference was found (p = 0.684).

5.2. B. Histopathological evaluation

In group 1 (1st week), no significant difference was found between the subgroups (1a, 1b, 1c) regarding histopathological characteristics (PNL infiltration, lymphocyte infiltration, fibroblast proliferation, surface epithelium loss, vascular proliferation, presence of superficial cilia) (p > 0.05) (Fig. 7).

In the subgroups of groups 2, 3 and 4 receiving active substance (2a,

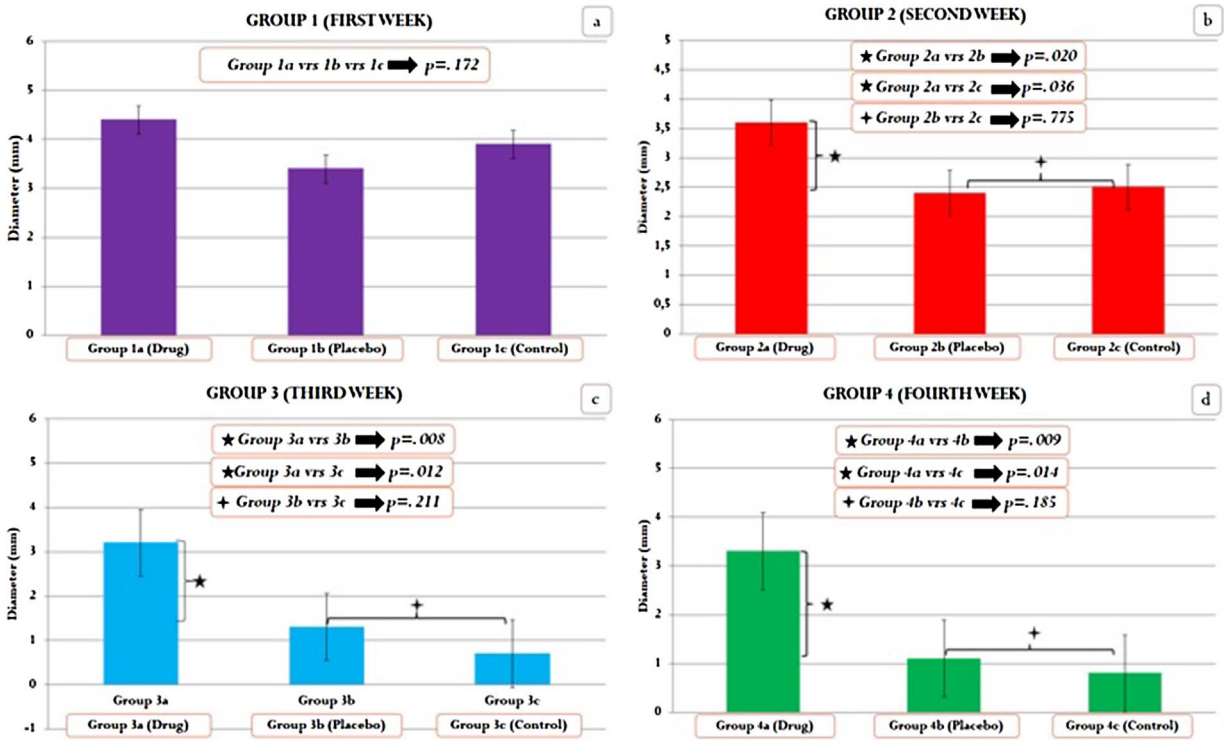


Fig. 3. Ostium diameter-Weekly comparison of groups and subgroups.

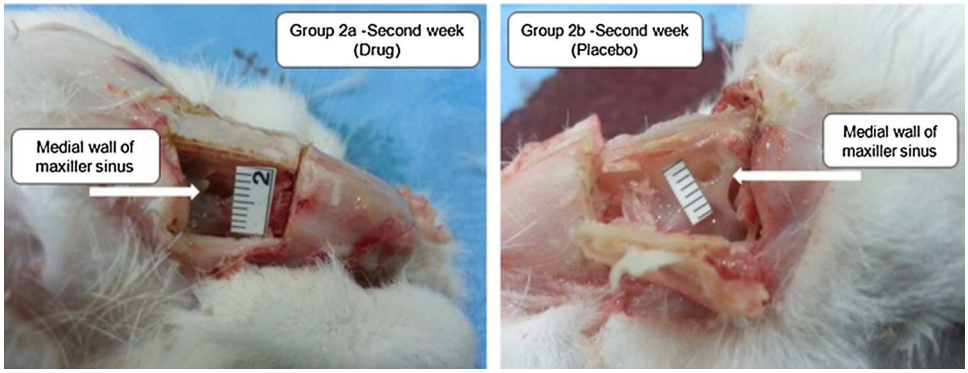


Fig. 4. Image of the maxillary sinus medial wall ostium in the drug-using subgroup and placebo subgroup of Group 2.

