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CHITOSAN - ZINC / STRONTIUM COMPOSITE MEMBRANE FOR BONE REGENERATION APPLICATION

Priyan I[1], Dr. Saravanan[2], Dr. Marina Francis[2]
[1]Undergraduate Resident, Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), 162, Poonamallee High Road, Velappanchavadi, Chennai – 600077, Tamil Nadu, India
152001006.sdc@saveetha.com
[2]Assistant Professor, Department of Prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), 162, Poonamallee High Road, Velappanchavadi, Chennai – 600077, Tamil Nadu, India
saravanan.sdc@gmail.com , marinafrancis26@gmail.com

ABSTRACT:
Bone regeneration, which may be observed during typical fracture healing and is involved in continual remodelling throughout adult life, is a complicated, well-orchestrated physiological process of bone creation. Avascular necrosis, atrophic non-unions, and osteoporosis are a few complex clinical conditions in which large amounts of bone regeneration are needed, such as for the skeletal reconstruction of large bone defects caused by trauma, infection, tumour resection, and skeletal abnormalities, or situations in which the regenerative process is impaired. Chitosan (Cs) (from Shrimp Shells, degree of deacetylation ≥75%) and collagen (Col) Type I, Insoluble (from Bovine Achilles Tendon) were purchased from Sigma-Aldrich. Hydroxyapatite (HA) was synthesized in our laboratory following the reaction:10Ca(OH)2 + 6 (NH4) 2HPO4 ——-> Ca10 (PO4) 6(OH)2 + 12NH4 OH + 6H
Chitosan/Collagen composite membranes (Cs/Col) were obtained varying collagen content into the chitosan solution. Briefly, collagen (0.15 and 0.75% w/v) was dispersed into a chitosan solution prepared as described above by mechanical stirred until a good collagen dispersion was obtained. Then, 25 g of solution was poured into Petri dishes and dried at room temperature.
The membranes with the highest hydroxyapatite and collagen content showed the highest cell adhesion, and no cytotoxicity was presented by any of the membranes prepared, suggesting that these materials have a significant potential to be used for bone regeneration .
Keywords: Human, Health, Disease, Illness, medicine.

1. INTRODUCTION

Bone regeneration, which may be observed during typical fracture healing and is involved in continual remodelling throughout adult life, is a complicated, well-orchestrated physiological process of bone creation.(1) Avascular necrosis, atrophic non-unions, and osteoporosis are a few complex clinical conditions in which large amounts of bone regeneration are needed, such as for the skeletal reconstruction of large bone defects caused by trauma, infection, tumour resection, and skeletal abnormalities, or situations in which the regenerative process is impaired.(2) The "gold standard" autologous bone graft, free fibula vascularized graft, allograft implantation, and use of growth factors, osteoconductive scaffolds, osteoprogenitor cells, (3)and distraction osteogenesis are just a few of the many current methods used to improve the impaired or "insufficient" bone-regeneration process.(4)The poly(ethylene glycol) (PEG), (5)multiwalled carbon nanotubes (MWCNT), and BKC-containing chitosan composite membranes were created by combining the membrane precursors and the antibacterial solution, then casting the mixture using an inverse phase process.Due to the rise in bone problems caused by the ageing population, increased obesity, and insufficient physical activity,(6) bone repair medicine research has received a lot of interest. The bone tissue cannot repair on its own when the bone problem is larger than the critical size defect (>2 cm), necessitating clinical therapy.(7) With more than two million bone graft surgeries performed each year worldwide, bone grafting is one of the most popular techniques for bone regeneration(8). In the past few decades, a variety of bone graft types have been used in bone tissue engineering; however, more focus is now being placed on the biomimetic approach to scaffold design, which achieves molecular, structural, and biological compatibility with complex native bone tissue.
The biomedical sector is very interested in bone regeneration since it has the potential to regenerate a variety of tissues, including cartilage, epithelial tissue, and bone(9). Tissue engineering attempts to eliminate the need to replace and repair damaged tissue in this setting(10). As a result, numerous studies look for ways to stimulate, direct, and promote the regeneration of various connective tissues, such as skin, cartilage, and bone, while minimising any negative effects.(10,11) As a result of their biological, chemical, physical, and mechanical characteristics as well as their ability to combine with other materials to create composites with improved capabilities, many ceramic and polymeric materials exhibit a significant potential for tissue regeneration.

2. MATERIALS AND METHODS :

Chitosan (Cs) (from Shrimp Shells, degree of deacetylation ≥75%) and collagen (Col) Type I, Insoluble (from Bovine Achilles Tendon) were purchased from Sigma-Aldrich. Hydroxyapatite (HA) was synthesized in our laboratory following the reaction:
10Ca(OH)2 + 6 (NH4) 2HPO4 ——-> Ca10 (PO4) 6(OH)2 + 12NH4 OH + 6H2O
Briefly, ammonium phosphate solution was applied dropwise to a Ca(OH)2 suspension in deionized water. The mixture was thoroughly stirred while being kept at a constant temperature of 25 °C. This solution's pH was raised to 10, and it was then let to stand at room temperature for 16 hours. The product was removed from the mother solution and rinsed repeatedly until the pH was neutral. The powder was produced and dried for 20 hours at 60 °C. By using X-ray diffraction (XRD) and Fourier transform infrared spectroscopy, the final material was evaluated (FTIR).

3. RESULTS AND DISCUSSION :

Chitosan membranes (Cs) were prepared by solvent casting method. Chitosan solution was obtained by dispersing 1.5% (w/v) in an aqueous solution of acetic acid at 2% (v/v). The solution was mechanically stirred until solubilization. (12)After that, 25 g of solution was poured into Petri dishes (diameter = 8.2 cm) and dried at room temperature (~a week).Chitosan/collagen composite membranes
Chitosan/Collagen composite membranes (Cs/Col) were obtained varying collagen content into the chitosan solution. (13)Briefly, collagen (0.15 and 0.75% w/v) was dispersed into a chitosan solution prepared as described above by mechanical stirred until a good collagen dispersion was obtained. Then, 25 g of solution was poured into Petri dishes and dried at room temperature.
Chitosan/hydroxyapatite composite membranes
Chitosan/hydroxyapatite composite membranes (Cs/HA) were obtained varying HA content into the chitosan solution.(14) Briefly, HA (0.15 and 0.75% w/v) was dispersed into a chitosan solution prepared as described above by mechanical stirring until a good dispersion of HA was obtained. Then, 25 g of solution was poured into Petri dishes (diameter = 8.2 cm) and dried at room temperature (~a week).

FIGURE 1 :

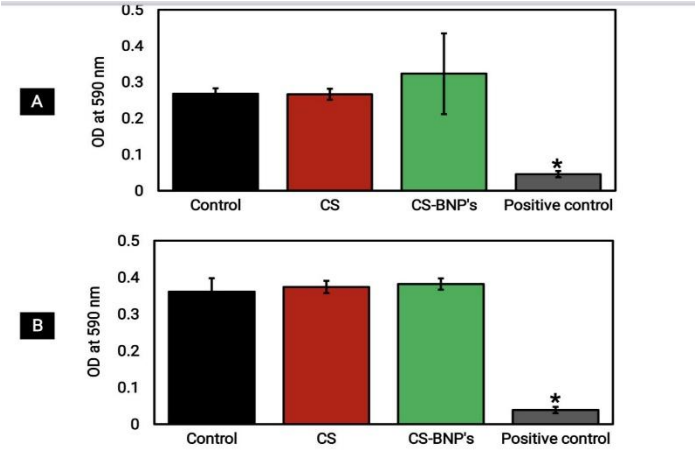

FIGURE 1 : Biocompatibility assessments of the prepared composite. (A) and (B)- 24 h and 48 h direct cytotoxicity assessment performed using MTT assay with human osteoblastic cells (MG-63). The membrane was found to cytofriendly. *-indicates significant differences compared to control, $p<0.05$, n=4. Positive control in MTT assay is 0.1% Triton-x-100.

