RESEARCH PAPER

Formulation and *In vitro* Evaluation of A Collagen Peptide-Based Dental Plug for Post-Tooth Extraction Socket Preservation

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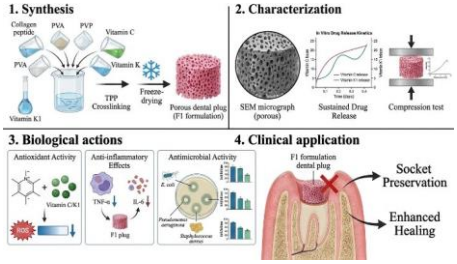
ABSTRACT

Post-extraction alveolar ridge resorption may affect future implant installation and prosthodontic restoration. The present research was undertaken to develop and evaluate a cross-linked collagen peptide based dental plug loaded with vitamins C and K1 using tripolyphosphate (TPP). The plug was developed by incorporating collagen peptide, polyvinyl alcohol, polyvinylpyrrolidone, vitamins C and K1 and TPP in a matrix which was freeze dried. Among all the developed formulas, the F1 formula showed the best overall results regarding a porous and structural stability of the matrix, presence of characteristic collagen peptide related IR bands, and the highest compressive strength. Swelling ratio of F1 was 476.54% at 15 min and 507.15% at 180 min. Residual mass was reduced from 43.32% on 3rd day to 29.21% on 7th day and 24.41% on 14th day which showed comparatively slow rate of degradation. At 24 hours, the cumulative release was 94.16% for vitamin C and 94.64% for vitamin K1. F1 had a rapid release phase followed by slow-release phase. Hemolytic activity of F1 was very low with mean optical density about 1.97, and it showed concentration dependent antioxidant activity with the range of 28.65% to 88.31% from 100 to 1000 µg/mL. The antimicrobial study revealed that the inhibition zones were 23.0, 17.5, and 29.0 mm against *Escherichia coli* and *Staphylococcus aureus*, respectively. It indicates that F1 can be considered an interesting multifunctional dental plug for socket protection after extraction.



Keywords: Collagen peptide, Dental plug, Health care, Socket preservation, Vitamin C, Vitamin K1

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INTRODUCTION

Alveolar ridge resorption for tooth extraction is one of the most challenging clinical situations since the unavoidable resorption of hard and soft tissue will make it difficult to place an implant due to the prosthetic requirement as well as to restore aesthetics in both the posterior and anterior regions. According to quantitative studies and systematic reviews, within 3-6 months post extraction, there could be up to 29-63% horizontal resorption of the alveolar bone with a mean reduction of about 3-4 mm in ridge width, while the vertical resorption could be around 11-22%, leading to a decrease in height of the ridge of 1-2 mm especially in the thin buccal plate.[1-3] In order to counteract such unfavourable consequences, alveolar ridge preservation (ARP) or socket preservation techniques have been developed as scientifically proven ways to minimize bone loss after extraction and keep the ridge dimensions unchanged until the moment of rehabilitation procedures. The meta-analysis of scientific literature suggests that ARP through socket grafting and socket sealing techniques could help avoid 1.5-2.4 mm of horizontal bone loss and 1-2.5 mm of vertical mid-buccal bone loss in comparison to natural healing, although it is impossible to restore fully the anatomy prior to extraction.[4,5] Still, the application of graft material together with barrier membranes or socket sealer materials has been proven to be associated with decreased ridge contraction, better quality of the sites for implants and reduced number of subsequent grafting procedures.[6] In this situation, there is widespread usage of collagen-based products such as sponges, plugs and membranes, as they are bioresorbable,[7,8] biocompatible and can stabilize blood clots and stimulate granulation tissue formation where collagen sponge and plugs made up of Type I collagen from bovine sources provide wound protection,[9] local hemostasis, and granulation tissue formation.[7] Collagen sponges and plugs have been used in various ARP protocols, either in isolation or in combination with bone grafts.[10,11] Clinically and histologically, it has been observed that insertion of collagen plugs into extraction sockets enhances the process of socket healing, increases the amount of bone formation when compared to empty sockets, and reduces the chance of developing complications such as a dry socket due to the stabilization of the clot.[12] Conventional collagen sponges and plugs face certain disadvantages such as rapid resorption (within 2-6 weeks), mechanical weakness, and inability to support the socket shape during the critical phase of healing.[13] Moreover, expensive cost, hard handling, and unpredictable degradation behavior of some xenograft membranes may be considered another challenge when employing collagen sponges and plugs.[12,13] The group of collagen peptides (bioactive fragments of collagen) is one of the newly developed biomaterials that are able to overcome the abovementioned drawbacks because of good processability, tunable physicochemical properties, and biologic activity linked to regulation of cell proliferation, matrix metalloproteinase activity, and tissue engineering.[14] Indeed, *in vitro* experiments with collagen peptides have shown increased proliferation of fibroblasts and production of extracellular matrix proteins, as well as regulation of collagenases MMP 13.[15] Through the development of collagen peptides in the form of a porous plug structure and the use of appropriate crosslinking methods, dental plugs can be developed with controlled levels of swelling, mechanical stability and degradation behavior to better suit the needs of socket healing and ridge remodeling. It has been demonstrated that Tripolyphosphate (TPP) crosslinking can be used as an effective and non-toxic method for stabilization of collagen or chitosan matrices, extended resorption period and retention of interconnected pores necessary for vascularization and cell migration in guided bone regeneration and tissue engineering.[16] In order to overcome the above limitations, the current study suggests a new formulation of a collagen peptide-based dental plug containing vitamins C and K1 that have been cross-linked with tripolyphosphate (TPP).[17] The use of collagen peptides has certain benefits over collagen matrixes due to their contribution towards cell proliferation, regulation of matrix metalloproteinase enzymes like MMP-13 inhibition and fast tissue healing.[18,19] The use of Vitamin C (ascorbic acid) is essential since it serves as a cofactor of prolyl and lysis hydroxylases that promote stable collagen formation and osteoblasts differentiation. Furthermore, Vitamin K1 assists in rapid hemostasis by contributing towards the coagulation process.[20,21] Collagen peptide dental plug crosslinking through TPP represents a safe method to enhance collagen peptide dental plug stability, prevent their premature degradation, and retain porosity important for angiogenesis and cell infiltration, eventually correlating the resorption of the collagen peptide dental plug with bone formation.[22] This multifunctional design is hypothesized to provide superior socket preservation compared to spontaneous healing or conventional materials by maintaining alveolar dimensions, enhancing osteogenic activity, and ensuring controlled biodegradation.[23,24] This work focuses on the determination of the feasibility of using a porous collagen peptide dental plug impregnated with vitamins C and K1 and crosslinked with tripolyphosphate (TPP) as a multifunctional biomaterial for socket preservation after tooth extraction. The main research questions include if TPP cross-linking would allow achieving plug stabilization and degradation control without decreasing porosity, if the plug would have enough capacity for swelling and mechanical strength to adapt to the socket and stabilize blood clot and if the vitamin C and K1 could be delivered in a controlled way during early healing