3a, 4a), surface epithelium loss was significantly less than in placebo and control groups, while the presence of superficial cilia was significantly greater ($p < 0.05$) (Fig. 7) (Fig. 8).

In the subgroups of groups 2, 3 and 4 receiving active substance (2a, 3a, 4a), fibroblastic proliferation and vascular proliferation were significantly greater than in placebo and control groups ($p < 0.05$) (Fig. 7) (Fig. 9).

In the subgroups of groups 2, 3 and 4 receiving active substance (2a, 3a, 4a), chronic inflammation parameters (PNL and lymphocyte infiltration) were significantly lower than in placebo and control groups ($p < 0.05$) (Fig. 7) (Fig. 10).

6. Discussion

In cases of chronic sinusitis not responding to medical therapy, functional endoscopic sinus surgery is used as an alternative treatment method. However, despite the minimally invasive surgical approach, a number of problems arise in the recovery of the nasal mucosa and with the restenosis of the widened ostia.

Limited progress has been achieved with the development of a nasal packing minimizing post-FESS scarring and formation of adhesions, improving wound healing and quickly restoring normal sinus functions [12,5,6,28]. FESS results directly depend upon the quality of healing of the nasal mucosa [13]. Wound healing is a highly organized process, regulated by fibroblasts, leukocytes, and epithelial cells; as a result of the activities of a wide range of growth factors and cytokines, it includes processes of inflammation, ECM deposition and remodeling, cell migration, multiplication and differentiation [13].

The aim of our study was to accelerate post-FESS wound healing, prevent closing of newly created ostia, and improve the success of the intervention and patient satisfaction by creating a biocompatible in situ combination of substances whose effectiveness in various stages of wound healing was proven.

In our study, we used an in situ combination that can be sprayed at room temperature and turns into a gel in the nasal cavity. As the solubility of MF and LH in water is low, these substances are found in available nose sprays as suspension. Yet drugs in suspension are dissolved slowly, hence the onset of their effect is delayed. Due to mucociliary clearance (within ca. 15 min), they are quickly eliminated from the nasal cavity, which makes MF and LH unsuitable for nasal application. With conventional gel formulations, nasal application is difficult and a sensitive drug dosage may not be achievable [29]. On the other hand, mucoadhesive powders do not allow for sensitive drug dosage, and as they can cause irritation in the nasal mucosa and a gritty sensation, they are not a preferred way of drug delivery. For these reasons, in order to increase patient compliance, recently a new dosage mode has become attractive in the application of nasal drugs, namely, in situ or in vivo gels (environment-sensitive gels). In comparison with liquid nasal formulations, nasal in situ gels are solutions with low viscosity that, in contact with the nasal mucosa, change their polymer structure and form a gel. Not only do in situ gels extend the contact period between the nasal mucosa and the drug, but they also guarantee a slow and continuous release of the drug [29].

6.1. Mucoadhesion properties

In order to improve bioadhesive properties of in situ gels, bioadhesive polymers such as polyacrylates (Polycarbophyl®, Carbopol®), cellulose derivatives (e.g., hydroxypropyl methyl cellulose, hydroxypropyl cellulose, methyl cellulose, and carboxymethyl cellulose), and natural polymers (e.g., chitosan, alginate) can be combined with them. Polyacrylates have many advantages especially in nasal drug delivery systems, due to their excellent mucoadhesive and gel forming capability. It was reported in the literature that the increase in gel adhesiveness caused by an increase in Carbopol 974P NF concentration might be attributed to the greater ability of polyacrylic acid to chemically interact with the probe [30].

In the same way, one of the bioadhesive polymers, Carbopol 974P NF was used in the formulation composition in our study. Therefore, it can be predicted that the formulation in our study shows bioadhesive properties based on literature.

In our study, we mostly intended to submit the clinical study data in

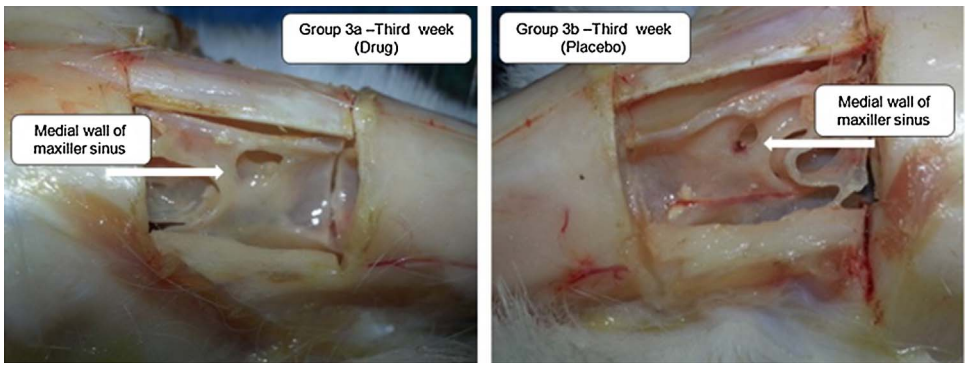


Fig. 5. Image of the maxillary sinus medial wall ostium in the drug-using subgroup and placebo subgroup of Group 3.

