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Novel Tri-Phasic Bioactive Collagen-Chitosan-Nanohydroxyapatite Membrane in Treating Intrabony Defect: A Randomized Clinical Trial

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Abstract: Aim: This study seeks to evaluate clinically and radiographically the effects of collagen-chitosan-nanohydroxyapatite membrane in the treatment of periodontal intrabony defects.

Materials & methods: Thirty-six patients participated in this study and were divided into three groups, each consisting of 12 cases: Group I received open flap debridement (OFD) only, Group II got OFD with collagen, chitosan, and nanohydroxyapatite membrane, and Group III underwent OFD with collagen type I membrane. Data were obtained at baseline, 3 months, and 6 months regarding plaque index (PI), modified sulcular bleeding index (MS), probing depth (PD), clinical attachment level (CAL), and radiographic measurements were statistically analyzed and compared.

Results: There were no significant differences in PI, MS, or CAL at baseline, 3 months, or 6 months among the three groups. There was no significant difference in PD at baseline or 6 months between the three groups. There was no significant change in PD between groups I and II at 3 months. At 3 months, group I's PD decreased significantly as compared to group III's. At 3 months, group II's PD decreased much more than group III's.

Conclusion: Despite limitations, this study suggests that collagen-chitosan-Nanohydroxyapatite membrane improve the clinical attachment level and pocket depth.

Keywords: *Chitosan, flap debridement, intrabony, nanohydroxyapatite.*

Introduction

Periodontitis is an inflammatory disease affecting the supporting tissues of the teeth. In the presence of risk factors, it is a multifactorial and multietiological infectious disease process induced by periodontal bacteria and

the host immunological response (Aas Jørn et al., 2005). Removing pathogenic biofilms and suppressing inflammation can reduce periodontal tissue degeneration. However, the ability to restore lost tissues is dependent on multiple factors, including the extent of tissue defect, overall health, and age (Könönen et al., 2019).

Periodontal therapy relies heavily on scaling and root planing (SRP). Its main objective is to remove both soft and hard microbial residues from the diseased root surfaces. It reduces clinical inflammation and pocket probing depth remarkably well (Cobb & Sottosanti, 2021). However, the efficacy of SRP can be restricted because it is incapable of completely eradicating all bacteria in cases with deep pockets, root concavities, grooves, furcation involvement, and invasion of root surface abnormalities (Vohra et al., 2018).

In order to overcome this limitation, other treatment modalities began to gain traction as supplementary to mechanical procedures. Pharmacological disinfectants and antibiotics as adjuncts to SRP may facilitate resolution of the inflammation (Patangia et al., 2022). Yet, because of the risk of bacterial resistance and the effect on the entire microbiota composition of the human organism that is deeply connected to systemic antibiotic administration, antibiotic use must be critically questioned, especially in terms of its potential added benefit and adverse side effects for the patient (Donos et al., 2020).

Different studies have suggested that guided bone regeneration (GTR) is an effective treatment modality for the management of intrabony defects (IBD). GTR was associated with the shallowest remaining defects and the best results in improving clinical attachment level (CAL) gain, reducing pocket depth (PD), less increase in gingival recession, and more gain in hard tissue probing at re-entry surgery when compared to open flap debridement (OFD) or the use of bone grafts alone (Murphy & Gunsolley, 2003).

The current research has been conducted to evaluate the clinical effects of chitosan, nanohydroxyapatite, and collagen membrane in the treatment of periodontal intrabony defects.

Materials and methods

Patients' selection and enrollment:

The present randomized controlled study included 36 patients aged from 25 to 55 years selected from the outpatient clinic of the Oral Medicine and Periodontology Department, Faculty of Dentistry, October 6 University with stage II and III periodontitis and their own written informed consent of participation in the study. The study protocol was approved by the ethic committee of the scientific research faculty of dentistry, October 6 University Egypt, with approval number RECO6U/26-2022 obtained in its meeting held on November 12, 2022. All subjects who participated in this study signed a written consent and fully agreed to participate in this work.

Inclusion criteria involved patients having stage II and III periodontitis with probing pocket depths ≥ 5 mm and radiographic evidence showing intrabony defects, patients with Grade B periodontitis, and systemically healthy individuals. Patient with probing depth more than 5 mm after phase 1 therapy, pocket depth is measured, and if more than 5 mm, the surgery will be done (Sanz et al., 2020). And compliant patients are willing to follow oral hygiene instructions. While exclusion criteria were patients received periodontal or antibiotic therapy within the previous 6 months preceding the study (Newman et al., 2018), smoking, pregnant and lactating females, or patients on medications known to affect bone turnover.

