Multi-Drug Loaded Chitosan/Gelatin Composite Sponge for Dental Application

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Abstract: The extraction of impacted third molar teeth can significantly lead to postoperative pain, swelling and trismus which delay hospital discharge. Dexamethasone steroid and Diclofenac are commonly prescribed to prevent inflammatory sequelae and relieve the associated pain. In this study, gelatin / chitosan blend was used for the preparation of dexamethasone- diclofenac sponge to be implanted directly to the socket benefiting from (1) the biodegradable nature of the polymers used as taking out the sponge after treatment always causes new damage to the wound. (2) The interaction between positively charged chitosan and negatively charged gelatin. The conditions expected to affect the stability of gelatin foams were optimized and the sponge was prepared by the lyophilization of stable foams generated by stirring a mixture of 7% aqueous gelatin and 2.5% chitosan in 1% acetic acid in a ratio of 1:5 respectively at 1000 rpm for 10 minutes. Scanning Electron Microscopy (SEM) was used to elucidate the internal structure of the developed sponge where drug crystals were detected on the lamellae and within pores of the sponge. In general, sponge formulae D, E and F showed higher and fast absorption behaviour reaching up to 2023% of its weight after 15 minutes only.

Keywords: Biodegradable polymers, Dexamethasone, Diclofenac, Sponge, wisdom teeth.

I. INTRODUCTION

In the 21st century, the pharmaceutical industry is trapped between the downward pressure on prices and the increasing cost of successful drug discovery and development. The average cost and time for the development of a new chemical entity are much higher than those required to develop a novel drug delivery system by which an existing drug molecule can get a new life, and thus, increase its market value and competitiveness [1].

Novel drug delivery systems offer optimization of the duration of action of the drug, decreasing dosage frequency, controlling the site of release, and maintaining constant drug levels. In addition, it provides a number of safety benefits such as reducing adverse effects and decreasing the number of concomitant medications a patient must take.

Sponges are patented polymeric delivery systems consisting of porous spheres that can entrap a wide range of active ingredients such as anti-infective, anti-fungal, and anti-inflammatory agents. This results in a large reservoir within each sponge, which can be loaded with up to its own weight of active agent [2].

In surgical operations, dental problems such as pain, edema, and trismus (a masticatory muscle inflammation) are very common. The dental provider appreciate that NSAIDS are effective anti-inflammatory agents for managing most cases of dental pain and regarded as first-line agents. However, there are several indications for which corticosteroids are preferable or may be considered when NSAIDS prove ineffective e.g. in Mucosa lesions such as severe aphthous ulcerations or lichens planus, they are immune mediated and require the added immunosuppressant actions of glucocorticoids [3]. Both NSAIDS and glucocorticoids are effective for prophylaxis of postoperative surgery. In other cases, steroids should be chosen when the event is anticipated to be severe, such as swelling following difficult third molar impactions [4].

Clinical trials have confirmed the advantage of the preoperative administration of both NSAIDS and glucocorticoids over either agent alone. NSAIDS likely have a greater influence in reducing postoperative pain, whereas the glucocorticoids have a greater tendency to reduce postoperative swelling. Ideally, regimens should be initiated preoperatively and coverage extended postoperatively for the duration swelling is anticipated. This may be only a day or two for minor procedures, or as long as a week for more traumatic procedures. It should be clarified that this abbreviated use of glucocorticoids has not been found to increase the risk of postoperative infection, and the addition of antibiotic coverage solely for this purpose is unwarranted [5].

Implantation of biodegradable materials are of great interest as taking out the implantable system after treatment or surgery always causes new damage to the wound . Chitosan is commonly used biodegradable polymer in the pharmaceutical preparations and is FDA approved food additive [6]. It is deacylated derivative of chitin and forms the exoskeleton of arthropod. Structurally chitosan is a linear polysaccharide consisting of β(1-4) linked D-glucosamine

with randomly located N-acetylglucosamine groups depending upon the degree of deacetylation of the polymer [7].