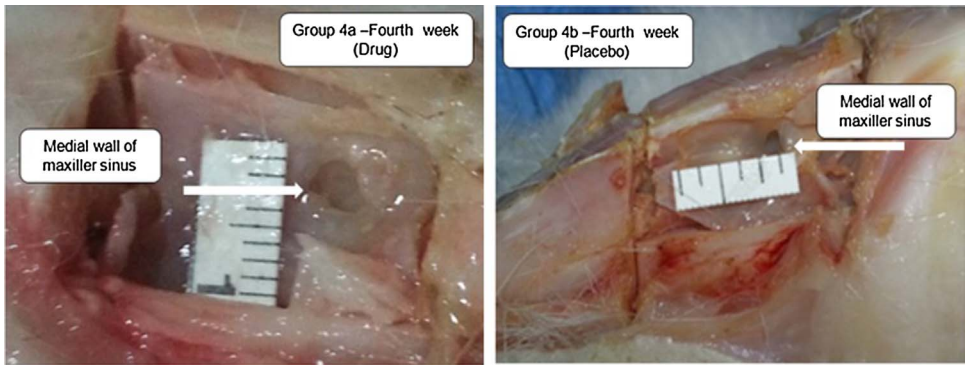


Fig. 6. Image of the maxillary sinus medial wall ostium in the drug-using subgroup and placebo subgroup of Group 2.

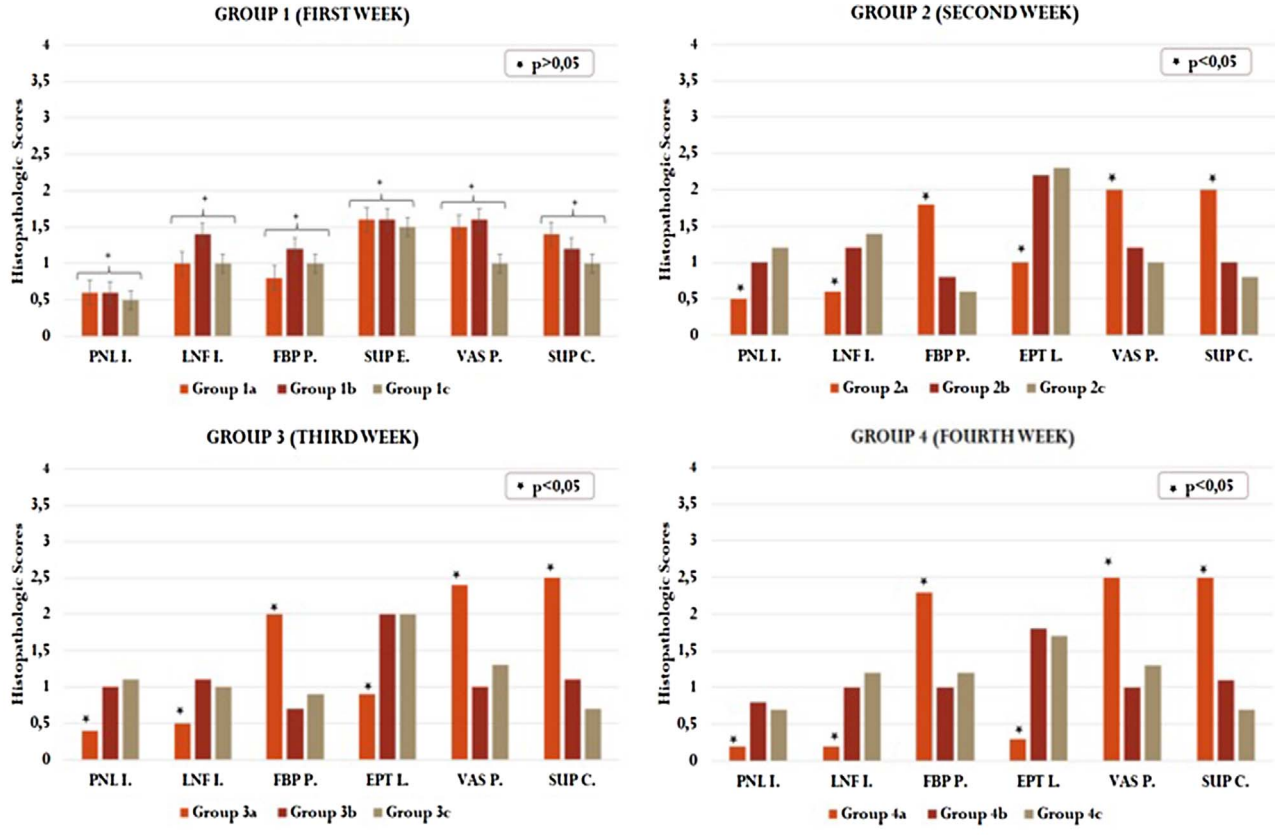
this manuscript, so we did not perform a mucoadhesion test. Also, the results of this study require extensive additions so we think that it would be better if we present them in another subsequent article. If you approve, we cited regarding the mucoadhesion properties in the discussion part of our manuscript.