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stages. Besides that, the hemocompatibility, antioxidant and anti-inflammatory properties, and cytocompatibility of the plug with osteoblast cells (MG-63 line) will be studied. Thus, the objective of the work is the preparation and characterization of a TPP-crosslinked collagen peptide dental plug and investigation of its physicochemical, mechanical, degradation, delivery properties and biological activity *in vitro*. The development approach involves the use of supportive polymers such as polyvinyl alcohol and polyvinyl pyrrolidone, freeze-drying method to obtain a porous structure and TPP cross-linking in order to provide appropriate balance between swelling properties, compressive strength and proper biodegradation during the 3-6 months critical period of alveolar ridge modeling. *In vitro* evaluation involves investigations of surface morphology by means of scanning electron microscopy, FTIR analysis to test chemical compatibility, X-ray diffraction for assessing crystallinity, compression strength tests, swelling and biodegradation, drug release profile determination, hemocompatibility studies, antioxidant and anti-inflammatory activity as well as antimicrobial activity against pathogenic bacteria of oral cavity.

2. MATERIAL AND METHODS

Materials: Poly vinyl alcohol (Mol Wt. 1,25,000), Collagen peptide was obtained from Hi media. Vitamin C (Drug A) and Vitamin K1 (Drug B) were obtained from Apex Pharmaceutical. Polyvinyl Pyrrolidone was obtained from Srlchem. All chemicals and reagents used were of analytical grade and pharmaceutical grade. Deionized water was used for all experiments.

2.1 Preparation of Collagen Peptide-Based Dental Plug

Collagen peptide was dissolved in deionised water under continuous stirring to obtain a clear collagen phase. Separately, polyvinyl alcohol (PVA) solution and polyvinylpyrrolidone (PVP) solution were prepared and allowed to hydrate fully. The polymeric solutions were then combined and mixed until a uniform blend was obtained. Vitamin C and vitamin K1 were incorporated sequentially into the homogeneous polymeric matrix, followed by addition of tripolyphosphate (TPP) as the crosslinking agent to improve network stability. The resulting dispersion was degassed to remove entrapped air and then cast into dental plug moulds. The filled moulds were subjected to deep freezing at −80°C to facilitate pore formation. Subsequently, the frozen samples were freeze-dried for 24–48 h to yield a porous collagen peptide dental plug structure, after which the dried plugs were sterilized and stored as final porous collagen dental plugs for extraction socket preservation. This processing approach is consistent with established collagen-based collagen peptide dental plug fabrication by freeze-drying, where porous architecture is generated to support tissue infiltration, fluid transport, and healing, and with socket-plug concepts designed to preserve alveolar dimensions after tooth extraction.[25]

2.2 SEM analysis

Using a scanning electron microscope (FE-SEM IT800, JEOL) with gold sputter coating, the surface morphology of the plug was analysed. The samples were mounted on the brass stubs using carbon tape. Further, they were placed in a vacuum chamber for sputtering, and at this stage, the samples were coated with platinum for 40 seconds. The images were captured at different magnifications. The average diameter of the plug was determined by ImageJ software (National Institutes of Health (NIH), USA).[26,27]

2.3 Fourier Transform infrared Spectroscopy

Infrared spectra of the collagen peptide-based dental plug as well as its constituents were analyzed through Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR; Bruker ALPHA II). The lyophilized samples were then placed directly against the ATR crystal, and a background spectrum was obtained in similar conditions before sample measurement. The spectra were recorded in the range of 550–4000 cm^{-1} with a resolution of 4 cm^{-1} for 64 scans per sample. The distinct collagen peaks such as amide A, amide B, amide I, amide II, and amide III were identified and compared for the pure collagen peptide, placebo, and TPP-cross-linked preparations. Any change in terms of shifts or variations in the intensity of amide I, amide II, and amide III peaks were noted to determine the interaction, if any, between the collagen peptides and PVA/PVP, vitamin C, vitamin K1, and TPP. The amide I peak at 1600-1700 cm^{-1}, amide II peak at 1550-1600 cm^{-1}, and amide III peak at 1230-1240 cm^{-1} were used as indicators of collagen peptide bonds and structures.[28]

2.4 Compressive strength analysis

Compressive strength was measured using a Universal Testing Machine (Instron E3000, Electro Puls, USA) at a crosshead speed of 1 mm/min. Samples **were** compressed along the longitudinal axis until failure, and the maximum load was recorded. Compressive strength was calculated using $CS = 4P/\pi d^{1}$, where P is the failure load and d is the mean specimen diameter.[29-31]