FIGURE 2 :

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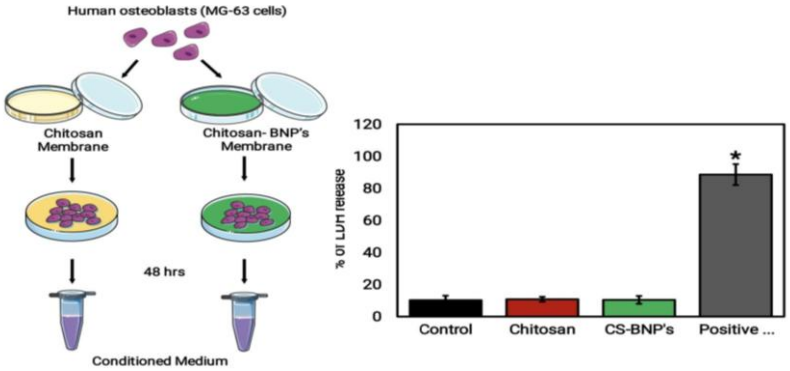

FIGURE 2: Biocompatibility assessments of the prepared composite. Lactate dehydrogenase release assay performed using the medium obtained from mouse mesenchymal stem cells grown on the composite membrane for 48 hours. The membrane was found to cytofriendly. *-indicates significant differences compared to control, p<0.05, n=4. Positive control =1% H2O2 treatment.*

FIGURE 3 :

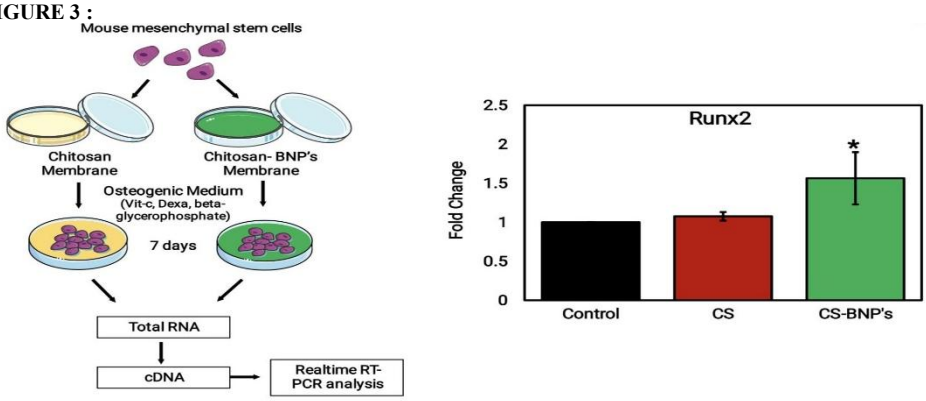

FIGURE 3: Role of prepared Biocomposite membrane on osteoblast differentiation *in vitro*. Mouse mesenchymal stem cells were grown onto the membrane under osteogenic conditions and total RNA was isolated, converted into cDNA and subjected to gene expression analysis of Runx2. *-indicates significant differences compared to control, p<0.05, n=4. Positive control =1% H2O2 treatment.

3.1 Chitosan/collagen/hydroxyapatite membranes: Chitosan/Collagen/Hydroxyapatite membranes (Cs/Col/HA) were obtained, varying HA and collagen content into the solution. Briefly, HA (0.15 and 0.75% w/v) and collagen (0.15 and 0.75% w/v) were dispersed into a chitosan solution prepared as described above by mechanical stirring until a good dispersion of HA and collagen was obtained. Then, 25 g of solution was poured into Petri dishes (diameter = 8.2 cm) and dried at room temperature (~a week).

Figure 4: SEM Micrograph and Elemental Mapping of the Chitosan-Composite Membrane. Scanning Electron Microscopy (SEM) image showing the surface morphology and particle distribution within the membrane matrix. The white rectangular box indicates the specific area ("Spectrum 1") selected for Energy-Dispersive X-ray (EDX) analysis.

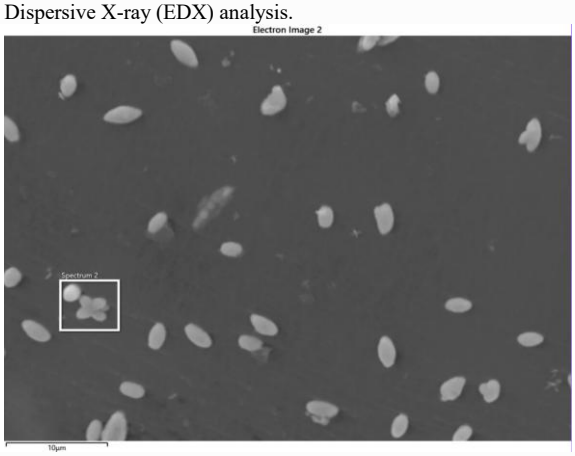

Figure 5: EDX Spectrum of the Chitosan-Copper/Strontium Composite. Energy-Dispersive X-ray (EDX) spectrum confirming the elemental composition of the membrane. The peaks and the data table verify the presence of **Carbon (C)** and **Oxygen (O)** from the organic chitosan/collagen matrix, along with **Strontium (Sr)** and trace **zinc** dopants used for enhanced bone regeneration.