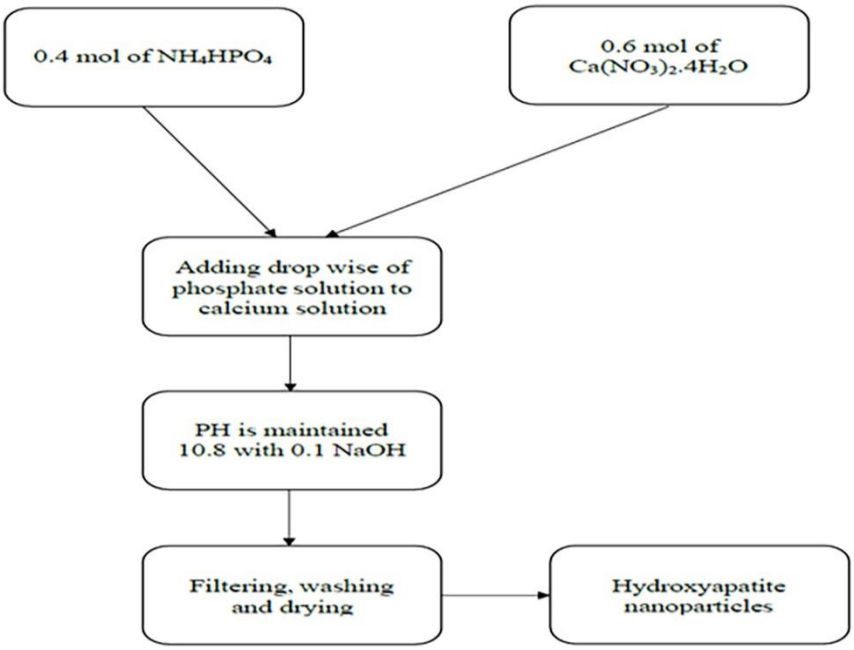
Sample size analysis:

A sample size of 36 (12 in each group) was calculated using G*Power with the power 80 and an error of 0.05 based on the study done by Babrawala et al. (Babrawala et al., 2019). The sample size calculation was performed considering 80% power, 5% significance level, and 1.0 as the standard deviation; 12 patients per group were needed. G*Power was used for sample size calculation (University of Düsseldorf, Düsseldorf, Germany).

Patients were fully informed about the steps of this study. After the initial examination, all patients were motivated to perform personal oral hygiene measures using soft toothbrushes and interdental cleansing devices. Each patient received full mouth supra and sub-gingival debridement. The patients were divided into

three groups as follows: Patients were randomly assigned into three groups: Group I: OFD only; Group II (test group): OFD with collagen-chitosan nanohydroxyapatite membrane; and Group III: OFD with collagen membrane.

Participants were blinded and randomly assigned using a computer-generated list of random numbers. either to the test or control group. Allocation was performed in blocks of 4 by a trial independent individual, and the allocation ratio was intended to be equal. The used materials were collagen type I, and chitosan was purchased with a low molecular weight (50.000 to 100.000 u) and degree of de-acetylation (DD 75%). Preparation of nano-Hap: 500 ml of 0.4 mol of diammonium hydrogen phosphate with pH-4.0 was vigorously stirred in a 2 L beaker at room temperature, and 500 mL of 0.6 mol calcium nitrate tetrahydrate with pH=7.4 was added drop-wise over 4 h.



The pH of the system was maintained at 10.8 throughout the stirring process by using 0.1 M sodium hydroxide. The mixture was allowed to remain stirred overnight. A white precipitate was formed. The precipitate was vacuum dried and cleaned with distilled water and ethanol simultaneously for three or four times. The prepared powder was used for further characterization **(Fig. 1)** (Chandrasekar et al., 2013).

Surgical procedures:

(Fig. 2).

All teeth, with intrabony pockets ≥ 5 mm in the same quadrant as the teeth selected for the study, were surgically treated. In all locations, under local anesthesia, an intracervical incision and full-thickness muco-periosteal flaps were reflected **(Fig. 2)**.

(Fig. 3)

(Fig. 4).

Surface of roots were scaled and planed only as Group I, but we put collagen, chitosan, and nanohydroxyapatite membrane **(Fig. 3)** in the defect as Group II; additionally, we put collagen membrane in the defect as Group III. Then an interrupted suture of the flap **(Fig. 4)**.

