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Original Article

Self-assembling peptide nanofibers and nanoceramics in a model of alveolar bone repair: Insights from in vivo experiments and clinical trial

Elahe Tahmasebi [a, b, 1], Sareh Azadi [c, d, 1], Samira Hajisadeghi [a, b], Hamidreza Barikani [e], Masoud Salehi [f], Mahdi Shafikhani [a, b], Fateme Mozaffari [a, b], Edris Nazarpour [a, b], Arman Torabizadeh [g, **], Ahad Khoshzaban [d, *]

[a] Research Center for Prevention of Oral and Dental Diseases, Baqiyatallah University of Medical Sciences, Tehran, Iran
[b] School of Dentistry, Baqiyatallah University of Medical Sciences, Tehran, Iran
[c] Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran
[d] Department of Medical Biotechnology, Iran University of Medical Sciences, Tehran, Iran
[e] Dental Implant Research Center, Tehran University of Medical Sciences, Tehran, Iran
[f] Department of Biostatistics, School of Public Health, Iran University of Medical Sciences, Tehran, Iran
[g] Oral and Maxillofacial Surgeon, Private Practice, Tehran, Iran

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ABSTRACT

Introduction: Tooth extraction initiates a cascade of homeostatic and structural modifications within the periodontal tissues, culminating in alveolar ridge resorption. To prevent ridge resorption following extraction and facilitate successful placement of an implant-supported prosthesis, alveolar ridge preservation was performed.
Methods: In this study, the biocompatibility of a nanocomposite consisting of self-assembling peptide nanofibers (organic phase) and tri-calcium phosphate-nano hydroxyapatite (mineral phase), was evaluated in rabbits. Subsequently, the nanocomposite was grafted onto a model of alveolar bone repair in patients.
Results: The in vivo findings revealed no significant differences in the irritation ranking score and average thickness of the reaction zone between the nanocomposite and control groups. Furthermore, there were no significant differences in the appearance of necrosis, granulation tissue, fibroplasia, neovascularization, and hemorrhage as well as in the number of neutrophils, mast cells, lymphocytes, macrophages, and giant cells between the two groups. The defect area was completely filled with newly formed bone trabeculae and cavities containing bone marrow, indicating angiogenesis, while remnants of the scaffold were observed in the deeper region of the defects, adjacent to the bone marrow, considered osteoinductive. The clinical trial findings (TRN: IR.IUMS.REC.1401.355) demonstrated robust bone regeneration after 3.5 months of socket preservation, whereas the bone in the control group experienced atrophy. The nanocomposite facilitated soft tissue healing without any signs of infection or other periodontal malfunction.
Conclusion: The application of nanotechnology has enhanced the bio-functionality of alloplastic materials, positioning this nanocomposite a promising alternative to autografts and allografts in alveolar bone repair.

1. Introduction

Tooth extraction is a final treatment for hopeless teeth. It is known that alveolar ridge modeling peaks within 4–6 weeks after extraction, but it may continue to atrophy at a slower rate over time. Replacing the missing tooth with a fixed, removable, implant, or tooth-supported prosthesis can pose a significant challenge

* Corresponding author.
** Corresponding author.
E-mail addresses: arman.torabi@gmail.com (A. Torabizadeh), akhoshzaban@gmail.com (A. Khoshzaban).
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[1] Authors to whom equally contributed.

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because of the resulting alveolar ridge reduction [1]. It has been observed that resorption of the alveolar ridge is a continuous and irreversible process that leads to various challenges in prosthodontics, aesthetics, and functionality while replacing missing teeth. Multiple studies in humans have confirmed that the alveolar process shrinks after the loss of one or more teeth [2].

After tooth extraction, resorption of the alveolar bone can limit the use of implants. Within 3–6 months of extraction, the alveolar bone can be absorbed to varying degrees, with most of the dimensional changes occurring within 2 weeks. This can lead to a loss of 29–63 % (2.46–4.56 mm) of the original width and 11–22 % (0.8–1.5 mm) of the original height of the alveolar ridge [3]. As a result, there can be a deficiency of alveolar ridge bone, affecting the long-term use and esthetic effects of implants. Therefore, preserving alveolar bone mass is a critical challenge in implant repair [4].

Alveolar ridge preservation (ARP) is used to reduce bone resorption and to maintain the shape of the alveolar bone after tooth extraction. This involves filling the socket with different biomaterials and sealing it with closure materials to prevent loss of the underlying biomaterial. It is important to tightly suture the wound to reduce the risk of infection and prevent early shedding of biomaterials, which could affect the contour of the bone tissue in the future [5,6].

There are many different ARP techniques and various types of materials, such as autografts, allografts, xenografts and alloplastic materials; each has unique properties [7,8]. Autograft grafting is currently considered the gold standard treatment for bone regeneration because of its ability to stimulate bone formation and provide structural support [9]. However, the use of autologous bone grafts is associated with certain disadvantages such as donor site morbidity and postoperative complications. This has led to the need for a new class of biomaterials with similar biological activities and better physical properties [10].

Alloplastic bone substitutes are synthetic materials that contain some of the essential chemical components found in natural bone, such as ceramics and organic phases. These substitutes are known to promote bone regeneration; however, they do not resemble the natural structure of bones [11]. The alloplastic bone substitute demonstrated acceptable potential in both research and clinical settings, with varying compositions and procedures, particularly in terms of vascularization and osteoinductive effects [12]. Xenograft bone substitutes have two main problems: long-term persistence and inflammation at the surgical site [13].