Successful release test is contingent on reliable drug transport from the test material through a membrane and into the receiving medium. Therefore, in identifying the optimal experimental parameters, physicochemical properties of drug, selection of the proper membrane, receiving medium and sampling schedule should be selected carefully for a useful test. Appropriate receptor medium such as aqueous buffer for water soluble drugs or a hydro-alcoholic medium for sparingly water soluble drugs" can be used. Hence, solvents that will provide sink conditions in a release test receiving vessel should be identified.

In our study, the formulation contains 3 active substances which have different physicochemical properties. Providing the sufficient solubilities of all these active substances in the release medium that meet the sink conditions as mentioned above is very difficult. Also, stabilization of these active substances in the same release medium during the experiment and making quantitative determinations of these substances with the same analytical method are quite difficult.

6.2. Toxicity

Mometasone furoate nasal spray (MFNS) (100 μg daily) is the optimal dosage that can provide significant efficacy and a favorable safety profile for children 3 to 11 years of age with seasonal and perennial allergic rhinitis. In addition, MFNS has a low potential for systemic



- PNL I. :Polymorphonuclear Leukocyte infiltration, LNF I. : Lymphocyte infiltration, FBP P. : Fibroblast proliferation, EPT L: Surface epithelium loss, VAS P. : Vascular proliferation, SUP C: Presence of superficial cilia
- To compare groups, Kruskal-Wallis variance analysis was used. To find the group causing difference, Mann-Whitney U-test was used. Significance level was set at p<0.05.

Fig. 7. Histopathological scores in groups.

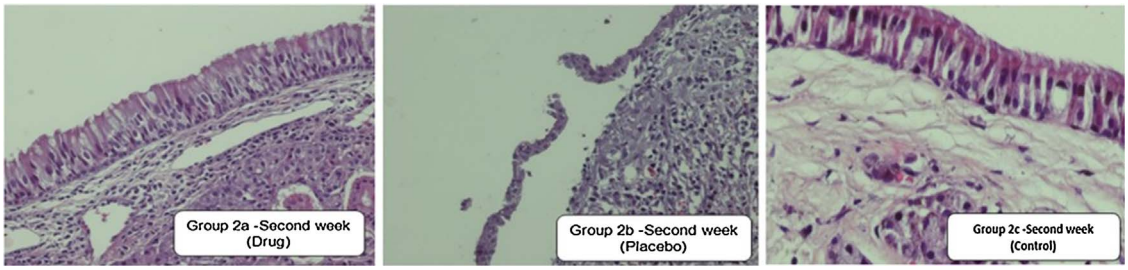


Fig. 8. Histopathological images showing the presence of superficial cilia and surface epithelium loss in the group treated with active substance and the placebo group in the second week.

effects because plasma concentrations of mometasone furoate in children were virtually undetectable in the 119 post dose samples tested (< 50 pg/mL). There was no evidence of HPA axis suppression in the patients tested with cosyntropin stimulation before and after treatment [31]. MFNS had no detectable effects on the hypothalamic- pituitary-adrenal (HPA) axis in adults, even when administered up to 20 times the recommended daily dose of 200 μg. In patients aged 12 years and older, MFNS also has been shown to be effective and well tolerated for prophylaxis and treatment of seasonal allergic rhinitis and the treatment of perennial rhinitis [32].

Levofloxacin hemihydrate, ocular irritation studies of temperature sensitive hydrogels containing levofloxacin hemihydrate was conducted on two male albino rabbits each weighing 2.25 kg. Temperature sensitive formulations were instilled twice a day for a period of 21 days and the rabbits were observed periodically for redness, swelling and watering of the eye. The results of the ocular irritation studies showed that all temperature sensitive hydrogels were non-irritant. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae were seen. No signs of redness, watering of the eye and swelling were observed throughout the study with both the formulations [33].