2.5 Swelling Ratio and Degradation Study

The capacity of water uptake of the plugs was observed by a swelling study. Into a 24-well plate with 2 mL of PBS (pH 7.4), the pre-weighed samples (W_1) were added in triplicate and incubated at 37° C to mimic human body conditions. The excess PBS was removed by blotting the plugs on tissue paper and weighed at 15, 30, 60, 120, and 180 minutes. The swelling capacity of the plugs was calculated using the formula.[32]

Individually weighed samples (1 × 1 cm) were placed in a 24-well TCPS plate containing 2 mL of PBS (pH 7.4) at 37 °C and incubated for 3,7 and 14 days. At predetermined intervals, the samples were removed, gently rinsed with deionized water, and vacuum-dried for 48 h. The *in vitro* degradation was evaluated by the loss of weight of the samples from their initial dry weight.[33]

2.6 Drug Release Kinetics

To analyze the kinetics of drug release, the cumulative drug release profile of vitamin C and vitamin K1 from collagen peptide-based dental plug were evaluated by applying zero order, first order, Higuchi and Korsmeyer-Peppas kinetics (R^1). The highest value of coefficient of determination was selected as best fitting and Korsmeyer-Peppas release exponent (n) was used for elucidating the release mechanism. [34]

2.7 Hemocompatibility

The haemolysis activity of the F1, F2, F3 plugs were measured by the released haemoglobin via UV−vis spectroscopy. Fresh human blood with EDTA was collected from the Institute hospital with the Institutional Ethical and Biosafety Committee approval (IHEC/SDC/FACULTY/24/PHARM/218). The dental plugs matrix with and without drug loading was cut into small pieces (1 cm × 1 cm dimension) and equilibrated for 30 min at 37 °C in 5 mL of PBS. Centrifugation was used to separate the RBCs from the entire blood for five minutes at a speed of 1500 rpm. To prevent platelets and other contaminants from being introduced by pure RBCs, the precipitated RBCs were washed with new PBS three times. A stock solution was prepared by mixing 10 mL of PBS with 100 mL of RBCs 100 mL of the stock solution was incubated with collagen peptide for 30 min at 37 °C in the dark.

Incubated RBCs were centrifuged again, and the supernatant from each sample was collected and transferred into the 96-well plate to analyse the released haemoglobin at a 540 nm wavelength using a microwell plate reader. The released haemoglobin from the treated sample well was compared with the negative (PBS) and positive control (distilled water), respectively.[35,36]

2.8 Antioxidant Activity and Anti-inflammatory Activity

The antioxidant activity of the samples was estimated. DPPH radical Scavenging activity was detected at an absorbance of 517 nm, and antioxidant activity was expressed as IC_{50} values.[34] The collagen peptide dental plug anti-inflammatory activity was estimated by the protein denaturation inhibition assay. In the egg albumin denaturation assay, the reaction mixture contains egg albumin, phosphate-buffered saline, and different concentrations of the collagen peptide dental plug. The reaction mixture was incubated at 37 °C and later heated at 70 °C, after which absorbance was recorded at 660 nm. The reference standard was diclofenac sodium. The absorbance of the test and control samples was used to determine the percentage of inhibition of protein denaturation. [37]

2.9 Antimicrobial activity

The antimicrobial activity of the collagen peptide dental plug and placebo was evaluated by the disk diffusion method against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli* ATCC strains. Briefly, bacterial suspensions were standardized to 0.5 McFarland turbidity, streaked uniformly over Mueller–Hinton agar plates to form a lawn culture, and the UV-sterilized the collagen peptide dental plug and placebo were then applied separately onto the agar surface; after incubation at 37°C for 18–24 h, the plates were examined for zones of inhibition, and the diameters were measured using an antibiogram scale in duplicate for reproducibility. This method follows the principle of agar disk diffusion susceptibility testing, where a standardized inoculum, Mueller–Hinton agar, and measurement of inhibition zones are used to assess antimicrobial potential. [38]

3. RESULT AND DISCUSSION

The collagen peptide-based dental plug, prove F1, showed the most favourable overall performance for socket preservation, because it combined higher swelling, strong compressive behaviour, good antioxidant activity, acceptable anti-inflammatory activity, low haemolysis, and the slowest *in vitro* degradation among the tested formulations. In the context of post-extraction socket preservation, these properties are relevant because a socket plug should stabilize the clot, maintain local moisture, support tissue integration, and resorb gradually during early healing.

3.1 SEM analysis

Collagen peptide plugs possessed a highly porous surface structure in all concentrations examined, as seen from the SEM analyses depicted in Figure 1 below where some morphological differences were clearly noticeable between the control group and the formulation group. The lower concentration of formulations possessed an open structure while the high concentration samples were denser in structure. The collagen control had the fibrous porous structure common to native collagen structures. Significantly, all the formed plugs were structurally stable and thus easy to handle as well as to place in the extraction sockets. The observed surface irregularities and porosity would be a good indicator of favourable conditions for fluid absorption, clot trapping and cell penetration during use.[39]

