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Formulation Of Lyophilized Membrane Using Cnt/Hap For Soft Tissue Regeneration

Pavithra Sekhar[1], Dr. Bhargavi[2]

[1] Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai – 600077, India

Email: pavithrasekhar97@gmail.com

[2] Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai – 600077, India.

Address: No. 162, Poonamallee High Road, Velappanchavadi, Chennai – 600077, India.

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ABSTRACT

Soft tissue regeneration remains a major challenge in regenerative medicine and periodontal therapy. