Preparation and Characterization of Periodontal Chips from Egg Shell Membrane

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Abstract

The present study was performed to prepare periodontal chips from egg shell membrane (ESM) and evaluates its physical, biodegradation and absorption properties.

ESM was extracted using acetic acid (1%). The membranes were cut into chips of 6×6 mm2. Thickness and morphology of the chips were evaluated using scanning electron microscopy (SEM). The chips were tested for biodegradability towards trypsin. Absorption test was done on artificial saliva AS and sterilized distilled water SDW. Ninety ESM chips were weighed and immersed in 90 Eppendorf tubes contained 0.5 (ml) of trypsin, artificial saliva and distilled water (30 for each),for 21 days. Chips were weighed and scanned by (SEM) on day7,14 and 21 to evaluate the biodegradation and absorption process. A profilometer was also used to evaluate the surface roughness of the ESM chips. The data were statistically analysed with a one-way ANOVA, Student's t-test, regression analysis and Chi-square test.

The mean weight values have been significantly decreased over time within the group of trypsin (baseline: 1.6 ± 0.452, day 21: 0.683 ± 0.16).While the weights have been significantly increased over the time within the groups of AS (baseline: 1.503 ± 0.371, day 21: 3.453 ± 0.657) and SDW (baseline: 1.77 ± 0.304, day 21: 3.14 ± 0.686). Statistical analyses showed a significant difference in thickness values of ESM during the biodegradation test (baseline: 55.3000 ± 8.35165, day 21: 7.7429 ± 5.08064).There were no significant different in the mean thickness values of the ESM chips during the absorption test by AS and SDW. The SEM revealed that the thickness was begun to reduce during biodegradation test on day 14 until day 21.

ESM chips were found to be biodegradable, exhibiting characteristic within the periodontal chips standard features.

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Introduction

Periodontitis is a common term defining particular diseases impacting the alveolar bone, the gingiva, and the supporting connective tissue which connecting the teeth in the jaws. It's leading to losing of connective tissue, affecting the alveolar bone and development of periodontal pockets, and ultimately to the loss of teeth.[1,2] Also, found a strong connection between periodontal disease and susceptibility to systemic disease (e.g., cardiovascular disease, bacterial pneumonia, infective endocarditis, diabetes). It is important thus to control and manage periodontal disease and its influences.[3]

The rate of development of this disease is dependent on the intensity or severity of the plaque bacterial infection and the efficiency of the individuals' systemic and local immune-inflammatory responses. The periodontal health critically depends on the balance of the bacterial plaque challenge and the inflammatory response of the immunity. However, there is a clear positive relationship between the periodontal inflammation and immune compromised patients.[4]

There are many risk factors establish for a periodontal disease. Among these risk factors are smoking, diabetes mellitus.[5] Other factors might attribute to periodontal disease like poor

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diet and stress, but the relationship not clear whether has a physiological basis or is because simply to the fact that persons suffer from stress are not good regular oral hygiene.[6]

There is a great variety of treatment options that are available such as antiseptics irrigation and rinse with a chlorhexidine gluconate, mechanical removal of plaque and calculus and antibiotics therapy. There are two types of antibiotics, systemic antibiotics; such as amoxicillin or clindamycin, and locally applied, antiseptics and antibiotics by chips such as atridox and periochipes.[7] Recently, the most technique has usually used for the treatment of periodontitis is a mechanical disruption of the bacterial flora by scaling and root debridement. On the other hand, some trials presented such mechanical disruption is not enough in changing the structure of flora to prevent infection from a return at the sites. So these studies have been recommended that the utilized of an adjunctive localized antimicrobial treatment may avoid the chance for secondary surgical involvement and advance clinical results.[8]

Different modes of administration, local or systemic have been used to determine the antimicrobial agents to the infected sites. These antimicrobial agents could carried by solid dosage structure or so-called local delivery system. PerioChip considers a pioneer in the field of the biodegradable delivery systems. The PerioChip can be introduced easily through the periodontal pocket because of the natural liquid containment that immersed in the gingival cervical fluid of the pocket. This fluid provides a filtering medium for the drug of the delivery system to be released. PerioChip is advised to be an adjunctive treatment to scaling and root surface instrumentation.[9,10]

PerioChip is a small, thin, bullet shaped, orange-brown chip effective against a broad spectrum of bacteria. The main component of these chips is chlorhexidine gluconate in a biodegradable matrix of hydrolysed gelatine (cross-linked with glutaraldehyde).