The results reveal that the collagen peptide dental plug contains all the necessary structural features required for socket preservation, which include appropriate porosity, collagen peptide dental plug integrity, and

surface characteristics that favor the early stabilization of the wound. The use of collagen-based plugs is common in the dental field since it acts as hemostatic collagen peptide dental plug, promoting clot stabilization and acting as a biocompatible matrix for tissue regeneration after tooth extraction. This is important especially in avoiding the early bone resorption process that hinders further prosthetic and implant placement in the socket. The more open porous nature in low percentages favors rapid absorption of blood, cellular infiltration, and nutrient transportation, while high percentage collagen peptides dental plug provides enhanced mechanical strength and retention in the socket. The balance between porosity and strength is very important because too much density of the collagen peptide dental plug impedes tissue integration process, and too porous structure hampers its mechanical strength. These findings correlate well with the current scientific literature, showing that collagen plugs play an important role in better healing and maintenance of the socket dimensions. Clinically it has been shown that the use of collagen plugs, especially when used in conjunction with platelet-rich fibrin (PRF), can lead to better ridge preservation results than PRF alone, specifically for socket width maintenance. Reviews have emphasized the importance of collagen plugs for haemostasis, angiogenesis and cell migration, further emphasizing their importance in socket extraction management. The present collagen peptide-based formulation is consistent with these findings and might provide additional benefits owing to its peptide form which can help in better processibility and biological interactions compared to whole collagen matrix. However, more research work is required to study the degradation rate, swelling and mechanical behavior, and *in vivo* biocompatibility of this formulation.[40]

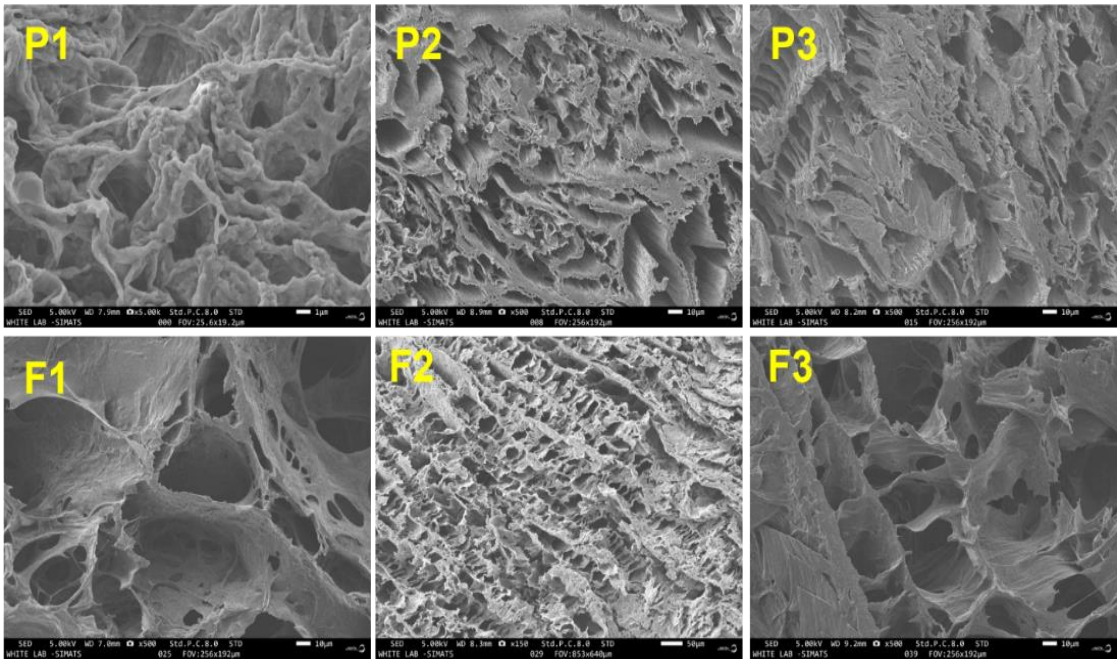

Figure 1: SEM Analysis of collagen peptide-based dental plug

3.2 FTIR analysis

It can be observed that the FTIR spectrum of formulation F1 exhibited characteristic absorption bands belonging to the collagen peptide and the excipients used in the preparation as depicted in figure 2 and no evidence of any destructive interaction can be observed.[41-43] The integrity of the characteristic bands indicates that the physical integration of the formulation has taken place rather than the chemical degradation, which is very much essential for the maintenance of biological activity. This is significant in the sense that the collagen peptide based dental plug is supposed to maintain its biological activities along with the antioxidant or anti-inflammatory agent.[41]