Retinyl palmitate: Retinol is accepted and recognized as generally safe (GRAS) in foods as a nutrient and dietary supplement. It has been reported that topical application of retinol does not result in changes in constitutive plasma levels of retinol and, therefore, does not increase the risk for developmental toxicity [34]. Two lots of moisturizer containing 0.1% retinyl palmitate were evaluated for dermal irritation in albino rabbits. Samples of 0.5 ml were applied to the back of each rabbit and a control sample to the other side of the back daily for 4 days. Results showed that all lots were slightly irritating, including the controls [35]. In a clinical study, two male volunteers reacted positively to all-trans-retinoic acid but showed negative results when patch-tested with 0.1% retinol or retinyl palmitate in petrolatum [36].

These are the chemical substances contained in our combination with roles in wound healing:

1. Mometasone furoate (MF): Intranasal topical corticosteroids show their effects by inhibiting the release of cytokines and reducing inflammatory cells, thus decreasing inflammation in the nasal mucosa [37]. In a recent study, it has been shown that MF on its own used in a nasal spray against acute rhinosinusitis leads to clinical improvement [38]. It has been found that the use of topical dexamethasone reduces the stromal thickness without preventing the formation of granulation tissue and epithelial differentiation, thus contributing to mucosal wound healing [39]. In our study, the ostium diameters in the group receiving the active combination were significantly greater than in the placebo and control groups, which can be thought to be an effect of MF. In their clinical study, Jorissen and Bachert reached the conclusion that mometasone furoate, which is topically applied after functional endoscopic sinus surgery, increases wound healing [40]. In the study of Murr et al., mometasone furoate impregnated bioabsorbable stents were applied after functional endoscopic sinus surgery [41].It was revealed that the applied steroid impregnated stent protects sinus structure and speeds up wound healing, and reduces inflammation and adhesions [41]. In our study, it was revealed that the combination which MF is also included both accelerates wound healing and reduces adhesions, thus our findings are in line with the literature.
2. Levofloxacin hemihydrate (LH): An antimicrobial member of the quinolone group, LH is the L-isomer of racemic ofloxacin. As a chiral fluoro-carboxy-quinolone, LH, the racemic drug constituent, chemically is the (S)-enantiomer of ofloxacin. It is a broad-spectrum antibiotic, effective against gram-(+) and gram-(–) bacteria. Fluoroquinolones inhibit DNA replication by inhibiting the DNA gyrase complex. Their use is indicated in acute sinusitis, acute exacerbation of chronic bronchitis, pneumonia, urinary system infections, prostatitis, skin and soft tissue infections, etc. [42] It can be assumed that the significant reduction of chronic inflammation parameters (PNL infiltration and lymphocyte infiltration) observed in our study in the group receiving the active combination compared to the placebo and control groups resulted from the use of LH.
3. Retinyl palmitate (RP): Vitamin A and all its active derivatives are known as retinoids. It is accepted that they are important mediators in the growth and function of respiratory epithelium. In one study, a positive effect of retinoic acid on the proliferation of mucociliary epithelium in the paranasal sinus was found. It was shown that the topical use of retinoic acid gel in the maxillary sinuses of a rabbit whose mucosa had been surgically removed increased the regeneration of ciliary epithelium compared to a control group not receiving medication [43]. In the experimental study of Kim et al., alkali burn was formed in the corneas of rats. It was demonstrated that it accelerated healing in the corneas of the group which retinyl palmitate +levofloxacin was applied for treatment [44]. In the test

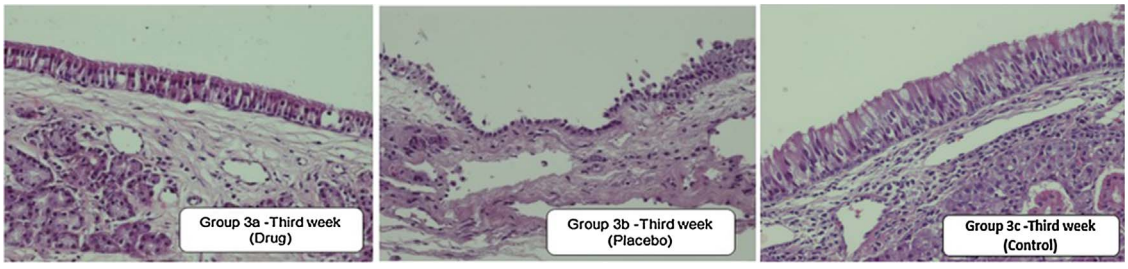


Fig. 9. Histopathological images showing fibroblastic proliferation and vascular proliferation in the group treated with active substance and the placebo group in the third week.