The common advantages of alloplastic bone substitutes are the standardized product quality and absence of infectious disease risk compared with allograft and xenograft grafts [14]. The main components of the dental alloplastic bone sub-architecture are tricalcium phosphate (Ca3(PO4)2, β-TCP), calcium phosphate (Ca–P), and hydroxyapatite (Ca10(PO4)6(OH)2, HA), which is a crystalline form of Ca–P. Nano-HA is the main mineralized bone tissue and exerts osteoconductive effects when grafted into the defect. Synthetic calcium phosphate ceramics (β-tricalcium phosphate [β-TCP] and HA) can be altered to autogenous grafts, allogenic grafts, and xenogenic grafts, and used as blocks, cement, pastes, powder, granules, and putty type with carboxymethyl cellulose or hyaluronic acid [15–18]. In contrast to allogeneic bone, alloplastic bone substitutes only have the ability to support osteo-conduction; their regenerative abilities are generally weak [19].

Self-assembling peptide nanofibers (SAPs) are widely used as scaffolds in tissue engineering. These nanomaterials can be administered in hydrogel form, providing injectability, filling spaces between grafted bones, and excellent biocompatibility [20–23]. These nanofibers consist of alternating hydrophilic and hydrophobic amino acid residues, or hydrophilic heads linked to hydrophobic tails. Upon exposure to an external stimulus, SAPs spontaneously self-assembled into nanofibrous structures. These structures form stable β-sheets at the nanoscale level and can self-assemble into twisted nanofibers and/or flat sheets [24]. They can attach to various biological motifs, including osteogenic, angiogenic, and antibacterial motifs, to enhance their biological activity.

Furthermore, SAPs have been tested in clinical trials, and many are being studied, showing their promising safety and therapeutic potential [24]. These nanofibrous scaffolds are made of synthetic amino acids without animal-derived components or biological or toxic contaminants and do not elicit any adverse immune responses or inflammatory reactions [25–27].

The purpose of this study was to conduct a randomized controlled clinical trial to evaluate the effectiveness of alveolar ridge preservation using the nanocomposite containing self-assembling peptide nanofibers and nanoceramic and compare it with natural healing. The nanocomposite is composed of synthetic anti-inflammatory, angiogenic, and osteogenic self-assembling peptide nanofibers and nano-ceramics, which serve as scaffolds [28]. The nanoceramics utilized in this study included tricalcium phosphate and spherical nano-hydroxyapatite in powder form. We opted for the powder variant of these nanoceramics because it exhibits faster biodegradation compared to granule forms, and our experiments indicated a greater efficacy in promoting bone regeneration. The nanoceramics is combined with self-assembling peptide nanofibers to create a putty that can be implanted at the site of a defect or injury. It is the first alloplastic in which two main mineral and organic phases are at the *nanoscale*, mimicking the natural structure of bone. The morphology of its nano-hydroxyapatite is *spherical* revealing higher biocompatibility than needle-like nanoceramics. When the nanocomposite comes in contact with the bone, it mineralizes the bone surface, exposing osteogenic biomolecules on the bone surface, triggering angiogenesis, and promoting robust osteogenesis. The evaluation of socket preservation consisted of clinical assessment of soft tissue healing, changes in alveolar dimensions, and CBCT analysis of bone regeneration and density taken 3.5 months after tooth extraction. In accordance with the ISO 10993 guidelines for the advancement of medical devices, it is imperative to conduct biocompatibility assessments and evaluate safety concerns prior to initiating clinical trials for these devices. Consequently, the current research undertook an implantation test following the ISO 10993-6 guideline, and subsequently assessed the osteogenic impact of the nanocomposite in a clinical trial.

2. Materials and methods

2.1. Morphological evaluation of nano-hydroxyapatite

FESEM (TESCAN, MIRA 3, USA) was used to evaluate the morphological characterization of nano-hydroxyapatite. A few amounts of ceramic were placed in gride and then was coated using gold sputtering method and scan using FESEM instrument.

2.2. Implantation in rabbit based on ISO 10993-6

The objective of this study was to evaluate the biocompatibility of nanocomposite implanted subcutaneously into the femur of New Zealand rabbits for a period of 30 days (Ethical code: 1400-3-99-22545). ISO 10993-6, which is part of the biocompatibility standards established by the International Organization for Standardization, provides guidelines for conducting implantation studies and evaluating local effects. According to ISO 10993, it is essential to perform biocompatibility examinations and evaluate safety concerns before medical devices proceed to clinical trials. Implantation tests were carried out to examine the impact of the implant on

living tissue at both macroscopic and microscopic levels. The biocompatibility histopathology report was obtained from the Department of Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences. Six naïve male New Zealand White rabbits were kept in a controlled environment with regular access to food, water, and a 12-h light/dark cycle. The rabbits were randomly assigned to two groups, with three rabbits per group. Group 1 received the nanocomposite material, while group 2 received high-density polyethylene (HDPE)4.

2.3. Inflammation and tissue response score

2.3.1. Irritant ranking score

After anesthetizing and preparing the skin of six inexperienced male New Zealand White rabbits, the samples were implanted subcutaneously in the femur. Each animal received four samples. The irritant ranking score was determined by subtracting the mean group combined inflammation and tissue response scores for the control material from the corresponding scores for the test material. Irritation was evaluated 30 days after implantation, following the guidelines outlined in ISO 10993-6.