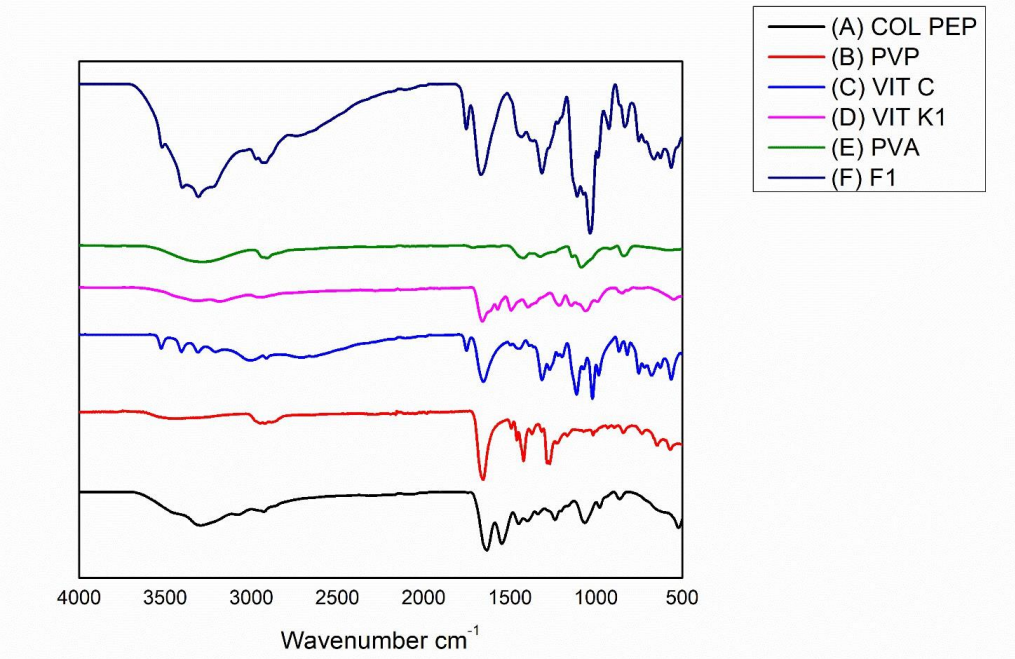

Figure 2: FTIR Plot of collagen peptide-based dental plug

3.3 Compressive strength analysis

The graph shows that formulation F1 had the highest value for maximum force of all samples, with much higher compressive strength from the very beginning compared to other samples in figure 3. It means that the material is capable of withstanding mechanical forces in the mouth when placed into the socket without being displaced from it. The sample with sufficient compressive strength would help retain space in the socket and provide the right conditions for clot formation during the first stages of alveolar ridge healing process.[44,45]

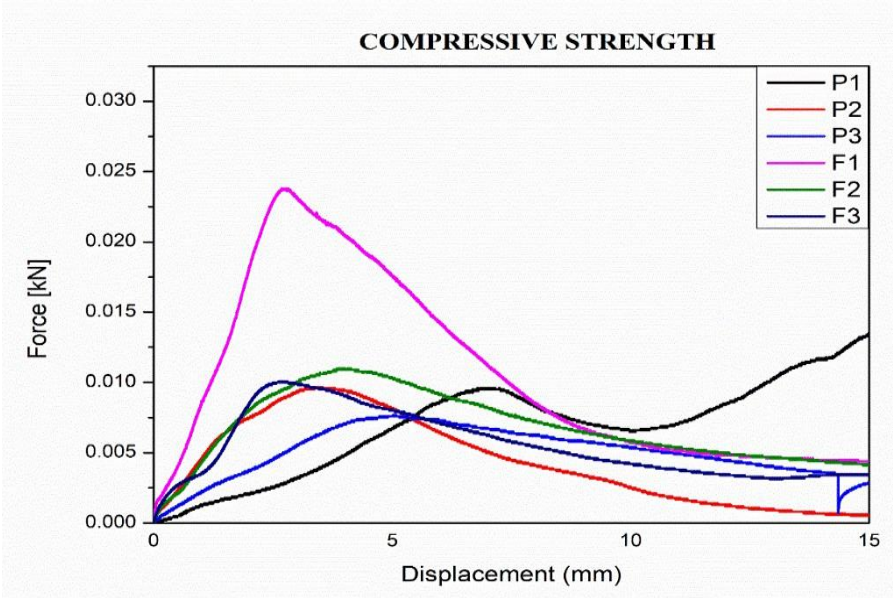

Figure 3: Compressive strength of collagen peptide-based dental plug

3.4 *In vitro* degradation and Swelling behaviour

Figure 4a shows the mass degradation of the formulations, where F1 maintained a higher mass compared to P1, P2, and P3 after 3, 7, and 14 days. Based on the results we have obtained from our raw data, F1 decreased from around 43.32% on day 3 to 29.21% on day 7 and 24.41% on day 14, showing a more prolonged break-down process compared to the

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placebo group. This is an indication of a superior structure in terms of structural integrity. This feature is preferred during the process of socket preservation since the barrier/plug must stay intact until the protection of the blood clot and the development of granulation tissue is accomplished.[46-47]

From the above swelling values, it is clear that formulation F1 showed the highest amount of water uptake compared to the others, being 476.54% at 15 min and 507.15% at 180 min. This was greater compared to other formulations and placebos in figure 4(b). Swelling is an important characteristic for the post-extraction plug since it aids the material in adhering to the socket walls, absorption of the exudates, and clot stabilization. Increase in swelling is an indication of a hydrophilic matrix which is important for moisture environment creation for fibroblasts migration.[48-50]

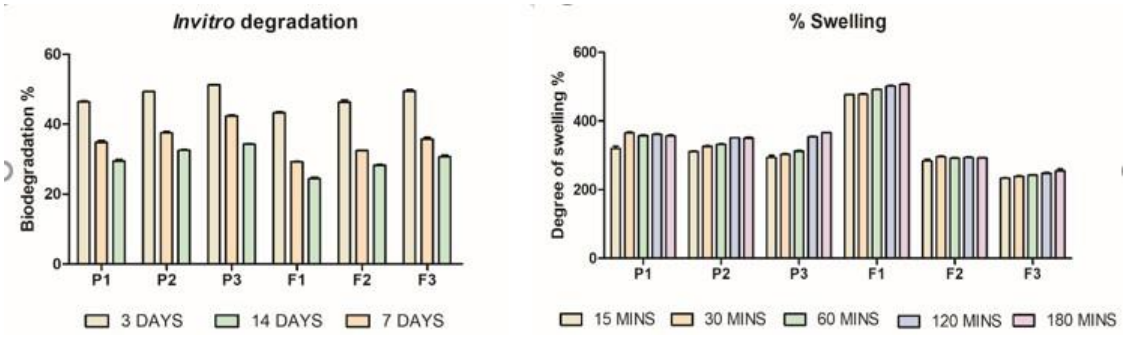
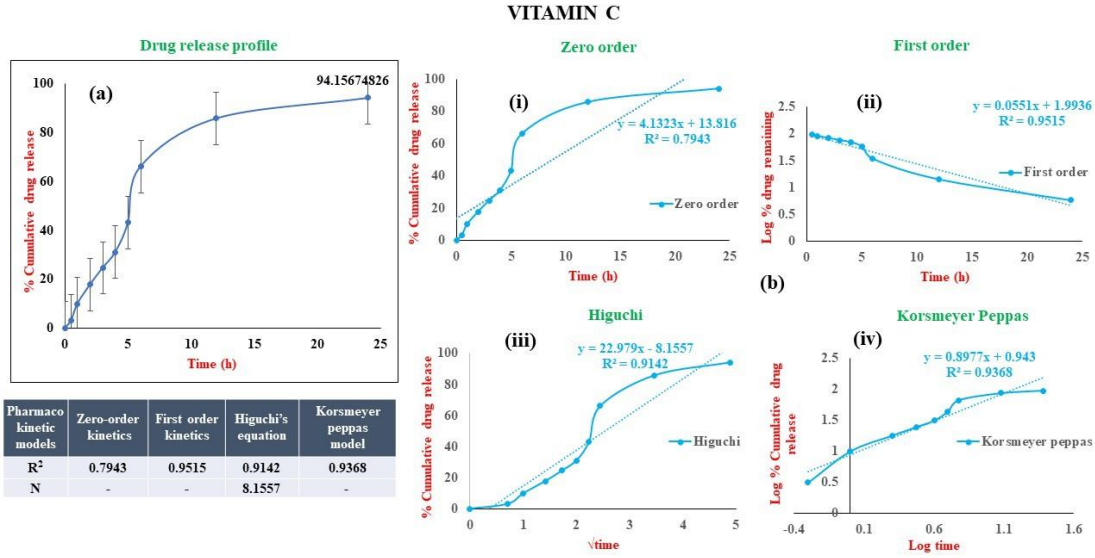