Method of clinical evaluation:

The study evaluated clinical parameters before and after surgery for patients, including modified sulcular bleeding index (MS) (Bangera et al., 2022), plaque index (PI) (Pilloni et al., 2021), clinical attachment level (CAL) (Newman et al., 2018), and pocket depth (PD) (Glavind & Löe, 1967).

(Fig. 5).

(Fig. 5) using a customized surgical stent. Radiographic examinations were performed at baseline and 6 months using digital periapical X-rays and intraoral radiographs captured using a paralleling technique with film. These measurements were taken using an image plate and a dental X-ray machine set at 70 KVP, 8 mA, and 0.08 - 0.04 s.

Statistical analysis was done by SPSS v26 (IBM Inc., Armonk, NY, USA). Quantitative variables were presented as mean and standard deviation (SD); the two groups were compared utilizing unpaired Student's t-test and compared between different readings utilizing the ANOVA (F) test. Qualitative variables were presented as frequency and percentage (%) and were analyzed utilizing the Chi-square test. A two-tailed P value < 0.05 was considered statistically significant.

Results

This research included 36 patients who were divided into three groups, each with 12 cases: Group I received only OFD, Group II received OFD with collagen, chitosan, and nanohydroxyapatite membrane, and Group III received OFD with collagen type 1 membrane. Plaque index (PI), modified sulcular bleeding index (MS),

probing depth (PD), clinical attachment level (CAL), and radiographic measures were collected and evaluated statistically at baseline, 3 months, and 6 months, respectively.

The study found no significant difference in PI between baseline and 3 months in Group I, 3 months, and 6 months, as illustrated in **Table (1)**. However, PI decreased significantly in Group II at 3 months, 6 months, and 6 months compared to baseline, suggesting factors influencing PI. In Group III, PI decreased at 3 months and 6 months, but no significant difference was observed between 3 and 6 months. Intragroup comparison revealed similar PI at baseline, no significant change in PI across the three groups after 3 months, and no significant change in PI after 6 months.

Table 1: Comparison between the three studied groups according to Plaque index (PI)

PI				
	Group I	**Group** II	**Group** III	
	$\overline{X}$ **± SD**	$\overline{X}$ **± SD**	$\overline{X}$ **± SD**	
Baseline	1.83 ± 0.75	2.17 ± 0.58	2.20 ± 0.92	
3 months	1.17 ± 0.75	0.92 ± 0.09	1.00 ± 1.05	
6 months	0.67 ± 0.82	0.25 ± 0.62	0.60 ± 1.07	
Within group comparison				
		MD	**p-value**	**Sig**
Group I	**Baseline vs 3 months**	0.66	0.14	**NS**
	Baseline vs 6 months	1.16	0.005	**S**
	3 months vs 6 months	0.5	0.21	**NS**
Group II	**Baseline vs 3 months**	1.25	0.001	**S**
	Baseline vs 6 months	1.92	0.001	**S**
	3 months vs 6 months	0.67	0.005	**S**
Group III	**Baseline vs 3 months**	1.2	0.001	**S**
	Baseline vs 6 months	1.6	0.001	**S**
	3 months vs 6 months	0.4	0.19	**NS**
Between group comparison				
		MD	**p- value**	**Sig**
Baseline	**Group** I **vs** II	-0.34	0.65	**NS**
	Group I **vs** III	-0.37	0.62	**NS**
	Group II **vs** III	-0.03	0.99	**NS**
3 months	**Group** I **vs** II	0.25	0.85	**NS**
	Group I **vs** III	0.17	0.93	**NS**
	Group II **vs** III	-0.08	0.97	**NS**
6 months	**Group** I **vs B**	0.42	0.95	**NS**
	Group I **vs** III	0.07	0.98	**NS**
	Group II **vs** III	-0.35	0.61	**NS**