2.3.2. Reaction zone thickness

The thickness of the reaction zone around each implant site was determined using a calibrated ocular micrometer. Measurements in microns were taken from regions representing the minimum, maximum, and median thicknesses after 30 days. The thickness of the reaction zone was measured at 12 sites within each group and the average values were recorded.

2.3.3. Histology study

After a period of 30 days following implantation of nanocomposite in implantation site, rabbits were sacrificed and the tissues processed for histopathological examination using hematoxylin and eosin (H&E) staining. The nature and extent of cellular reactions to implants can be evaluated at the microscopic level.

2.4. Clinical trial

The present study was a prospective, randomized controlled clinical trial performed in accordance with the World Medical Association Declaration of Helsinki and was reviewed and approved by the Institutional Ethics Committee (TRN: IR.IUMS.REC.1401.355). The research protocol was approved by the Iranian General Directorate of Medical Equipment and Device (ISO 11737-1, ISO 11737-2 and registered at the US Provisional Patent Application Ser. No. 62/374,928. Patients requiring extraction of premolars and molars were recruited from Dr. Shokri Dental Hospital at the Baqiyatallah University of Medical Science, Tehran, Iran, between January 2023 and November 2023. All patients were screened for inclusion and exclusion criteria, and eligible patients were enrolled after assuring verbal understanding and obtaining written informed consent.

2.4.1. Inclusion criteria

Patients eligible for the study required one or more molar tooth extractions followed by implant-supported restoration based on precise diagnosis and treatment planning. The following inclusion criteria were used to sequentially enroll the patients:

- Age from 18 to 70 years old
- Both sexes (male and female)
- Healthy patients with indication for dental extraction
- Patient consent approval and signing

2.4.2. Exclusion criteria

- Smokers and current alcohol or drug abuser
- Pregnant and breast-feeding females
- Bad oral hygiene.
- Patients on medications such as chemotherapy, anticoagulants, corticosteroids, bisphosphonates, drugs immunosuppressants, and autoimmune disorders.
- Patients with decompensated chronic diseases (e.g., hypertension, diabetes, rheumatic diseases, and renal and hepatic diseases)

Patients with bone diseases or metabolic disorders (such as osteomalacia, hypocalcemia, and hypercalcemia).

2.4.3. Surgical procedure

Prior to tooth extraction, all patients underwent clinical and radiographic examinations and received periodontal treatment, as needed. All the surgical procedures were performed by an experienced dentist. After local anesthesia, flapless atraumatic tooth extraction was performed. Following extraction, the sockets were thoroughly debrided to remove any granulation tissue within the socket. After tooth extraction, the patients were randomized into one of the two groups. Furthermore, the patient and clinician responsible for the clinical measurements were blinded to the nature of the treatment that was rendered.

- I. Socket preservation using nanocomposites ($n = 12$).
- II. Tooth extraction without socket preservation: Control group ($n = 6$).

2.4.4. Soft tissue and bone density evaluation

Clinical and radiographic measurements and statistical analyses were performed blinded to the treatment assignments. The surgical site was visually inspected, and wound healing outcomes, including persistent swelling, especially pain or signs of infection, spontaneous bleeding, and ulceration, were assessed 1 and 14 days after ARP. The efficacy of nanocomposites was determined by measuring linear and volumetric changes on cone-beam computed tomography (CBCT) (Carestream Kodak CBCT 9600 3D, CS9600, France) imaging after implantation of the nanocomposite (immediately after extraction) and 3.5 months after ARP.

2.5. Statistical analysis

The sample size calculation was conducted using data obtained from a previous study by Tosta et al. [29]. Descriptive statistics such as mean and standard deviation (SD) were used to accurately represent the distribution of the data. For statistical data analysis, p-values were calculated using either a Mann-Whitney or Student's t-test, depending on whether the data were parametric or nonparametric, respectively. All statistical analyses were performed using GraphPad Prism software (version 3.0; GraphPad, La Jolla, California, USA). Statistical significance was set at $P < 0.05$.

3. Results

3.1. Morphological study

FESEM was used to evaluate the morphological structure of the nano-hydroxyapatite. Results showed spherical nano-hydroxyapatite less than 50 nm (Fig. 1a).