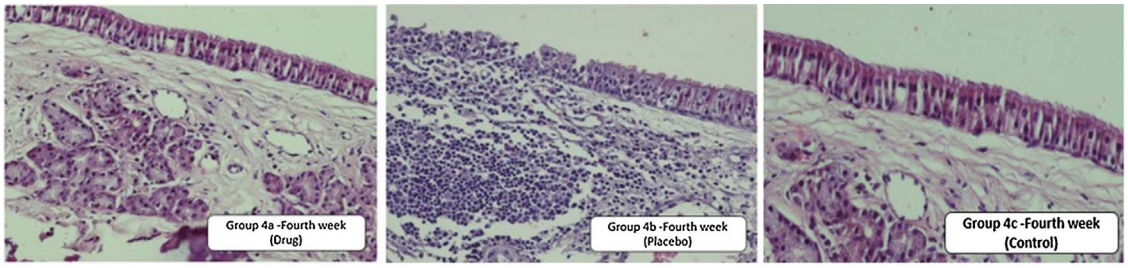


Fig. 10. Histopathological images showing chronic inflammation parameters (PNL and lymphocyte infiltration) in the group treated with active substance and the placebo group in the fourth week.

study carried out by Jain et al. on rabbits, they examined the effects of different topical agents in paranasal mucosa healing [45]. In the study, the effect of new generation acitetrin, mometasone furoate, lactoferrin and salyclic acid was evaluated. [45]. They reached the conclusion that acitretin reduces collagen storing and increases ciliary healing more effectively than the other substances [45]. In the study of Maccabee et al., it was demonstrated that retinoic acid which is applied as a topical jel increases ciliary regeneration in paranasal sinus mucosa [46]. In the study of Ericson et al., it was demonstrated that topical retinoic acid which is placed inside demucosalized maxillary sinus produces functional cilia [47]. This finding demonstrated that retinoic acid might be effective in functional and morphological healing of cilia regeneration [47].

Retinoic acid is also available in the combination we formed in our study and the findings we reached are in line with the literature.

In our study, the group receiving the active combination, compared to the placebo and control groups, showed a significantly lower epithelium loss and a higher rate of superficial cilia regeneration, which shows that RA has a beneficial effect on the proliferation of mucociliary epithelium.

According to the literature, the mixture of mometasone furoate, levofloxacin hemihydrate, and retinyl palmitate has been created and used for the first time in our study. In the experimental application, histopathologically the combination has not caused any toxic effect on the nasal mucosa. This result shows that our combination is histopathologically safe. Our findings make us believe that the combination presents the four characteristics required for a drug improving the healing of nasal mucosa specified in the literature. With the in situ gel system we used, the drug can be delivered adequately. The gelation of the combination in the nasal environment ensures that the substance remains in the nose for a sufficiently long period as required for local absorption. According to positive results of histopathological evaluation, it was found that the combination is biocompatible and supports healing without scarring.

The product we developed is a new combination that is liquid at room temperature, gelates in the nasal cavity due to the warmth inside the nose, and has a mucoadhesive polymer ingredient, which enhances drug exposure. In the literature, no product is found that was developed in a dosage form aimed at accelerating post-FESS wound healing in the nose. Due to its ingredients, the formulation extends the drug's time period inside the nose that is mainly counteracted by mucociliary clearance. Thus, the drug remains inside the nasal cavity for a longer time and is released over an extended period in a controlled manner.

As soon as the effectiveness of our product has been proven in experimental and clinical studies, if it is introduced into the market, it will be the first application promoting mucosal healing and preventing occlusion of neo-ostia with a special delivery system. We believe that this product will increase post-FESS patient satisfaction and the success of the operation, preventing potential complications. Thus, post-operative systemic drug use will no longer be necessary, as effective results will be achieved by topical application and complications originating from systemic use will be avoided.

7. Conclusion

The combination of MF + LH + RP in an in situ gel formation application has been proposed in this study for the first time in the literature. This will prevent side effects caused by systemic use of the drugs and provides a mechanism, which reaches the target region in high concentration. Our in situ gel combination prevents stenosis of new ostia and actively supports wound healing. In order to introduce this combination for clinical use, further experimental and clinical studies are required.