PerioChip is expensive and shown many side effects in previous experiences like bitter taste, Brown discoloration of teeth and restorative materials have reported when chlorhexidine has utilized as mouth rinses. To overcome these side effects, the World Health Organization (WHO) advised using natural products.[11]

Natural remedy has been taking the attention recently because it is cheap, affordable, and fewer side effects.[12] ESM is one of the most inexpensive antibacterial agents because it has been available in homes and fast food and restaurants industries and can easily collect in plenty. ESM is a thin layer between the egg white and the eggshell was designed by nature with the perfect matrix of compounds to nourish and protect the precious egg components.[13]

Disposal of eggshell waste contributes to environmental pollution. There are many challenges related to placement of eggshells like cost, odour, flies and insects and availability of disposal sites, so instead of wastage, an ESM can be processed into saleable products like fertilizer utilized in fields, human and animal supplements and to produce collagen from the membrane.[14]

The Egg Shell Membrane is considering a source of many natural nutrients, such as hyaluronic acid, chondroitin, glucosamine, collagen and amino acids that play a vital role in healing process.[15]

There is a lack of studies regarding the usage of The Egg Shell Membrane in the management of periodontal disease and develop ESM periodontal chip, so the present study will focus on formulating and characterization of the periodontal chip from ESM which is cheap and natural.

Materials and methods

Egg Shell Membrane Extraction

Thirty farm fresh chicken eggs were cleaned with soap and water and arranged on eggs rack until dry. Eggshells this Eppendorf tube with a small hole by sterilizing small knife, empty the albumen and yolk into a clean storage container and thoroughly were washed with sterilized distilled water several times to be sure that all residues are entirely gone. The clean empty eggs were returned to the egg rack to be completely dry. The eggs were filled with 1% acetic acid solution (prepared 1% acetic acid by mixed in sterilized baker (1 ml) of acetic acid (reagent 99%) with (100 ml) of distilled water for 10 min. After those membranes had been extracted manually, the membranes were rinsed with sterilized distilled water and were put on lane free cloth at room temperature for 24h.

The method was estimated using the previous study with some modifications.[16] To produce ESM chips, round shape 6 square millimetres (mm²), the membranes were cut into sheets by scissors to making easy punch the membrane by a paper punch to produce the chips. One Hole Paper Punch Pliers 6×6 mm2 (A hole puncher is a standard office tool that is used to create holes in sheets of paper), was utilized. The chips then individually packed in sterilized container and were kept at room temperature.

Biodegradable Test for ESM

Thirty chips were weight by Top pan weighing balance after adjusted it on milligram (mg). All data was recorded to make the comparison with the resulting data that got after biodegradation test to evaluate the extent of degradation. 0.5 ml of Aqueous trypsin (100 ml), 2.5% (10x, 25000.0 mg/L) was pipetted out into Eppendorf tubes with plastic transfer pipettes size 1ml.

Thirty ESM chips were immersed into these Eppendorf tubes. Then arranged these Eppendorf tubes on to a tubes rack and Incubated at particular condition (37°C, without exposure, to direct sunlight) for seven, fourteen and twenty-one days. After a duration of 7, 14 and 21 days, the chips were removed from the Eppendorf and left on filter paper to dry.[17] The next step begins with calibration and adjustment of an analytical balance (top pan weighing balance) on milligram (mg), the chips were placed one by one using Forceps on the top of the balance pan to record the weight of each chip, and then the data was recorded. The definition of the biodegradation degree is the relation between non-degraded mass and initial mass of the samples.[18]

Artificial Saliva Absorption by ESM chips

To evaluate the saliva absorption, thirty chips were weight by Top pan weighing balance, and all data was recorded to make the comparison with the subsequent data that got after saliva absorption test to evaluate the extent of weight gained after absorption process. Then 0.5 ml of freshly prepared artificial saliva was added in eppendorf tubes by Plastic Transfer Pipettes. After that, the chips were brought and immersed into these tubes, and left it at room temperature 23°C for seven, fourteen and twenty-one days.