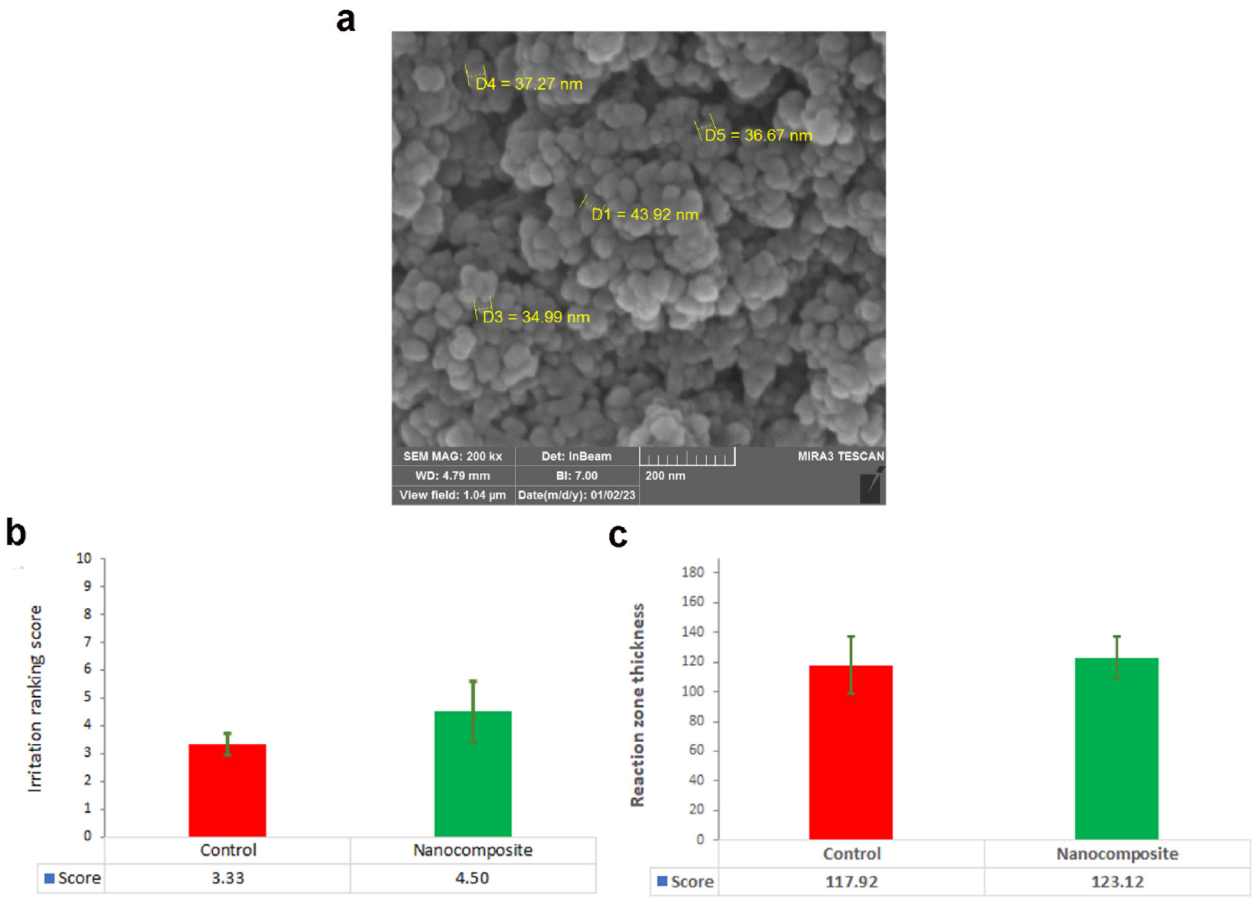

Fig. 1. Morphological and biocompatibility evaluation based on ISO 10993-6 in rabbits. a) Morphological evaluation of nano-hydroxyapatite using FESEM b) Irritation ranking score c) reaction zone thickness. There was no significant difference between groups and nanocomposite was biocompatible.

3.2. Biocompatibility evaluation based on 1SO 10993-6

3.2.1. Irritation ranking score

The assessment of implant biocompatibility relies heavily on the irritation ranking score. In accordance with the ISO 10993-6 guideline, irritation was conducted after a 30-day period after implantation. The collected data revealed no significant difference in the irritation ranking score between the nanocomposite and control group ($p = 0.0775$). Furthermore, irritation caused by the nanocomposite material was negligible, as shown in Fig. 1b.

3.2.2. Reaction zone thickness

A calibrated ocular micrometer was used to ascertain the thickness of the reaction zone surrounding each implant. The irritation ranking score, which ranges from 0 to 4, indicates the severity of the reaction as follows: 0, absence; 1, slight irritation, 2 representing mild irritation, 3 signifying moderate irritation; and 4, severe irritation. The results revealed that the average thickness of the reaction zone in the control group was approximately 1.044 times greater than that in the nanocomposite group. However, statistical analysis demonstrated no significant disparity in tissue responses between the control and nanocomposite groups ($p = 0.4607$) (Fig. 1c).

3.2.3. Histology study

Histological evaluation was conducted using H&E staining to assess tissue responses including necrosis, granulation tissue, fibroplasia, neovascularization, and hemorrhage. The responses were carefully evaluated and scored. The findings revealed no significant difference in tissue response between the control group and nanocomposite group ($p > 0.05$) (Fig. 2a). Additionally, inflammation in the tissue was assessed by quantifying the number of neutrophils, mast cells, lymphocytes, macrophages, and giant cells. The results demonstrated no significant difference in the tissue response between the control group and nanocomposite group ($p > 0.05$) (Fig. 2b). In summary, the total tissue response in both the control and nanocomposite groups did not exhibit any significant variation ($p > 0.05$) (Fig. 2c). The defect area was completely filled with newly formed bone trabeculae, whereas some remnants of the scaffold were observed in the deeper regions of the defects adjacent to the bone marrow. Notably, no foreign-body inflammatory response was detected around the scaffold residues. The existing compact bone at the edges of the defect displayed a normal structure (Fig. 3).

3.3. Clinical trial findings

3.3.1. General observation

Nine patients (male and female) ranging in age from 18 to 70 years were included in the study, with 18 alveolar sockets. Caries, root fractures, and periodontal disease are the main causes of dental extraction. During follow-up, no changes were observed in the original clinical trial. There were no signs of acute pain or local complications following the extractions in the nanocomposite group, whereas two sockets in the control group experienced pain for 2 weeks.