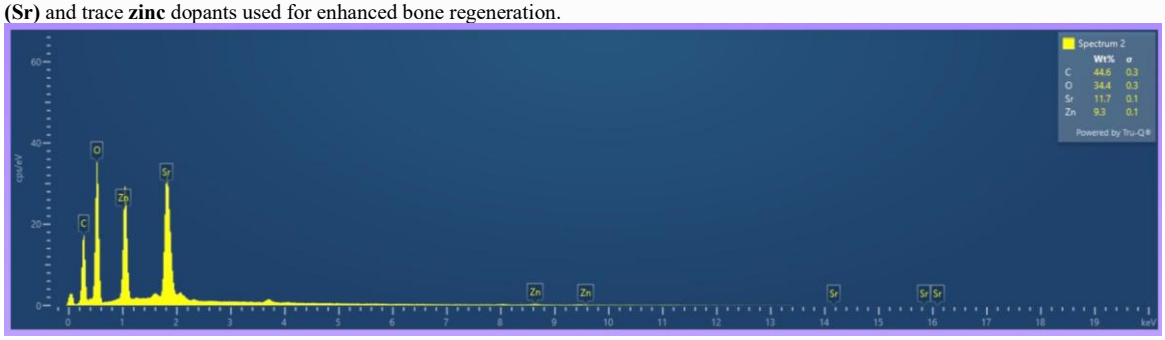

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Figure 6: Low-Magnification SEM Surface Analysis. SEM micrograph at 91.4x68.6µm magnification showing the general topography and uniformity of the solvent-casted chitosan membrane. The scale bar represents 50micrometre.

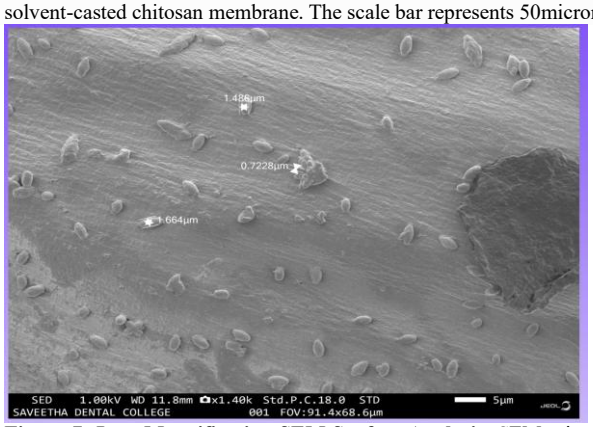

Figure 7: Low-Magnification SEM Surface Analysis. SEM micrograph at 985x738µm magnification showing the general topography and uniformity of the solvent-casted chitosan membrane. The scale bar represents 50micrometre.

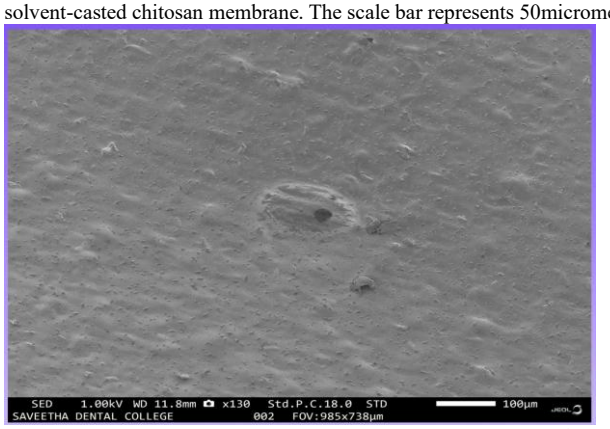

Figure 8: Low-Magnification SEM Surface Analysis. SEM micrograph at 160x120µm magnification showing the general topography and uniformity of the solvent-casted chitosan membrane. The scale bar represents 50micrometre.