The percentage of weight gain of the chips was measured by removed all the chips, dried with a lane free cloth, and weight by Top pan weighing balance. The data was recorded on the seventh, fourteenth and twenty-first days to evaluate the extent of the absorption process.[17]

Water Adsorption by the ESM Chips

At the beginning of the test, thirty chips were weight by Top pan weighing balance after, and all data was recorded to make the comparison with the resulting data that got after absorption test to evaluate the extent of weight gained after absorption process. Eppendorf tubes were prepared by adding 0.5 ml of sterilizing distilled water by Plastic transfer pipettes. Then the weighted chips have immersed into these Eppendorf tube at room temperature, 23°C for seven, fourteen and twenty-one days.

The percentage of weight gain of the chips was measured by removed all the chips, dried with a lane free cloth, and weight by Top pan weighing balance. The data was recorded on the seventh, fourteenth and twenty-first days to evaluate the extent of the absorption process.[17]

Data Analysis

Data collection was analyzed by using Statistical Package for the Social Sciences (SPSS) version 22.0 by using paired t-test and ANOVA. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test. The significance of the obtained results was judged at the 0.05 level.

Results

Assessment the effect of the Trypsin media (degradation) over the ESM thickness values through time intervals. The thickness of chips were measured by using SEM (figure 1), As shown in Table 1.1, the mean thickness values of SEM were significantly decreased throw the time of the degradation teste by trypsin (between days 0-21). One way ANOVA statistical test proved that the rate of the decreased in the mean thickness values of ESM

chips become increased so fast after day 14 and reaches to the minimum mean values on day 21(7.7429 ± 5.08064).

	Thickness (µm) Mean ± SD	P-value
Baseline	55.3000 ± 8.35165	<0.001
Day 7	52.5143 ± 7.29187	
Day 14	45.7286 ± 7.28165	
Day 21	7.7429 ± 5.08064	

***(One-way ANOVA) Significant difference versus the control group under 95% confidence interval (P< 0.05).**

Table 1. The effect of the Trypsin media (degradation) over the ESM thickness values through time intervals.*

Assessment the effect of the Artificial saliva media (absorption) over the ESM thickness values through time intervals.

As shown in Table 2, the mean thickness values of SEM were no significantly changed throw the time of the absorption teste by AS (between days 0-21). One way ANOVA statistical test proved that the mean value of the ESM chips thickness significantly no changed appeared in the absorption test by AS.

	Thickness (µm) Mean ± SD	P-value
Baseline	55.3000 ± 8.35165	0.192
Day 7	57.0000 ± 6.23565	
Day 14	63.0857 ± 7.79731	
Day 21	61.2286 ± 6.72030	

***(One-way ANOVA) No significant difference versus the control group under 95% confidence interval (P = 0.192).**

Table 2. The effect of the Artificial saliva media (absorption) over the ESM thickness values through time intervals.*

Assessment the effect of the Distilled water media (absorption) over the ESM thickness values through time intervals.

As shown in Table 3, the mean thickness values of SEM were no significantly changed throw the time of the absorption teste by SDW (between days 0-21). One way ANOVA statistical test proved that the mean value of the ESM chips thickness significantly no changed appeared in the absorption test by SDW.

	Thickness (µm) Mean ± SD	P-value
Baseline	55.3000 ± 8.35165	0.194
Day 7	53.9143 ± 5.61469	
Day 14	55.7571 ± 5.98494	
Day 21	66.1429 ± 4.19319	

*(One-way ANOVA) No significant difference versus the control group under 95% confidence interval (P = 0.194).