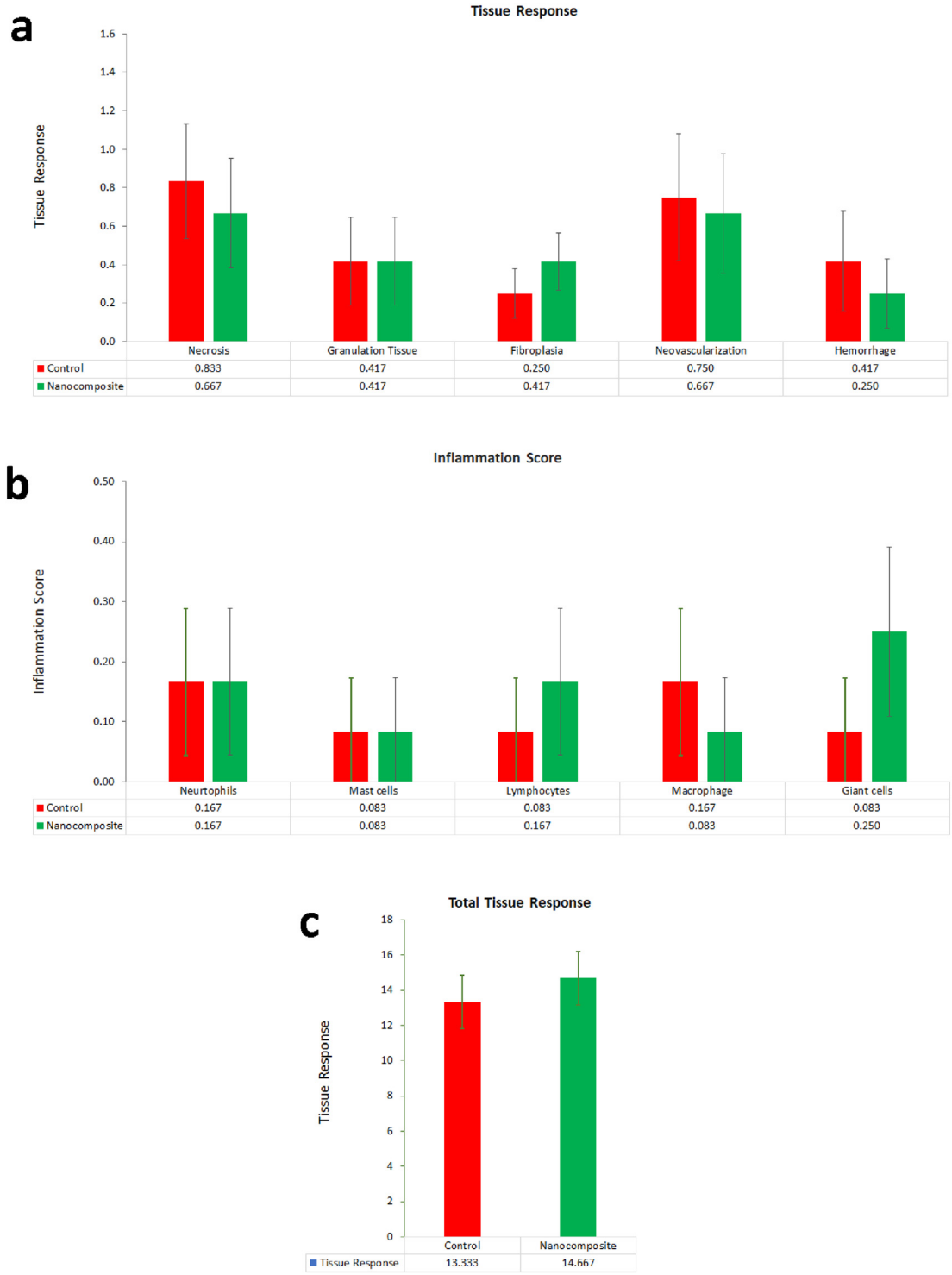

Fig. 2. Biocompatibility evaluation based on ISO 10993-6 in rabbits. a) Tissue response b) inflammation score c) Total tissue response. There was no significant difference between groups and nanocomposite was biocompatible.

3.3.2. *Soft tissue healing*

A clinical trial was conducted on 18 patients, as described below. Twelve patients were placed in the treatment group and six patients in the control group without the tested substance. The factors under investigation were infection and soft-tissue formation. None of the patients in either the control or nanocomposite group showed any signs of infection during tooth extraction or 2 weeks later. There was no significant difference in soft tissue formation between the control and nanocomposite groups ($p < 0.05$).

3.3.3. *CBCT analysis*

Reconstructed images of patients demonstrated a distinct pattern of bone repair in the alveolar socket of the nanocomposite group compared with the control group after a duration of 3.5