Figure 4a: *In vitro* biodegradation profile of collagen peptide-based dental plug; Figure 4b: Swelling behaviours of collagen peptide-based dental plug

3.5 Drug Release Kinetics

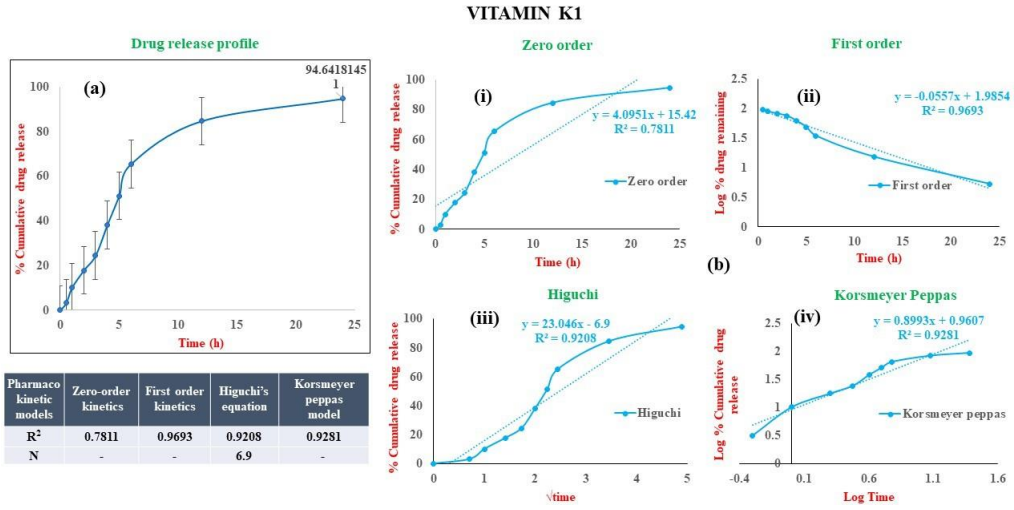
The collagen peptide-based dental plug showed a biphasic release pattern, with an initial faster release followed by a slower sustained phase over 24 hours. Vitamin C reached 94.16% cumulative release at 24 hours and vitamin K1 reached 94.64%, indicating efficient delivery with prolonged release during the healing period in Figure 5a & 5b.

This behavior is relevant for post-extraction socket preservation because early stabilization of the socket and local delivery of healing-supportive agents are important for reducing soft tissue collapse and supporting ridge maintenance. Collagen plugs are already used in socket preservation because they are biocompatible, resorbable, and clinically useful for protecting the extraction site and supporting healing. Vitamin C can support collagen synthesis and fibroblast activity, while vitamin K1 may contribute to tissue remodelling and bone-related healing, so combining both in a collagen matrix may create a favourable microenvironment for socket repair.[51]



Pharmaco kinetic models	Zero-order kinetics	First order kinetics	Higuchi's equation	Korsmeyer peppas model
R^2	0.7943	0.9515	0.9142	0.9368
N	-	-	8.1557	-

Figure 5a: Drug release profile for vitamin C collagen peptide-based dental plug



Pharmaco kinetic models	Zero-order kinetics	First order kinetics	Higuchi's equation	Korsmeyer peppas model
R^2	0.7811	0.9693	0.9208	0.9281
N	-	-	6.9	-

Figure 5b: Drug release profile for vitamin K1 collagen peptide-based dental plug

3.6 Hemocompatibility

The hemolysis assay demonstrated that formulation F1 produced very low haemolytic activity, with mean OD values around 1.97 and results comparable to the other formulations shown in figure 6. Based on these findings, F1 did not exhibit a strongly haemolytic response and may be considered blood-compatible for direct contact with the fresh extraction socket, where early interaction with blood is expected. For a dental plug intended for immediate post-extraction placement, this is an important property because hemocompatibility is required to minimize adverse blood-material interactions. Standards used in dentistry emphasize the biological evaluation of materials that contact oral tissues and body fluids, including blood-contacting applications.