Table 3. Effect of the Distilled water media (absorption) over the ESM thickness values through time intervals.*

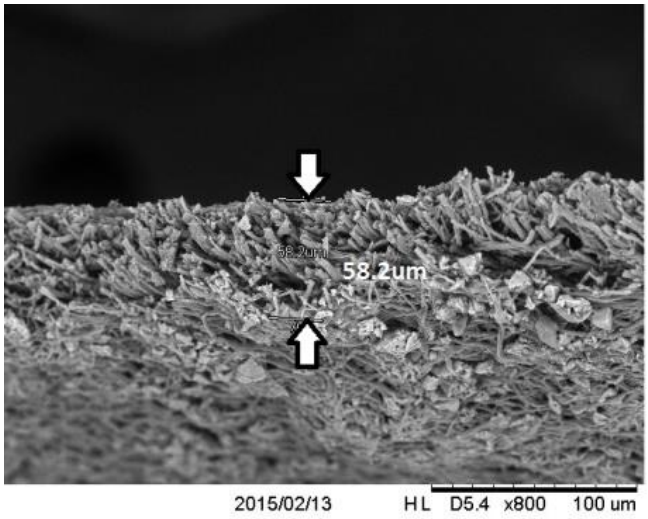

Figure1. Thickness of the ESM.

(Figure 3, Table 4, 5, 6 and 7).

Parameter	Time	N	Weight (mg) * Mean ± SD
Abs AS	Baseline	30	1.503 ± 0.371
	Day 7	30	2.39 ± 0.598
	Day 14	30	3.46 ± 0.697
	Day 21	30	3.453 ± 0.657
Abs SDW	Baseline	30	1.77 ± 0.304
	Day 7	30	1.813 ± 0.46
	Day 14	30	2.847 ± 0.636
	Day 21	30	3.14 ± 0.686
Deg. Trypsin	Baseline	30	1.6 ± 0.452
	Day 7	30	1.267 ± 0.292
	Day 14	30	0.773 ± 0.155
	Day 21	30	0.683 ± 0.16

***(Kolmogorov-Smirnov test) Weight (mg) departs from a uniform distribution (P <0.001).**

Table 4. Mean and SD of the ESM weight values for the absorption and degradation medias through time intervals.

General Descriptive Analysis

Biodegradation test of ESM chips study done by using trypsin. Absorption test was done using artificial saliva and distilled water. Weight of the chip immersed in the solution was measured every 7 days. All the chips changed their shape after several days. Statistical analysis showed significant difference in chips weight. One way ANOVA showed that the average weight (mg) varies across values of biodegradation media. Kolmogorov-Smirnov test ,Post Hoc t-test of weight by biodegradation media within groups showed clear significant differences of the trypsin comparing to other media

	Trypsin Mean ± SD	P-value
Baseline	1.6 ± 0.169	< 0.001
Day 7	1.267 ± 0.109 *	< 0.001
Day 14	0.773 ± 0.058 *	< 0.001
Day 21	0.683 ± 0.06 *	< 0.001

***(One-way ANOVA) Significant difference versus the control group under 95% confidence interval (P< 0.05).**

Table 5. The effect of the degradation media over the ESM weight values through time intervals.

	Weight (mg) Mean ± SD	P-value
Baseline	1.503 ± 0.139	< 0.001
Day 7	2.39 ± 0.223	
Day 14	3.46 ± 0.26	
Day 21	3.453 ± 0.245	

*(One-way ANOVA) Significant difference versus the baseline group under 95% confidence interval (P< 0.05).

Table 6. The effect of the Artificial saliva (AS) media (absorption) over the ESM weight values through time intervals.*

	Weight (mg) Mean ± SD	P-value
Baseline	1.77 ± 0.114	< 0.001
Day 7	1.813 ± 0.172	
Day 14	2.847 ± 0.237	
Day 21	3.14 ± 0.256	

***(One-way ANOVA) Significant difference versus the control group under 95% confidence interval (P< 0.05).**

Table 7. The effect of the Sterilized Distilled water (SDW) media (absorption) over the ESM weight values through time intervals.*

Regression Analysis

For the scatter diagram of Trypsin Figure 2, the ESM chips weight values decreased by -0.333 mg in the first seven days intervals. The coefficient of the weights decrease is almost -0.9mg, based on that the degradation of ESM was increased with average time progressively until the ESM chips were completely degraded.