Chitosan is soluble in weekly acidic solutions resulting in the formation of a cationic polymer with a high charge density and can therefore form polyelectrolyte complexes with wide range of anionic polymers. Chitosan was used to develop injectable thermo-sensitive carrier material for biomedical applications. Due to the mild gelling conditions, the hydrogel has been found to be a potential delivery vehicle for growth factors, small molecular weight drugs and cells for localized therapy [8].

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Thermal or chemical dissociation of collagen polypeptide chains forms products known as gelatin. Gelatin is commonly used for biomedical applications due to its biodegradability and biocompatibility in physiological environments, in contact with living tissues. Two different types of gelatin can be produced depending on the method in which collagen is pretreated, prior to the extraction process [6]. Absorbable gelatin sponge is an official monograph in USP as haemostatic and coagulant in surgical procedures because it is capable of absorbing and holding within its meshes many times its weight of whole blood [7]

In this study, biocompatible, biodegradable chitosan –gelatin sponge containing both diclofenac as NSAID and Dexamethasone steroid for the management of pain and edema in tooth extraction was developed.

II. EXPERIMENTAL

MATERIALS

Diclofenac sodium, Dexamethasone were kindly donated from Sigma Pharmaceutical Co. (Cairo, Egypt). Gelatin Type B (225 bloom) was obtained from Sigma Chemical (St. Louis, MO, USA). Low molecular weight chitosan (degree of deacetylation 85%, viscosity of 45 cps (1% solution in 1% acetic acid) was purchased from Aldrich Chemical Co.(St. Louis, MO). Glacial acetic acid and glutaraldehyde (25% solution) were purchased from Merck, (Darmstadt, Germany). Other materials used in this study were of pharmaceutical or analytical grade and were used as received.

METHODS

1. Optimization of Manufacturing Conditions of Gelatin Foams

Various conditions expected to affect the generation of stable foams such as gelatin concentration and its pH on foam formation were studied.

1.1 Optimization of Gelatin Solutions

100 g of various concentrations of freshly prepared gelatin solutions 1, 3, 5, 7,9, and 11% (w/w) adjusted to pH values of 4.5, 5.5,and 6.5 (before, at, and after its isoelectric point ,respectively) using diluted solutions of acetic acid and ammonia were prepared for foam generation.

1.2 Optimization of Whipping Conditions

Gelatin foams were produced by the whipping method. The used homogenizer (VirTis, Gardiner,,NY) consists of double-jacketed thermostatic stainless steel tank and a speed controllable stirrer with a special blade that enhances air bubbles incorporation and foam stability. Different gelatin solutions were transferred into the foam apparatus tank and generated into foams under different conditions. The effect of foam operation temperature (25, 35, 40, 50˚C), whipping duration (5, 10, 15, and 30 min), and speed of stirring (500, 1000 rpm) were studied.

1.2 Characterization of Gelatin Foams

The resulting foams were evaluated with respect to foam uniformity (defined as the homogeneity of air bubbles examined under an electron microscope), foam stability at 25°C (defined as separation of the liquid part from derived foams in a cylinder along 60 minutes after finishing foam generation) and apparent foam density (estimated by dividing the foam weight (mg) in a cylinder over the foam volume (ml) in the same cylinder). Finally, the foam volume which is the reciprocal of the apparent foam density was also calculated and the optimum conditions for generation of stable foams were determined.

2. Generation of Chitosan-Gelatin Foams

Gelatin was used as a foam builder for the preparation of chitosan-gelatin foams. 7% (w/w) aqueous gelatin solutions and 2.5% (w/w) low molecular weight (MW) chitosan solution in 1% acetic acid were used. Different chitosan-gelatin mixtures of 1:1, 1:5, and 1:10 (Fig. 1) were whipped into foams using different speeds of 1000, 1500, and 2000 rpm for 15 minutes each. The apparent foam density and foam stability were compared.