A hemolysis test is widely used as an initial screening method for biomaterial blood compatibility, since excessive red blood cell lysis indicates poor hemocompatibility. In the present context, the low haemolytic response of F1 suggests that the formulation is unlikely to cause clinically relevant erythrocyte damage when used as a dental plug. This is consistent with reports showing that dental biomaterials with hemolysis values below accepted thresholds can be regarded as having good blood compatibility. Therefore, the observed hemolysis profile supports the safety of F1 for immediate post-extraction use as a local dental plug.[52,53]

Highly Hemocompatible (≤5% Hemolysis)
Hemocompatible (10% Hemolysis)
Non Hemocompatible (≥20% Hemolysis)

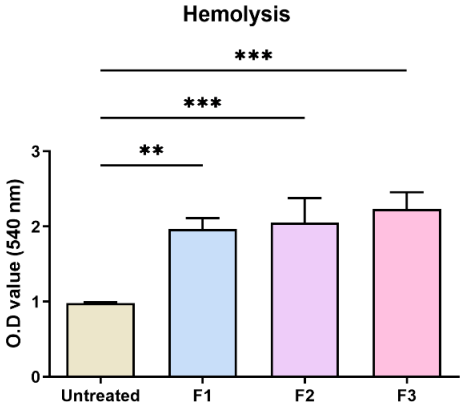


Figure 6: Hemolysis profile of collagen peptide-based dental plug

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3.7 Antioxidant activity

F1 demonstrated substantial antioxidant activity in the DPPH radical scavenging assay, with inhibition increasing from 28.65% at 100 µg/mL to 88.31% at 1000 µg/mL, indicating a clear concentration-dependent response drawn in figure 7. This strong free radical scavenging behavior suggests that the formulation may contribute to the control of oxidative stress in the post-extraction socket. Since production of reactive oxygen species can lead to increased inflammation and impaired tissue repair, antioxidation function could be an important additional function of a socket preservation plug, along with its supportive structural role.[54]

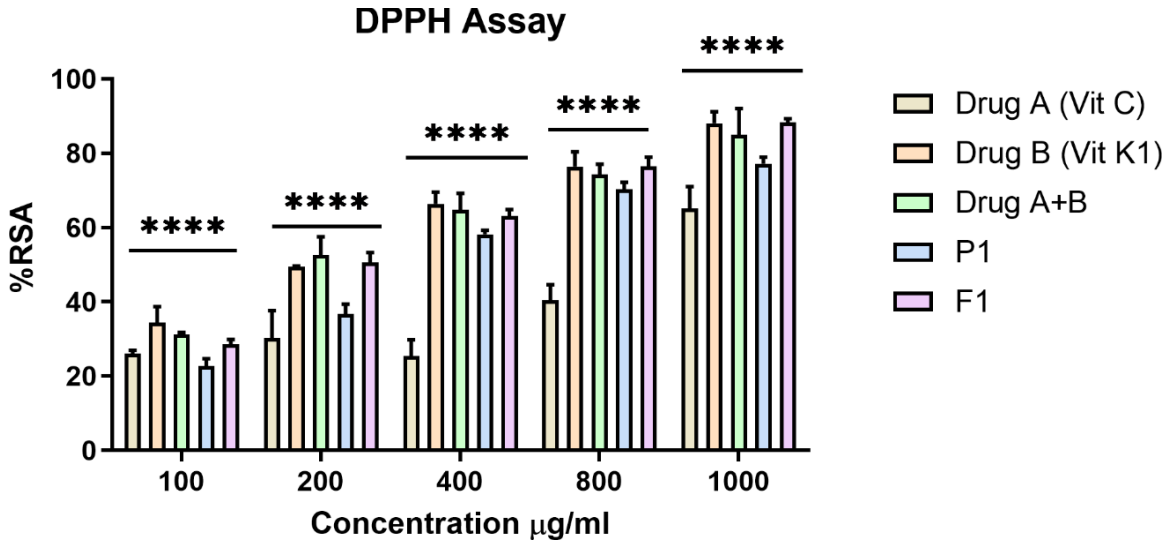

Figure 7: Radical scavenging assay of collagen peptide-based dental plug

3.8 Anti-inflammatory activity

The results of anti-inflammatory tests revealed that there was a concentration dependent inhibitory effect by F1, which attained about 61.31% at 1000 µg/mL, which are shown in Figure 8 below. This implies that the drug will play an important role in controlling the inflammatory process after tooth extraction. Excessive or prolonged inflammation can impair granulation tissue formation, delay epithelial closure, and contribute to delayed alveolar repair. Because the formulation also contains collagen peptide with bioactive additives, F1 may provide dual functionality as a local dental plug: first, by offering mechanical support to stabilize the extraction socket, and second, by delivering local bioactivity that may assist in controlling post-extraction inflammation and supporting tissue regeneration. This concept is consistent with prior reports showing that collagen-based socket dressings can reduce postoperative pain, promote bone healing, support granulation tissue formation, and aid alveolar ridge preservation after extraction.[55,56]

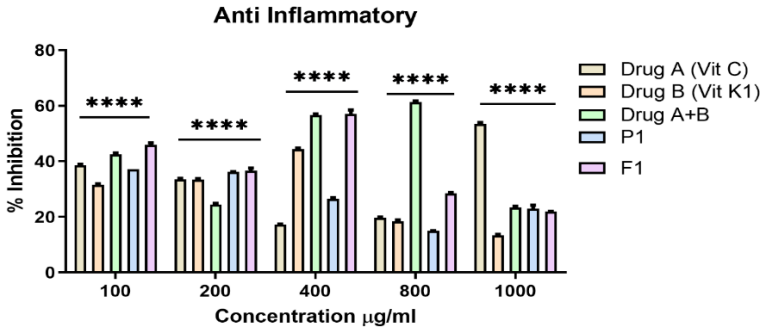

Figure 8: Anti Inflammatory assay of collagen peptide-based dental plug

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3.9 Antimicrobial Activity of Collagen Peptide Dental Plug Applications

The antimicrobial efficacy of the developed collagen peptide dental plug, intended for use as a dental plug, was evaluated using the disc diffusion method against common oral and wound-associated pathogens, namely *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus.* The zones of inhibition (ZOI) were measured and compared between the test collagen peptide dental plug and placebo control shown in table 1 and figure 9.