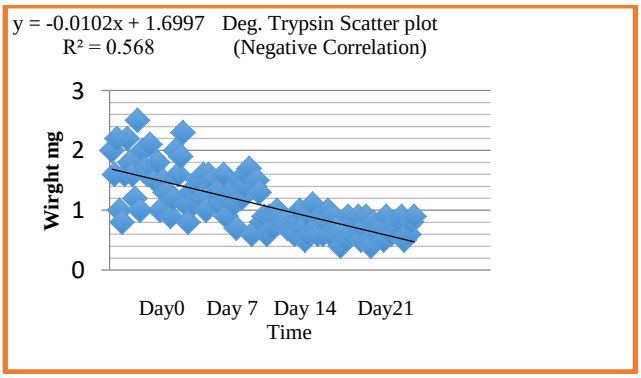

Figure 2. Scatter plot of the weight values of ESM chips within the duration of the biodegradation test by trypsin.

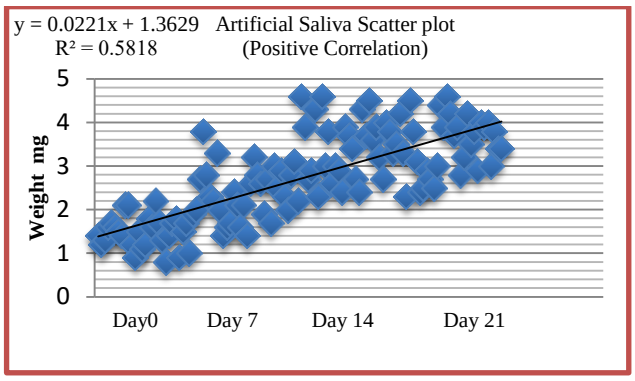

Figure 3. Scatter plot of the weight values of ESM chips within the duration (days) of the absorption test by AS.

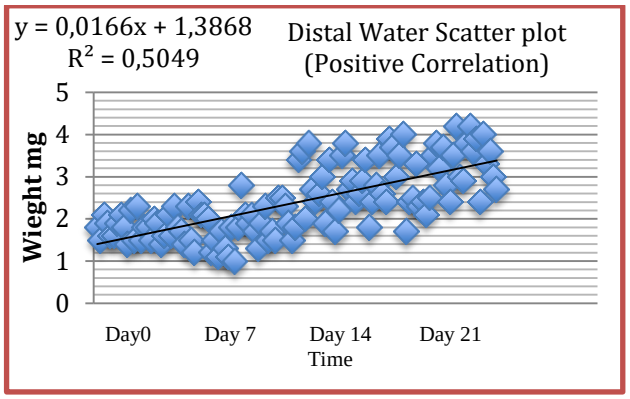

Figure 5. Scatter plot of the weight values of ESM chips within the duration of the absorption test by SDW.

For the scatter diagram of AS Figure 3, the ESM chips weight values increased by 0.877 mg in the first seven days intervals. The coefficient of the weights increase is almost 1.95 mg, based on that the AS absorption process of ESM was increased with average time progressively until the SEM chips completely saturated.

For the scatter diagram of SDW Fig4, the ESM chips weight values increased by 0.043 mg in the first 7 days intervals. The coefficient of the weights increase is 1.077 mg on the second 7 days, on the third 7 days the coefficient of the weight values increased by 1.37 based on that the SDW absorption process of ESM was increased with average time progressively until the SEM chips completely saturated.

Discussion

Periodontitis is a dental disease, which is an inflammatory response to bacteriological infections. Periodontitis destroys the attachment apparatus of teeth resulting in the periodontal pocket formation and alteration of normal osseous anatomy. Local application of antimicrobials has been indicated in localized forms of periodontitis and non-responding and recurrent sites. Earlier studies demonstrated that biodegradable chlorhexidine chip could be utilized as adjunct to conventional periodontal therapy. In the current study, new biodegradable chips were prepared and characterized by egg shell membrane.

In the present study, the experimental chips dimensions were standardized before the experimental procedures. The average size of ESM chips were 6×6 mm2. Many studies were deals with the preparation of periodontal chips.[9,19] In their studies, the periodontal chips were prepared in average size was 5×6 mm2 which is clearly within the average of the chips dimensions of the present study.

Al-Bayaty et al.[9] and Jensen et al.[19] were showed in their studies that the average values of chips thickness were within 0.02mm to 0.135mm and the average values of the weights were within 5.8mg to 10mg. In the present study, the ESM chips mean thickness values were 0.055 mm and mean weight values were 1.6 mg. Based on that, the present study findings regarding the weight proved that the ESM chips are lighter in weight than the chlorohexidine chips. Hence, the ESM chips could be inserted into the periodontal pocket without introducing pressure on the gingiva and occupying the space between the gingival tissue and the tooth.