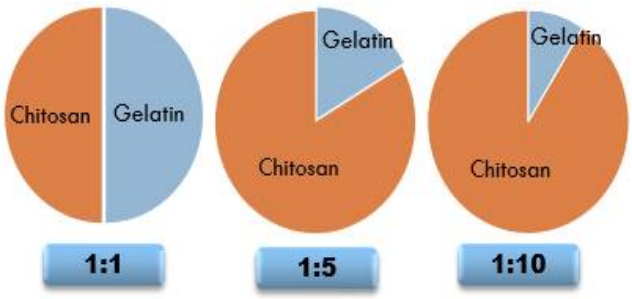

Fig. 1 Different chitosan-gelatin mixtures

3. Preparation of Chitosan-Gelatin Cross Linked Medicated Sponge

Diclofenac and Dexamethasone each of 25 mg were incorporated into 10 g of 1:5 gelatin:chitosan mixture. The mixture was whipped into stable foam at 25°C with a stirring rate of 1000 rpm for 15 minutes. The resulting foams were then cross-linked with 0.1% glutaraldehyde solution.

Sponge	Chitosan: Gelatin ratio	Stirring speed (rpm)
A	1:1	1000
B	1:1	1500
C	1:1	2000
D	1:5	1000
E	1:5	1500
F	1:5	2000
G	1:10	1000
H	1:10	1500
I	1:10	2000

Continuous stirring for another 15 minutes was necessary to enhance glutaraldehyde cross-linking effect. The cross-linked foams were poured into ELISA plate and freeze dried (Freeze dryer, CHRIST, LYO CHAMBER GUARO,ALPHA 1-4 LSC) at −80°C, under a vacuum of 33 × 10–3 mbar.

Table 1 Different prepared sponges.

4. Characterization of the Sponges

4.1 Scanning Electron Microscopy (SEM)

A thin piece of a sponge (0.5 mm) was fixed on a SEM sample holder with double-sided adhesive tape and coated with a layer of gold of 150 Å for 2 min (Sputter coater, S-150A, Edwards, Crawley, England) in a vacuum of 3 × 10−1 atm. of argon gas. The sample was then examined (Scanning electron microscope, JSM T20, Jeol, Tokyo, Japan).

4.2 Dissolution Medium Uptake Capacity

The sponge was accurately weighed and placed in a small bottle containing 30 ml of Sorensen's phosphate buffer (pH 7.4) at 25°C. The bottle was turned up and down twice to ensure complete wetting of the sponge. The sponge was removed from the buffer solution after 0.25, 1, and 2 hr by means of a small forceps, allowed to drain by careful dropping on a filter paper, and reweighed. The increase in weight represents the weight of the buffer solution taken by the sponge, which was calculated as a ratio of the weight of absorbed buffer solution to the weight of the dry sponge at each period of time as follows:

Dissolution medium uptake capacity (g/g) =

$$(W_{wet} - W_{dry}) / W_{dry}$$

4.3 Determination of Drug Content

The sponges were soaked overnight at 25°C in 500 mL distilled water. After filtration through a cellulose acetate membrane (0.45 μm), aliquote were taken, diluted as necessary the concentration of diclofenac and dexamethasone in the solution were determined spectrophotometrically (1601-PC double beam spectrophotometer,Shimadzu, Kyoto, Japan) at 276 and 254nm respectively. The assay was run in triplicate.

III. RESULTS AND DISCUSSION

3.1 Optimization of Manufacturing Conditions of Gelatin Foams

The best conditions to get stable homogeneous gelatin foam with small air bubbles were by whipping 7% gelatin solution with high shear at a speed of 1000 rpm for 10 minutes at 25°C.

3.1.1 Characterization of Gelatin Foams

Gelatin foams prepared at all whipping speeds (1000, 1500, and 2000 rpm) showed stable foam conditions after 60 minutes of preparation. The same froth height and stability at time zero and after 60 minutes.

As shown in Table 2, Gelatin prepared at the fastest whipping speed showed the least density while the intermediate one (1500 rpm) showed the highest weight with highest density.