References

[1] H.L. Verwoerd-Verhoef, P.G. ten Koppel, G.J. van Osch, et al., Wound healing of cartilage structures in the head and neck region, Int. J. Pediatr. Otorhinolaryngol. 43 (1998) 241–251.
[2] J.S. [2].Toung, R.C. Ogle, R.F. Morgan, et al., Insulin-like growth factor 1- and 2-augmented collagen gel repair of facial osseous defects, Arch. Otolaryngol. Head Neck Surg. 125 (1999) 451–455.
[3] K. Watanabe, C. Kiuna, Epithelial damage of nasal mucosa in nasal allergy, Ann. Otol. Rhinol. Laryngol. 107 (1998) 564–570.
[4] J.G. Harpel, C.N. Metz, S. Kojima, et al., Control of transforming growth factor–beta activity: latency vs activation, Prog. Growth Factor Res. 4 (1992) 321–335.
[5] D.J. Altman, S.L. Schneider, D.A. Thompson, et al., A transforming growth factor beta 2 (TGF-beta 2)-like immunosuppressive factor in amniotic fluid and localization of TGF-beta 2 mRNA in the pregnant uterus, J. Exp. Med. 172 (1990) 1391–1401.
[6] P.M. Bartold, A. Raben, Growth factor modulation of fibroblasts in simulated wound healing, J. Periodontal Res. 31 (1996) 205–216.
[7] D.W. Kennedy, S.J. Zinreich, A.E. Rosenbaum, et al., Functional endoscopic sinus surgery. Theory and diagnostic evaluation, Arch. Otolaryngol. 111 (1985) 576–582.
[8] H. Moriyama, K. Yanagi, N. Ohtori, et al., Healing process of sinus mucosa after endoscopic sinus surgery, Am. J. Rhinol. 10 (1996) 61–66.
[9] C.K. Shaw, A. Cowin, P.J. Wormald, A study of the normal temporal healing pattern and the mucociliary transport after endoscopic partial and full-thickness removal of nasal mucosa in sheep, Immunol. Cell Biol. 79 (2001) 145–148.
[10] D.A. Gudis, N.A. Cohen, Cilia dysfunction, Otolaryngol. Clin. North Am. 43 (2010) 461–472.
[11] J.M. Zahm, M. Chevillard, E. Puchelle, Wound repair of human surface respiratory epithelium, Am. J. Respir. Cell Mol. Biol. 5 (1991) 242–248.
[12] K. Forsgren, P. Stierna, J. Kumlien, et al., Regeneration of maxillary sinus mucosa following surgical removal. Experimental study in rabbits, Ann. Otol. Rhinol. Laryngol. 102 (1993) 459–466.
[13] J.B. Watelet, C. Bachert, P. Gevaert, et al., Wound healing of the nasal and paranasal mucosa: a review, Am. J. Rhinol. 16 (2002) 77–84.
[14] Q. Chen, G. Sun, Y. Wang, et al., The evaluation of two new hyaluronan hydrogels as nasal dressing in the rabbit maxillary sinu, Am. J. Rhinol. Allergy 26 (2012) 152–156.
[15] J.R. Perloff, F.H. Gannon, W.E. Bolger, et al., Bone involvement in sinusitis: an apparent pathway for the spread of disease, Laryngoscope 110 (2000) 2095–2099.
[16] D.B. Hurley, G.S. Smith, G.A. Vogler, et al., Leukotriene B4 levels in rabbit maxillary sinusitis: limitation of the current model, Am. J. Rhinol. 15 (2001) 47–48.
[17] J.E. Kerschner, M.J. Cruz, D.J. Beste, et al., Computed tomography vs magnetic resonance imaging of acute bacterial sinusitis in the rabbit model, Am. J. Otolaryngol. 21 (2000) 298–305.
[18] S.C. Marks, Acute sinusitis in the rabbit: a new rhinogenic model, Laryngoscope 107 (1997) 1579–1585.
[19] E.M. Gilbert, R.K. Kirker, D.S. Gray, et al., Chondroitin sulfate hydrogel and wound healing in rabbit maxillary Sinus Mucosa, Laryngoscope 114 (2004) 1406–1409.
[20] M. Proctor, K. Proctor, X.Z. Shu, et al., Composition of hyaluronan affects wound healing in the rabbit maxillary sinus, Am. J. Rhinol. 20 (2006) 206–211.
[21] R. Orlandi, X.Z. Shu, L. McGill, et al., Structural variations in a single hyaluronan derivative significantly alter wound-healing effects in the rabbit maxillary sinus, Laryngoscope 117 (2007) 1288–1295.
[22] I.R. Schmolka, Artificial skin I: preparation and properties of pluronic-127 gels for