In the present study, a chemical method by acetic acid was used to extract the ESM easily and without decomposition the fibers.[6,20] This method is useful because of the outer ESM is highly embedded into the egg shell. Hence, the general acidic treatment is needed to separate ESM completely from the egg shell without effects on the composition of the collagen fibers.[6,21] The current extraction method was avoided tearing membrane and collagen denaturation. This preservation of collagen fibres is essential to formulate the periodontal chips.

In vitro biodegradation test was done to evaluate the changes that will occur to the ESM within the present of the bacterial trypsin in the periodontal pocket. Chen et al.[22] suggested using the conventional technique to evaluate the biodegradability of the ESM. The present study was used the same conventional method with modifications. The samples were collected in Eppendorf's tubes to be more observable and measurable. The time intervals of the present study were modified from the conventional technique to be more adapted to the clinical aspects. Furthermore, the final findings of the conventional method were showed the same biodegradable behaviour for the present study which is increasing in the biodegradability in the last time intervals. The results show that there was significant decreasing for the mean weights of ESM chips progressively with the experiments duration. The significant decrease in the ESM weights values could be attributed to trypsin actions on the peptidic bonds (-CONH-) inside the amino acids Arginine and Lysine. The degradation rate of matrices was crosslinked with the same trypsin concentration, but with different reaction times (7, 14 and 21 days).Our result in agreement with study carried out by Fouad et. al.[21]

The SEM images as in were showed the degradation process begins after the seventh day of the experiment. Pre-experimentally, the fibres more defined and surrounded by a lot of porosities areas and the ESM chip mean thickness is (55.3000 ± 8.35165). While after seven days, there were no noticeable changes in the thickness, the fibres have become more compacted and thicker showed changes in the architecture of the ESM that mean the chips become clamped (52.5143 ± 7.29187). In day 14, the ESM chip thickness was begun to reduce to (45.7286 ± 7.28165).While in day 21, the chip thickness was declined apparently to show the lowest thickness values (7.7429 ± 5.08064).

The regression coefficient analysis revealed that, the gradient is -0.333 in the first seven days interval. That mean on averages, the weight (mg) values will be less by 0.333 mg for each seven days interval. While in the second and third seven days intervals, the gradient is near to be -0.9 (-0.827 and -0.917 in the second and third seven days intervals respectively). These mean that the weight (mg) values will be less by 0.9 mg for each seven days interval starting from the second week and continues in positive correlation form. The predicted weight

(mg) for day zero (baseline) is 1.6 mg; this implies that on the average weight (mg) of ESM showed decreasing behaviour at the time.

In the present study, most of the ESM chips were still presents until 21 days of the biodegradation experiment. This behaviour could be attributed to the types of the ESM fibres and how much its crosslinked throw the tissue. This crosslinking makes some difficulties for trypsin to diffusion into the fibres. Comparing with chlorohexidine gluconate chips, its completely degraded after ten days of the treatment.[23]

ESMs are consisting of protein fibres that are organized to produce a semi-permeable membrane. Therefore, the ESM have a complicated lattice network of stable and water-insoluble fibres. Therefore, it is quite essential to evaluate the ESM behaviour towards fluids absorption such as artificial saliva and distilled water.

The release of drug in the certain material could be affected by the material ability for water diffusion. In specific materials such as restorative materials, it's essential to enhance the aqueous solutions or diffusion of water to reach certain performance requirements. Like in glass inomer cement (GIC) restorative material, which is lead to release fluoride.[18] Clinically, the ESM chips will be directly attached with body fluids like saliva and water. So, the absorption experiment is important because the physical properties of the ESM chips fibres and its dimensional solidity may be affected by the absorption feature. To evaluate the clinical performance, the amount of water absorption must be calculated over a period, which is comparable with the proposed duration that been used in the oral environment.