$\overline{X}$ **: Mean** **SD: Standard deviation** **MD: Mean difference**

p va**lue: Probability value** **S: Significant** **NS: Non significant**

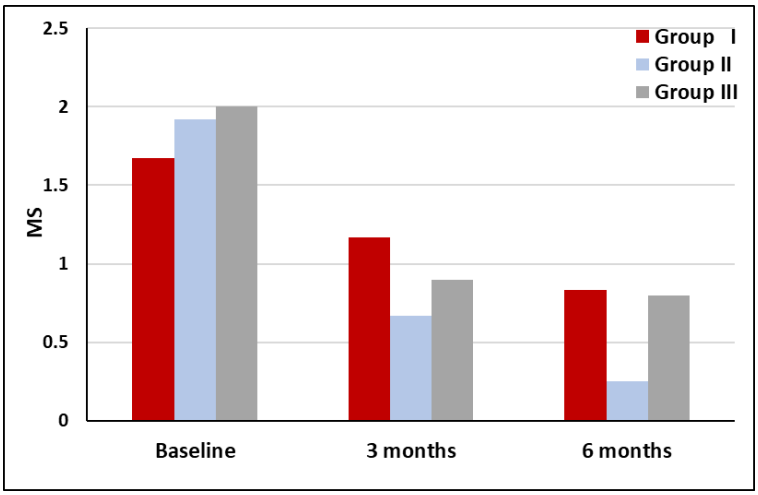


Figure (6). Mean MS at baseline, 3 months and 6 months of group I, II, and III.

According to **(Fig. 6)**, there was no significant difference in MS between baseline and 3 months in Group I and 6 months in Group II. However, at 3 months, there was a significant decrease in MS compared to baseline, and at 6 months, there was a significant decrease in MS compared to 3 months. In Group III, there was a significant decrease in MS at 3 months and 6 months, but no significant difference was observed between the three groups at any point in time.

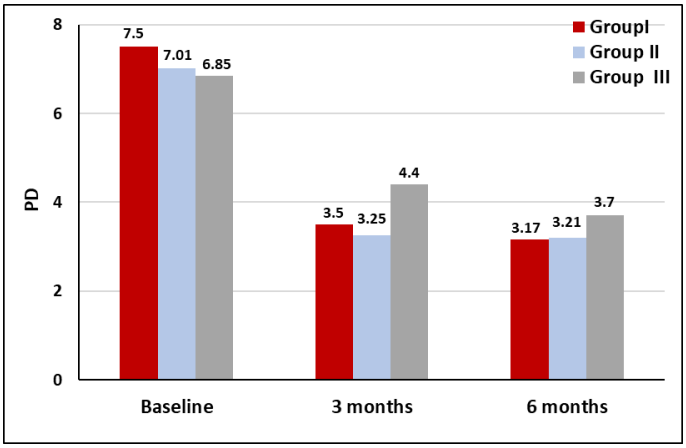


Figure (7). Mean PD at baseline, 3 months and 6 months of group I, II, and III.

In addition, the present investigation revealed a significant decrease in Parkinson's disease (PD) in Group I at 3 months and 6 months compared to the baseline, but no significant difference was observed between the two periods, as shown in **Fig. 7**. In Group II, PD decreased at 3 months, 6 months, and no significant difference was observed between 3 months and 6 months. In Group III, PD decreased at 3 months, 6 months, and 6 months compared to the baseline, but no significant difference was observed between the three periods. The study suggests that PD can be significantly reduced over time, with no significant difference observed between the three periods.

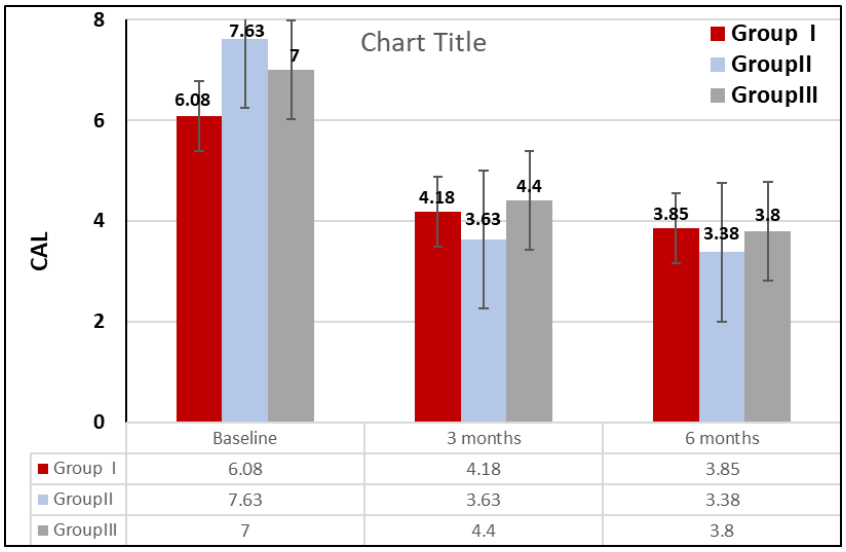


Figure (8). Mean CAL at baseline, 3 months and 6 months of group I, II, and III.