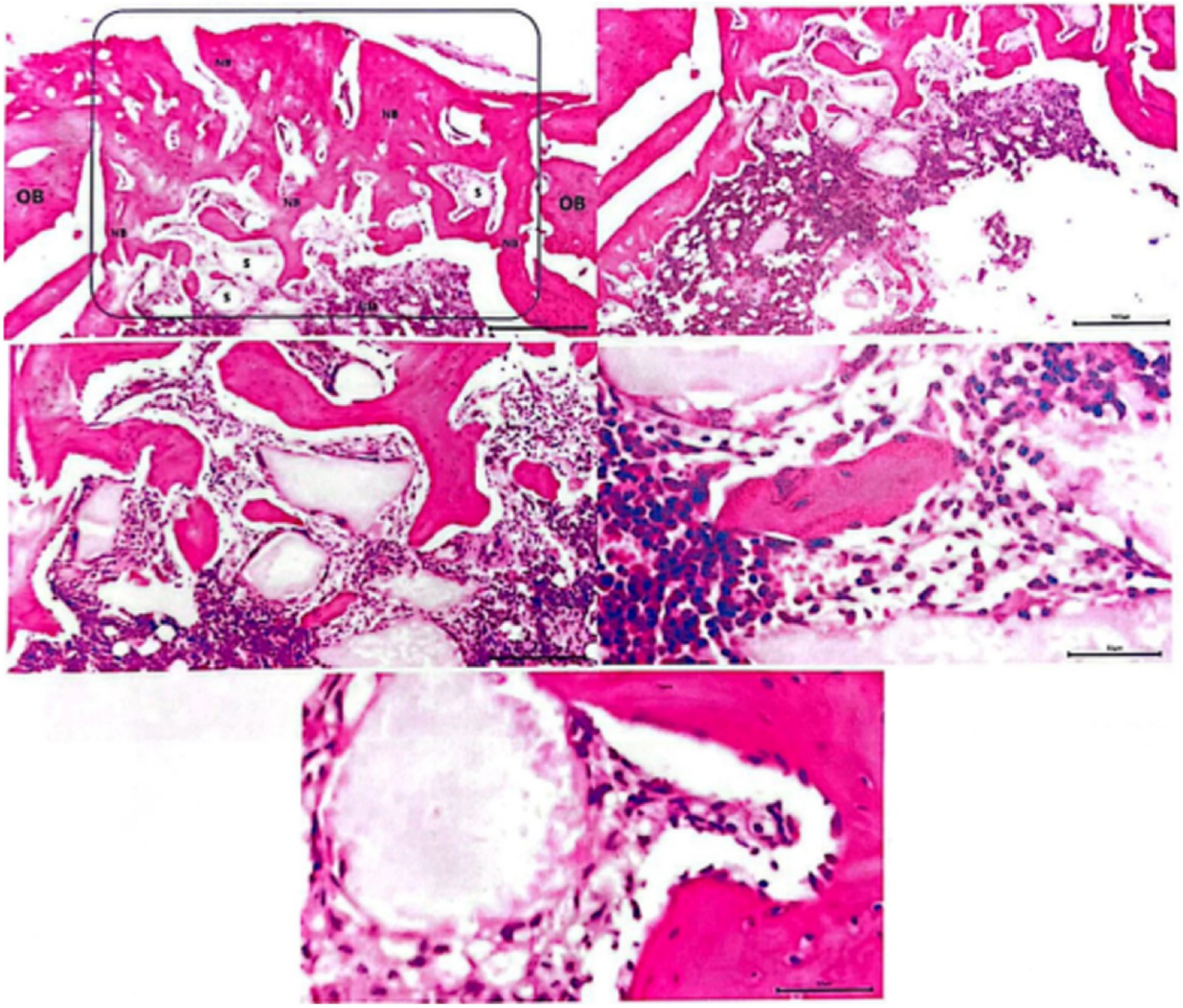

Fig. 3. H&E staining of rabbit specimens. Images showed bone regeneration in the site of implantation without foreign body reaction (40X).

months. This was further confirmed by morphometric analysis of the newly formed bone, which revealed notable differences between the two groups. The nanocomposite group exhibited 79.26% of new bone formation of 79.26 % (Table 1), whereas the control group displayed a statistically significant bone reduction in the alveolar socket ($p < 0.01$) (Fig. 4). Additionally, the nanocomposite groups exhibited an increase in bone density (77.04 %), surpassing that of the control group. The control group exhibited satisfactory soft healing at the extraction site; however, some degree of atrophy was observed in the alveolar socket. A significant difference was observed between the two groups in the extent of bone density ($p < 0.01$) (Table 1).

Furthermore, upon examination of the radiographs obtained during the initial socket preservation, the nanocomposite material appeared radiolucent (Fig. 4). However, at the 3.5-month mark, the radiographs of the nanocomposite group exhibited radiopacity, indicating bone formation (Fig. 4).

Table 1
Bone volume and density in sockets implanted with nanocomposites and the control groups 3.5 months post-treatment.

	Nanocomposite	Control
Bone volume	79.26 ± 6.9	−29.3 ± 5.5
Bone density	77.04 ± 17.11	−12.9 ± 5.7

4. Discussion

Tooth extraction causes homeostatic and structural changes in the periodontal tissues, leading to alveolar ridge atrophy. ARP is performed to avoid ridge resorption after extraction to perform a correct implant-supported prosthesis [30]. Over the past two decades, different procedures have been suggested to preserve bone volume after tooth extraction, and different biomaterials have been promoted for ASP surgical treatment.

The present study investigated the efficacy and safety of a self-assembled peptide cocktail nanofiber 3.5 months after ARP. In the case series shown here, an attempt was made to generate an advantage for the patient in terms of the quality of bone regeneration and volume preservation after a short time, all of which were higher in the peptide cocktail nanofiber groups than in the control group. Furthermore, true osteogenesis with the formation of woven or fibrous bone, which has already been partially transformed into mature lamellar bone without signs of inflammation or necrosis, was verified.