The purpose of the absorption test with artificial saliva was to evaluate the changes that will occur to the ESM within the periodontal pocket and evaluate the increasing in the chips weight to determine the possibility to use the ESM as a periodontal chip. Human saliva can vary in sufficiently great degree and is conditional on many factors like age and sex of the patient, medication and oral hygiene eating habits.[24] In this study, we selected artificial saliva because the results obtained must be standardized.

The adsorption efficiencies of ESM towards the artificial saliva was evaluated by comparing the ESM chips initial weights with ESM chips weights within the experiment duration. It is obvious from the results, by increasing the AS concentration, the mean weight values of ESM increased. The mean values of the weights within the group of AS in day 14 and 21 are relatively equal that demonstrate that the absorbency reaches to the equilibrium and there is no more absorption after day 21.

Based on the regression coefficient results (fig 4), the gradient is0.887 in the first seven days interval, that mean on average the weight (mg) values will be more by 0.877 mg for each seven days interval at the time. While in the second and third seven days intervals, the gradient is 1.95. This mean that the weight (mg) values will be more by 1.95 mg for each seven days interval starting from the second week and continues in positive correlation form. The predicted weight (mg) for day one (baseline) is 1.503 mg; this implies that on the average weight (mg) of ESM showed increasing behaviour with the time.

In the present study, the ESM chips thickness measurements were determined by SEM within all the absorption test duration. The thickness of the chip in baseline was (58.2± 0.11), on day seven, the fibres more defined and surrounded by a lot of porosities area and the chips thickness was (62.6± 0,14), the porosities and the black areas between the fibres were reduced within a period of the experiment. In day 21 of the experiment, the fibres became more compacted. That mean the AS molecules was interrupted within the ESM fibres lead to increase of the fibres size without effect on the ESM thickness.

In the present study, the absorption property by Sterilized distilled water (SDW) for ESM was evaluated to examine the physical and dimensional changes that have occurred to the ESM fibres during the absorption process. The techniques that have been utilized to study the absorption and correlated dimensional changes have shown to be simple but meaningful. Many materials are required to promote the absorption of aqueous solutions or water so as to reach certain performance requirements.

The adsorption efficiencies of ESM towards the SDW was evaluated by comparing the ESM chips initial weights with ESM chips weights within the experiment duration. The findings of the present study have shown that the mean values for the weights of the ESM have been significantly increased over time intervals

within the groups of SDW (Table 7). The mechanism of the absorption may be done by capillary action because the ESM consider rigid and porous structure so may absorb the SDW into porosities through this capillary action. This action may create a little or no dimensional changes but increase in ESM chips weights.

Based on the regression coefficient results (figure 5), the gradient is 0.043 in the first seven days interval. That mean on average the weight (mg) values will be more by 0.043 mg for each seven days interval at the time. While in the second and third seven days intervals, the gradient is slightly more than 1 mg (1.07 and 1.37 in the second and third seven days interval respectively). This mean that the weight (mg) values will be more by slightly higher than 1 mg for each seven days interval starting from the second week and continues in positive correlation form. The predicted weight (mg) for day zero (baseline) is 1.77 mg; this implies that on the average weight (mg) of ESM showed increasing behaviour at the time.

ESM chips thickness measurements were examined by one-way ANOVA statistical test and by SEM within all the absorption test duration. The results proved that there were no significant different in the mean thickness values of ESM chips during the absorption test by SDW (Table 3). The mean thickness of the chips in baseline was (55.3000 ± 8.35165), the fibres more defined and surrounded by a lot of porosities area and on day seven the mean thickness value was (53.9143 ± 5.61469). The porosities and the black areas between the fibres were reduced within 14 days of the experiment; the mean thickness value was (55.7571 ± 5.98494). In day 21 of the experiment, the fibres were more compacted and closed to each other leaving fewer porosities and cavities and the mean thickness value was (1429 ± 4.19319).

That mean the SDW molecules was intercepted within the ESM fibres lead to increase of the fibres size without effect on the ESM thickness. The present study demonstrates that the ESM is like sponge tissue, this conclusion lead to improving that the ESM can be used in the dental pocket without the increase in its size.

Conclusions

The present study findings proved that the characteristic features of the ESM chips are within the previous literature studies regarding the commercial chips characters. Hence, the ESM could be used to produce periodontal chips.

Declaration of Interest

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