Based on data recorded in **Fig. 8**, there was no significant difference in CAL levels between baseline and 3 months in Group I, Group II, and Group III. However, at 3 months in Group II and 6 months, there was a significant decrease in CAL, but no significant difference was observed between the three and six months. In Group III, there was a significant decrease in CAL at 3 months, 6 months, and 6 months, but no significant difference was observed between the three groups, suggesting that the treatment may not be effective in reducing CAL levels in the long run.

The baseline radiographic values for Group I and II were 6.67 ± 1.32 and 6.95 ± 1.77, respectively, while Group III had a mean of 7.12 ± 1.66. There was no significant difference in radiographic measurements between baseline and 3 months of Group I, but a significant decrease was observed at 3 months, 0.1 months, and 6 months **(Table 2)**.

Table 2: Radiographic measurements of groups I, II, and III at baseline, 3, and 6 months

Radiographic measurements (mm)			
	Group I	Group II	Group III
	$\overline{X} \pm SD$	$\overline{X} \pm SD$	$\overline{X} \pm SD$
Baseline	6.67 ± 1.32	6.95 ± 1.77	7.12 ± 1.66
3 months	6.62 ± 1.21	6.68 ± 1.66	6.91 ± 1.47
6 months	6.61 ± 1.20	4.95 ± 0.92	6.27 ± 1.24

Table 3: Comparison between the three studied groups according to radiographic measurements

Between group comparison				
		MD	p- value	Sig
Baseline	Group I vs II	-0.28	0.94	NS
	Group I vs III	-0.45	0.86	NS
	Group II vs III	-0.17	0.96	NS
3 months	Group I vs II	-0.06	0.99	NS
	Group I vs III	-0.29	0.92	NS
	Group II vs III	-0.23	0.93	NS
6 months	Group I vs II	1.66	0.01	S
	Group I vs III	0.34	0.82	NS
	Group II vs III	-1.32	0.02	S

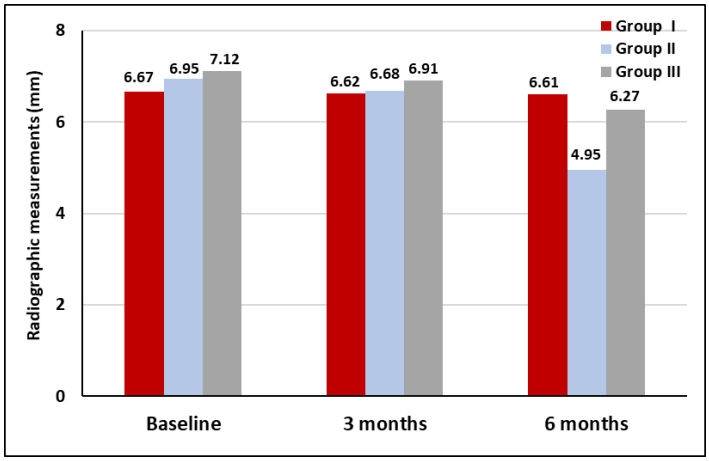


Figure (9). Mean radiographic measurements at baseline, 3 months and 6 months of group I, II, and III.

Figure (10). A) Radiographic measurements at baseline, B) 3 months and C) 6 months of group I, II, and III.

Table (3) and **Figs. 9**, and **10** pointed out that there was no significant difference in radiographic measurements at baseline between the three groups, but group II showed a significant decrease in measurements compared to group I and group C at 6 months, and no significant difference in measurements between group I and III at 6 months, indicating consistent radiographic measurements across all groups.

Discussion

Periodontitis is an inflammatory disease affecting teeth's supporting tissues, triggered by periodontal bacteria and host immune response. The primary goal of periodontal therapy is to improve gingival health and preserve remaining periodontal tissues (Petersen & Baehni, 2012). Natural materials, such as collagen-chitosan-nanohydroxyapatite membrane and collagen membrane, have been shown to speed up healing (Agrawal et al., 2023). The present study focuses on treating periodontal intrabony defects.