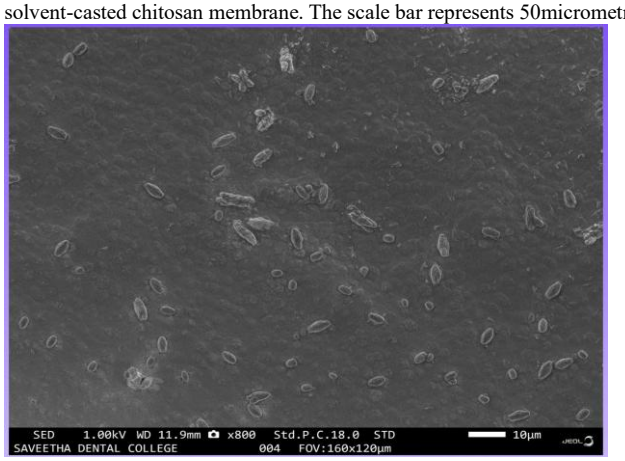

Figure 9: Low-Magnification SEM Surface Analysis. SEM micrograph at 171x128µm magnification showing the general topography and uniformity of the solvent-casted chitosan membrane. The scale bar represents 50micrometre.

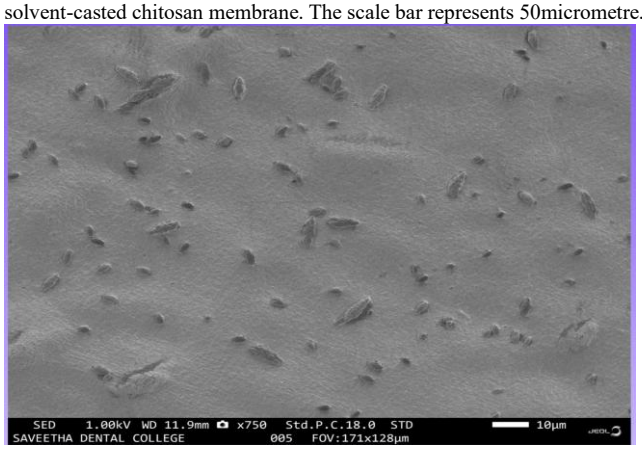

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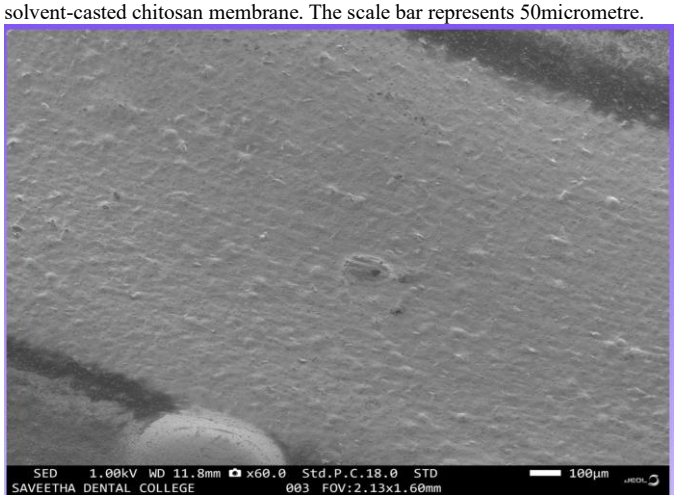
Figure 10: Low-Magnification SEM Surface Analysis. SEM micrograph at 2.13x1.60 mm magnification showing the general topography and uniformity of the solvent-casted chitosan membrane. The scale bar represents 50micrometre.



SEM analyses

The morphological characterization of the different synthesized samples and the seeded membranes was carried out in a Scanning Electron Microscopy (SEM) FEI Inspect F50 System attached with an Energy-Dispersive Spectrometer (EDX) EDAX Apollo.

The preparation of the seeded membranes for SEM analyses was performed modified method. Briefly, samples were fixed using 2.5% v/v glutaraldehyde in PBS for 1 h at 4 °C, then postfixed with 1% v/v OsO4 in PBS for 1 h at 4 °C and rinsed three times with distilled water. After, seeded membranes were dehydrated with a graded series of ethanol (50, 70, 80, 90, and 100% v/v), followed by a 5 min incubation with hexamethyldisilazane (HMDS. Sigma-Aldrich®) and left at room temperature for 2 min to dry. The samples were carbon/Pt coated in Balzers BA 510 evaporator.