The collagen peptide dental plug demonstrated enhanced antibacterial activity against all tested microorganisms. Against *E. coli*, the test group exhibited a ZOI of 23.0 ± 0.0mm compared to 18.0 ± 0.0mm in the control group. For *P. aeruginosa*, no inhibitory effect was observed in the control group, whereas the collagen peptide dental plug showed a measurable inhibition zone of 17.5 ± 0.71 mm, indicating its efficacy against this inherently resistant pathogen. As for *S. aureus*, an oral pathogen known to cause post-extraction infections, the collagen peptide dental plug yielded a greater ZOI of 29.0±1.41mm as opposed to the control group which yielded 10.5±0.71mm.

The better antibacterial efficacy exhibited by the collagen peptide dental plug may be due to the presence of vitamins C and K1 in it, which help inhibit bacterial protein synthesis and cell membrane. This quality is very helpful in dental cases, where it is important to prevent infections and dry sockets.

The results indicate that the collagen peptide dental plug has potent antibacterial activity against Gram positive as well as Gram negative organisms related to oral infections. This supports its potential application as a dental plug for post-extraction socket management, aiding in infection control, promoting wound healing, and reducing the risk of complications such as alveolar osteitis.[57-61]

Table1: Anti-Microbial activity of collagen peptide dental plug

S. No	Control	Test
Escherichia coli		
1	18 mm	23mm
2	18mm	23mm
Pseudomonas aeruginosa		
1	-	17mm
2	-	18mm
Staphylococcus aureus		
1	10mm	28mm
2	11mm	30mm

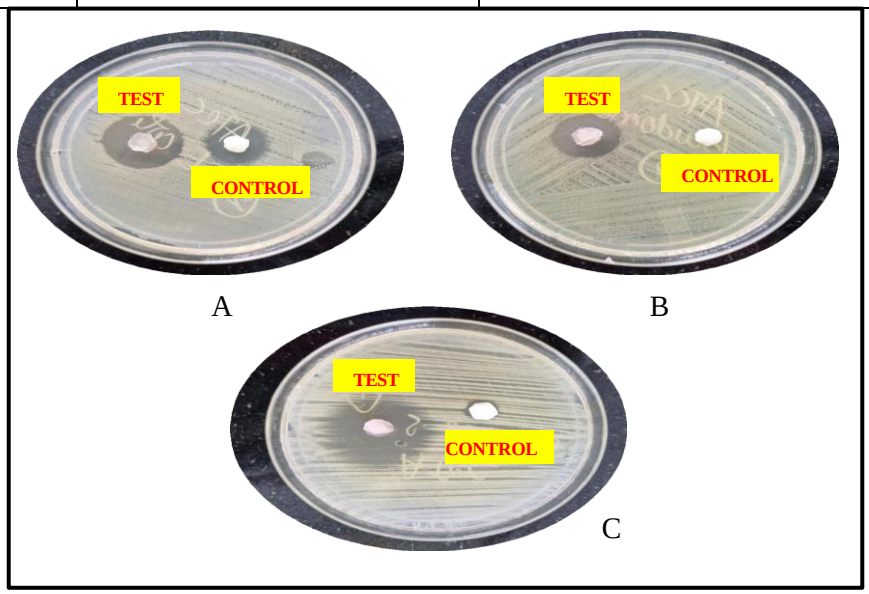

Figure 9: Antimicrobial Activity of collagen peptide dental plug

4. CONCLUSION

F1 collagen peptide–derived dental plug demonstrated a good combination of mechanical, physicochemical, and biological characteristics to be considered as a material for socket preservation after tooth extraction. The level of swelling was higher in the case of F1 material (≈476–507% within 15–180 min), which

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means that there is a possibility to fit well into the socket cavity and effectively absorb exudate to form a stable blood clot. *In vitro* degradation was slower compared with placebos (showed the highest mass at days 3, 7 and 14), which is indicative of structural durability and, at the same time, resorption after this period. SEM analysis showed that there is a porous and interconnected structure, which is good for blood clot retention and cell penetration, as well as concentration-dependent density to maintain a right balance between porosity and compressive stability. F1 also has a high compressive strength, which indicates a good retention in the mouth cavity during healing. Drug-release studies revealed the presence of a burst effect followed by a sustained one (vitamin C and K1 ≈94% at 24 h), which can be characterized as diffusion and erosion kinetics by Korsmeyer–Peppas equation. The collagen peptide dental plug was found to have significant antimicrobial zones against *E. coli*, *P. aeruginosa*, and *S. aureus*, dealing with infection concerns and dry socket formation. In light of the fact that early alveolar ridge resorption is highly prevalent in practice, where the horizontal width loss may be from several millimetres up to about 63% size reduction, which makes future prosthetic/implant placement problematic, such multifunctional dental plug could help to avoid these problems.

5. ABBREVIATIONS:

Alveolar ridge preservation (ARP), Tripolyphosphate (TPP), Fourier-transform infrared spectroscopy (FTIR), polyvinyl alcohol (PVA) solution and polyvinylpyrrolidone (PVP), Scanning Electron Microscope (SEM), zones of inhibition (ZOI), 2,2-diphenyl-1-picrylhydrazyl (DPPH)

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