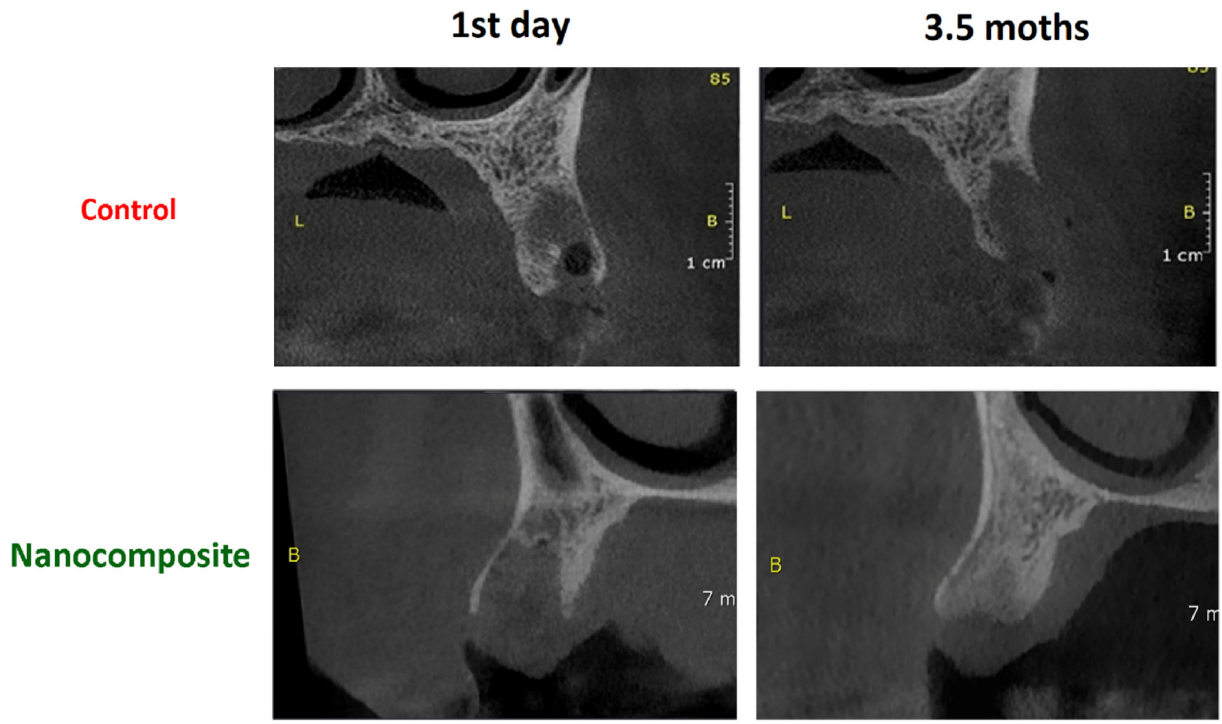

Fig. 4. CBCT in patient just after implantation and in 3.5 months. Images showed that the nanocomposite was radiolucent and enhanced bone regeneration in the site of socket preservation while control group showed reduced bone volume and density.

Bone substitutes used in grafting have the disadvantage of inducing new bone formation at a slow and unpredictable rate. However, with the help of innovative tissue engineering technologies, these materials can be modified to enhance and hasten their bone-inductive capabilities. Neiva et al. [31] demonstrated favorable outcomes using PepGen P-15 Putty (an organic bovine bone matrix (ABM) coupled with a synthetic cell-binding peptide), which showed a faster and more mature trabecular pattern of osteogenesis compared to the control. Our CBCT assessment also showed bone formation as early as 3.5 months.

A newly published article by Han et al. [32] has examined the effectiveness of rhBMP-2 combined with β-TCP (rhBMP-2/β-TCP) as a graft material for alveolar ridge preservation. The results showed that patients who received 0.5 mg/mL rhBMP-2/β-TCP had significantly greater bone augmentation than those who received β-TCP alone. Additionally, rhBMP-2/β-TCP provided a better ARP effect for both the height and width of the alveolar bone, while maintaining a good safety profile. In another randomized clinical trial by Jo et al. [33], two rhBMP-2 delivery systems for ARP were compared. The study evaluated the efficacy of 1.5 mg/mL rhBMP-2 for ACS and 1.0 mg/mL for β-TCP/HA. In this study, we evaluated the effectiveness and safety of a self-assembled peptide cocktail nanofiber at a low concentration (250μL/site), ready to preserve the alveolar ridge. In particular, considering that maintaining a sufficient volume of alveolar ridge through ARP will allow placement of implants at the optimal position with favorable angulation and enable functional and esthetic prostheses, the clinical application of peptide cocktail nanofibers is worth considering.

Notably, Bone mineral density in this context provides a measure of the extent of bone mineralization. As new bone becomes more mineralized as it matures, a higher bone mineral density represents a more mature bone. In this study, bone mineral density was higher at sites treated with peptide cocktail nanofibers compared the control group (77.04 ± 17.11 %). In contrast, other synthetic materials, such as inorganic bovine bone (BioOss), have shown delayed bone formation in extraction sockets [34].

Various bone graft products are commercially available worldwide. However, there is no clear consensus regarding the appropriate bone graft products for different clinical situations. In total, 87 alloplastic bone graft products were approved by the FDA from 1996 to December 2020:15 hydroxyapatite (HA), 21 β-tricalcium phosphate (β-TCP), 18 biphasic calcium phosphate (BCP), 5 calcium sulfate (CS), 5 calcium phosphate (CP:detailed composition was not confirmed), 11 bio-glass (BG), and 4 others (e.g., carbonate apatite) [8]. Ideal alloplastic bone substitutes are biologically stable and maintain their volume, allowing cell infiltration and remodeling [35]. The capabilities of such substitutes vary depending on factors such as the manufacturing methods, crystal structure, pore size, mechanical properties, composition, and absorption rate, all of which contribute to their osteoconductive properties [36].