3.1.2 Generation of Gelatin:Chitosan Foams

The optimum concentrations that could produce stable blend of Gelatin:Chitosan foam were 7% (w/w) aqueous gelatin solutions, 2.5% (w/w) low molecular weight (MW) chitosan solution in 1% acetic acid blended in 1:5 ratio respectively. 0.1% glutaraldehyde was used for crosslinking and whipped at 1000 rpm for 10 minutes at room temperature.

Table 2 Densities of different Gelatin foams.

Whipping Speed (rpm)	Weight (g)	Volume (ml)	Density (g/ml)
1000	8.871	20	0.4436
1500	9.114	20	0.4557
2000	8.773	20	0.4390

3.2 Characterization of the Sponges

3.2.1 Scanning Electron Microscopy (SEM)

Cross linked medicated 1:5 Gelatin:Chitosan was chosen because of the stability of its foam mixture where homogenous, uniform foam with small air bubbles were produced as shown in Fig. 2. On the other hand, as depicted from Fig. 3, non-cross linked medicated 1:5 chitosan: gelatin showed less homogeneity with large air bubbles.

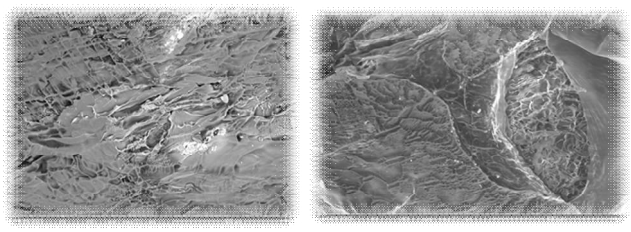

Fig. 2 Scanning Electron Micrographs of Cross-linked Medicated 1:5 Gelatin:Chitosan Sponge.

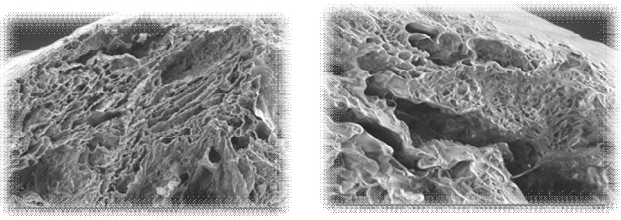

Fig. 3 Scanning Electron Micrographs of Non Cross-linked Medicated 1:5 Gelatin:Chitosan Sponge.

3.2.2 Dissolution Medium Uptake Capacity

Table 3 Percentages of sponge weight after 0.25, 1 and 2 hours of dissolution medium uptake capacity for the different sponge formulations.

Sponge	0.25 hr	1 hr	2 hr
A	5%	10%	13%
B	7%	8%	14%
C	6%	14%	15%
D	1445%	949%	7%
E	2023%	897%	6%
F	1155%	1255%	6%
G	2058%	1257%	4%
H	2030%	2143%	5%
I	5%	53%	8%

According to the different prepared sponges in table 1 and results in table 3, Formulations D, E and F, of 1:5 Gelatin:Chitosan ratio with different stirring speeds, show fast and high absorption behaviour reaching up to 2023% of its weight at time zero after 15 minutes only, and almost a complete dissolution with remaining only 6% of its weight after 2 hours.

Formulations G and H of 1:10 Gelatin:Chitosan ratio, show a similar behaviour to that of 1:5 formulations with also fast and high absorption rates and good dissolution after 2 hours of immersing sponges in buffer. While the third formulation (Sponge I) prepared according to the same Gelatin:Chitosan ratio shows very low absorption manner.

The least absorption capacity goes to 1:1 Gelatin:Chitosan ratio formulations as their weights decreased to 5, 6 and 7% of their weights before test after 15 minutes only.

3.2.3 Determination of Drug Content

The loading of both drugs in the sponges did not deviate markedly from the calculated amount where > 95% of both drugs were recovered

Conclusion

Chitosan –gelatin sponge was obtained after freeze drying cross-linked stable foam generated at room temperature by whipping 1:5 gelatin:chitosan solution at 1000 rpm for 15 minutes. The optimum formula is chosen for further in vitro release study, pharmacodynamics activity as well as clinical study

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