5. CONCLUSION:

Composite membranes of chitosan/collagen, chitosan/hydroxyapatite, and chitosan/collagen/hydroxyapatite were successfully prepared by solvent casting method. Membranes with micro and nanopores were obtained with good dispersion of hydroxyapatite in the organic matrix. The addition of collagen and hydroxyapatite to chitosan improves thermal stability and reduces thermal decomposition of the composites. The membranes with the highest hydroxyapatite and collagen content showed the highest cell adhesion, and no cytotoxicity was presented by any of the membranes prepared, suggesting that these materials have a significant potential to be used for bone regeneration .

6. REFERENCE:

1. Sivakumar P, Gurunathan D. Behavior of Children toward Various Dental Procedures. Int J Clin Pediatr Dent. 2019 Sep-Oct;12(5):379–84.
2. Ann Preethy N, Somasundaram S. Safety and physiologic effects of intranasal midazolam and nitrous oxide inhalation based sedation in children visiting Saveetha Dental College and Hospitals, India. Bioinformation. 2022 Jan 31;18(1):26–35.
3. Park KB, Park H, Jeon YD. Priming of Mesenchymal Stem Cells for Tendon Regeneration: Mechanisms and Clinical Translation. Stem Cell Rev Rep [Internet]. 2026 May 11; Available from: http://dx.doi.org/10.1007/s12015-026-11146-0
4. He JX, Lan SY, Ren LH, Zheng YN, Cao Y, Xu HK, et al. Isolation and Therapeutic Application of Extracellular Vesicles Derived from Self-Assembled Mesenchymal Stem Cell Aggregates. J Vis Exp [Internet]. 2026 Apr 24;(230). Available from: http://dx.doi.org/10.3791/70534
5. Sathiyamoorthy S, Gheena S, Jain RK. Prevalence of oral mucocele among outpatients at saveetha dental hospital, india. Bioinformation. 2020 Dec 31;16(12):1013–8.
6. Chen L, Chen Y, Xie X, Zhu Z, Zhang Y, Dong Y, et al. A bilayer hydrogel enables spatiotemporal delivery of distinct hepcidin forms to reprogram macrophage responses for repair of infected bone defect. J Control Release. 2026 May 8;115008.
7. Pradeep M, Balakrishnan N, Arvind TRP. Prevalence of usage of various removable appliances among undergraduate dental students in a private dental college. J Adv Pharm Technol Res. 2022 Dec;13(Suppl 2):S559–62.
8. Xie Q, Jiao H, Cao F, Ge B, Yi P, Song L, et al. Fabrication and evaluation of a peptide-magnesium bifunctional coating on 3D printed porous tantalum scaffolds for enhanced bone regeneration. Colloids Surf B Biointerfaces. 2026 May 7;266:115790.
9. Xu N, Xu Y, Ma W, Yang F. Bifunctional scaffolds: strategic bridges coordinating osteosarcoma therapy and bone regeneration. Front Bioeng Biotechnol. 2026 Apr 24;14:1825326.
10. Nakkaragundi MS, Kerketta AH, Jawahar PP, Jain K, Satapathy D, Singh A. Advances in Musculoskeletal Tissue Biology and Regenerative Techniques: Innovative Approaches to Bone Repair and Cartilage Regeneration in Orthopedic Surgery. Cureus. 2026 Apr;18(4):e106623.
11. Tarquino González S, Carbonell Medina B. Design and Standardization of a Mandibular Injury Model for the Study of Craniofacial Tissue Regeneration in the Ambystoma mexicanum Model. J Vis Exp [Internet]. 2026 Apr 24;(230). Available from: http://dx.doi.org/10.3791/69895
12. Cheng C, Qiang L, Yue G. Synergistic antibacterial and osteogenic activities of GO/GelMA hydrogel for regenerating periodontal bone defects. Front Bioeng Biotechnol. 2026 Apr 23;14:1744639.
13. Aliabadi MM, Jahani A, Moradi A, Jirofti N. Magnesium and Strontium-Doped Bioglass Nanoparticles as a Novel Formulation: Advancing Osteoconductivity and Enhancing Bone Healing Potential. Small Sci. 2026 Apr;6(4):e70263.
14. Dong Y, Zhang S, Xu T, Sun W, Liu X. Advances of piezoelectric biomaterials in bone defect repair: The role of direct and inverse piezoelectric effect. J Orthop Translat. 2026 May;58:101106.