The current investigation showed an improvement in MS, PD, and CAL in agreement with the previous study via Eshwar et al. (Eshwar et al., 2023), who reported the effect of scaling and root planing in treatment of intrabony defects. This may be due to the use of fucoidan containing chitosan hydrogel, which was effective in treatment of intrabony defect. Moreover, the present study revealed that there was no significant difference in CAL at baseline or after 3 or 6 months between the three groups, in agreement with other studies, which may be the same time of follow-up (Rahman et al., 2015).

On the contrary with this present finding, another study conducted Bandeira et al. (Bandeira et al., 2017) showed a significant difference in CAL at baseline, at 3 months, and 6 months between the three groups and revealed that the combined use of nano-hydroxyapatite and chitosan may have a synergistic effect, resulting in a more favorable clinical response, and increase bone mass using chitosan alone. This previous study gave a 12-month follow-up that may cause a superior result than our study, which tracked a 6-month follow-up. Correspondingly, another investigation by Bhardwaj et al. (Bhardwaj et al., 2018) contradicting our study showed a significant difference in CAL of 4.37 mm PD reduction and 3.08 mm CAL gain 12 months after using zinc-incorporated nano-HA.

Besides, our study revealed a significant decrease in CAL between baseline and three months, with an average difference of 2.6. After six months, the difference was 3.2, and at six months, it was 0.6, with a significant decrease compared to three months. Supporting our study, Eshwar et al. (Eshwar et al., 2023) reported that the mean CAL gain of 3.3 mm in a group treated by collagen membrane combined with bovine bone. These findings were similar to the present study, which revealed the mean ± SD CAL at baseline was 7.00 ± 1.97, and at 3 months it was 4.40 ± 0.74, and at 6 months it was 3.80 ± 0.67, but there were superior results than the present study. This can be due to the use of bovine bone combined with collagen membrane in the previous study.

Moreover, Ghousia Fatima et al. (Fatima et al., 2015), demonstrated the beneficial effects of an anorganic bovine-derived hydroxyapatite matrix/cell-binding peptide graft, in terms of CAL gain and PD reduction which in contrast with present study. Respectively, regarding PD, the current study demonstrated no significant difference in PD at baseline and at 6 months between the three groups, which disagree with the Rahman et al. (Rahman et al., 2015), which revealed a significant difference in PD at baseline between the three groups ($p < 0.001$).

Likewise, in agreement with the present study, another study, Haghgoo et al. (Moradi Haghgoo et al., 2023) observed the effect of collagen-chitosan membranes for intrabony defects and reported that the difference in probing depth between the test group and control group was statistically significant. It is reasonable for nanofibrous scaffolds to release tetracycline hydrochloride, which gives a superior effect in egeneration. Another study by Babrawala et al. (Babrawala et al., 2019) showed a reduction in probing depth with 15% chitosan gel for OFD. The outcomes at 6 months showed a PD reduction of 3.8 mm for 15% chitosan, which is superior due to the concentration of chitosan, which in the present study is 2%.

As regard the PI score recorded in the present study investigation, the results showed that there was a significant difference in the all three groups at the 3- and 6-month observation period when compared to baseline. This was clarified in a study conducted on hydroxyapatite Pandiyan et al. (Pandiyan et al., 2022), who showed antimicrobial activity against various gram-negative and gram-positive bacteria. There was no significant difference in PI at baseline between the three groups. Furthermore, there was no significant difference in PI at 3 and 6 months between the three groups.

Results obtained from the present study of PI, in group I revealed no significant difference PI at baseline, at 3 months, and at 6 months between the three groups ($p > 0.05$), which is similar to another experiment via Pandiyan et al. (Pandiyan et al., 2022), which might be to excellent patient motivation protocol and good oral hygiene measures at baseline, 3, and 6 months. Concerning MS, the present study detected that there was no significant difference in MS at baseline, at 3 months, and at 6 months between the three studied groups ($p > 0.05$), which is in agreement with another study made by Pandiyan et al. (Pandiyan et al., 2022), who explained that this could be due to excellent patient motivation protocol and good oral hygiene measures at baseline, 3, and 6 months.

Conclusion

Given the limitations of this investigation, it may be inferred that the collagen-chitosan-nanohydroxyapatite membrane improve the clinical attachment level and pocket depth. The clinical effects of different chitosan and nano-HAP concentrations are required to be investigated in more studies.

Authors' contributions:

Conceptualization, motivation, writing-original review, editing manuscript. All authors approved the final draft of the manuscript. All authors participated in the study design, performed the experiments, collected and analyzed the data, drafted the manuscript, and confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Competing interests

The authors declare that they have no competing interests.

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