treatment of burns, J. Biomed. Mater. Res. 6 (1972) 571–582.
[23] M.J. Bhandwalkar, A.M. Avachat, Thermoreversible nasal in situ gel of venlafaxine hydrochloride: formulation characterization, and pharmacodynamic evaluation, AAPS PharmSciTech 14 (2013) 101–110.
[24] S. Pund, G. Rasve, G. Borade, Ex vivo permeation characteristics of venlafaxine through sheep nasal mucosa, Eur. J. Pharm. Sci. 48 (2013) 195–201.
[25] M. Proctor, K. Proctor, X.Z. Shu, et al., Composition of hyaluronan affects wound healing in the rabbit maxillary sinus, Am. J. Rhinol. 20 (2006) 206–211.
[26] R. Orlandi, X.Z. Shu, L. McGill, et al., Structural variations in a single hyaluronan derivative significantly alter wound-healing effects in the rabbit maxillary sinus, Laryngoscope 117 (2007) 1288–1295.
[27] D. DePoortere, J.M. Kofonow, A.G. Chiu, et al., Enhanced post surgical re-mucosalization in a rabbit model, Otolaryngol. Head Neck Surg. 143 (2010) 129.
[28] G. Schultz, D.S. Rotatori, W. Clark, EGF and TGF-alpha in wound healing and repair, J. Cell. Biochem. 45 (1991) 346–352.
[29] S.C. Caoa, X. Rena, Q. Zhanga, E. Chena, F. Xua, et al., In situ gel based on gellan gum as new carrier for nasal administration of mometasone furoate, Int. J. Pharm. 365 (2009) 109–115.
[30] E. Altuntaş, F.G. Yener, Formulation and evaluation of thermoreversible in-situ nasal gels containing mometasone furoate for allergic rhinitis, AAPS PharmSciTech (March (9)) (2017).
[31] J. Dibildox, Safety and efficacy of mometasone furoate aqueous nasal spray in children with allergic rhinitis: results of recent clinical trials, J. Allergy Clin. Immunol. 108 (1) (2001) 54–58.
[32] Melvin D. Brannan, Jerry hi. Herron, Melton B. Affrime, Safety and tolerability of once-daily mometasone furoate aqueous nasal spray in children, Clin. Ther. 19 (1997) 6.
[33] P. Saxena, S. Kushwaha, Temperature sensitive ophthalmic hydrogels of levofloxacın hemihydrate with enhanced solubility and prolonged retention time, Int. J. Pharm. Pharm. Sci. 5 (2013) 3.
[34] G. Ries, R. Hess, Retinol: safety considerations for its use in cosmetic products, J. Toxicol. Cut. Ocular Toxicol. 18 (3) (1999) 15–169.
[35] H. Schaefer, Penetration and percutaneous absorption of topical retinoids. A review, Skin Pharmucol. 6 (1993) 17–23.
[36] W.P. Jordan Jr., M. Higgins, J. Dvorak, Allergic contact dermatitis to all-trans-retinoic acid; epicutaneous and leukocyte migration inhihition testing, Contuct Derm. 1 (5) (1975) 306–310.
[37] H. Derendorf, E.O. Meltzer, Molecular and clinical pharmacology of intranasal corticosteroids: clinical and therapeutic implications, Allergy 63 (2008) 1292–1300.
[38] E.O. Meltzer, D. Gates, C. Bachert, Mometasone furoate nasal spray increasesthe number of minimal-symptom days in patients with acuterhinosinusitis, Ann. Allergy Asthma Immunol. 108 (2012) 275–279.
[39] A.G. Beule, C. Scharf, K.E. Biebler, et al., Effects of topically applied dexamethasone on mucosal wound healing using a drug-realising stent, Laryngoscope 118 (2008) 2073–2077.
[40] M. Jorissen, C. Bachert, Effect of corticosteroids on wound healing after endoscopic sinus surgery, Rhinology 47 (3) (2009) 280–286.
[41] A.H. Murr, T.L. Smith, P.H. Hwang, N. Bhattacharyya, B.J. Lanier, J.W. Stambaugh, A.S. Mugglin, Safety and efficacy of a novel bioabsorbable, steroid-eluting sinus stent, Int. Forum Allergy Rhinol. 1 (1) (2011) 23–32.
[42] V.K. Shahwal, B.K. Dubey, M. Bhoumick, Preformulation study of levofloxacin, Int. J. Adv. Pharm. 1 (2012) 1–8.
[43] P.H. Hwang, J.M. Chan, Retinoic acid improves ciliogenesis after surgery of the maxillary sinus in rabbits, Laryngoscope 116 (2006) 1080–1085.
[44] E.C. Kim, T.K. Kim, S.H. Park, M.S. Kim, The wound healing effects of vitamin A eye drops after a corneal alkali burn in rats, Acta Ophthalmol. 90 (7) (2012) 540–546.
[45] R. Jain, R. Kim, S. Waldvogel-Thurlow, P. Hwang, J. Cornish, R. Douglas, The effects of topical agents on paranasal sinus mucosa healing: a rabbit study, Int. Forum Allergy Rhinol. 5 (4) (2015) 310–317.
[46] M.S. Maccabee, D.R. Trune, P.H. Hwang, Paranasal sinus mucosal regeneration: the effect of topical retinoic acid, Am. J. Rhinol. 17 (3) (2003) 133–137.
[47] V.R. Erickson, M. Antunes, B. Chen, N.A. Cohen, P.H. Hwang, The effects of retinoic acid on ciliary function of regenerated sinus mucosa, Am. J. Rhinol. 22 (3) (2008) 334–336.