GENESIS-BCP (DIO, Busan, Korea) consisted of 60 % of HA and 40 % of β-TCP. Human study level II was received by a prospective controlled clinical trial, which resulted in good outcomes in periodontal defects [37]. In horizontal augmentation, successful results were obtained with Nano-Bone (HA and silica gel matrix; Artoss GmbH, Warnemünde, Germany) in a case report, and it obtained a human study level IV [38].

It is preferable that alloplastic bone substitutes are completely resorbed. In a previous study, it was noted that non-resorbable products, such as HA sintered at high temperatures, are not commonly used for periodontal regeneration. This was due to concerns that residual bone graft materials may hinder the formation of periodontal tissue over the long term and lead to weak resistance against reinfection [11,39].

Allograft and xenografts are currently in high demand for periodontal and bone regeneration applications in the United States [40]. However, there are several advantages to using alloplastic bone graft substitutes, which include the absence of

potential infectious disease transmission, as well as the absence of ethical or religious controversies. When alloplastic bone substitutes are used together with growth factors and/or cell transplantation, osteoconduction, osteoinduction, and osteogenesis can be achieved. Therefore, alloplastic bone substitute products may be the preferred option for periodontal and bone regeneration therapy because of their safety and predictability [8].

Self-assembling peptide nanofibers are a new type of hydrogel-based scaffolds used in tissue engineering. They have great potential as injectable scaffolds for the repair of craniofacial and spinal fractures. In recent years, significant research has been conducted to develop highly biocompatible, osteoconductive/osteoinductive, and biodegradable in situ scaffolds for repairing craniofacial and spinal fractures. As a self-assembling core in sol form, it turns into hydrogel-based scaffolds with a β-sheet structure and nanofiber topography when exposed to ionic media and completely fills cavities and defect sites [25]. In our study, the characteristics of injectable self-assembled peptide cocktail nanofibers could help investigators easily apply bone substitutes to the extraction socket without the need for open surgery. In addition, peptide cocktails provide an angiogenic, anti-inflammatory, and osteogenic scaffold to enhance bone regeneration through their osteogenic biological motifs in the backbone of oligopeptides, self-assembling peptide nanofibers, and nano-ceramics.

A study conducted by Thompson et al. [41] demonstrated that PepGen P-15 228 FLOW PUTTY(containing PepGen P-15 particulate in a biocompatible hydrogel) produced significantly greater vital bone than Puros® and C-Graft 228 after 4 months in maxillary and human extraction sockets ($P < 0.01$). The amount of vital bone for FLOW PUTTY was 12-fold higher than that of C-Graft 228 and more than 4-fold higher than that of Puros®. Because the hydrogel carrier provides optimal spacing between the particles and enhanced cellular interaction for bone formation, PepGen P-15 Flow may substantially decrease the time required to place an implant. The effect of PepGen P-15 228 FLOW PUTTY suggested by these results was consistent with our results.

Regarding safety, there were no severe adverse events related to injectable nanofiber substitutes or procedures in our study. SAP scaffolds do not elicit any noticeable adverse immune responses or inflammatory reactions, as they are made of pure synthetic amino acids without animal-derived components or chemical, biological, or toxic contaminants and are subsequently degraded into amino acids, which are a group of naturally biodegradable molecules. It is worth mentioning that adding graft material may hinder osteogenesis by delaying healing or by requiring additional time for resorption due to residual scaffold material [42].

5. Conclusion

In vivo and clinical trial studies have demonstrated the synergistic potential of integrating self-assembled peptide nanofibers with Nanoceramics in a model of alveolar bone repair. This innovative approach holds promise for enhancing the regenerative capacity of alveolar bone, offering a potential solution for individuals suffering from bone defects or injuries. By harnessing the self-assembling properties of peptide nanofibers and the unique characteristics of Nanoceramics, researchers have aimed to develop a biomaterial-based strategy that promotes efficient bone regeneration. These findings pave the way for further exploration and optimization of this novel therapeutic approach in the field of bone tissue engineering. In other words, this clinical trial showed that nanocomposite containing nanoceramics and self-assembled peptide nanofibers provided a strong ARP effect for both the regeneration and density of alveolar bone with a safety profile. Therefore, the nanocomposite is a safe graft material that provides high alveolar bone preservation in patients undergoing dental extraction.

Author contributions

Elahe Tahmasebi, Arman Torabizadeh, and Ahad Khoshzaban contributed to study conception and design. All authors contributed to the data collection and analysis were performed by Elahe Tahmasebi, Sareh Azadi, Samira Hajisadeghi, Hamidreza Barikani, Mahdi Shafikhani, Fateme Mozaffari, Edris Nazarpour, Arman Torabizadeh, Ahad Khoshzaban. The first draft of the manuscript was written by Sareh Azadi, and all the authors commented on the previous versions of the manuscript. All the authors have read and approved the final manuscript."

Ethical statement

The present study was a prospective, randomized controlled clinical trial performed in accordance with the World Medical Association Declaration of Helsinki and was reviewed and approved by the Institutional Ethics Committee at Iran University of Medical Scinces (IR.IUMS.REC.1401.355) and (TRN: IR.IUMS.REC.1401.355).

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Declaration of competing interest

The authors declare no financial and nonfinancial conflict of interest.

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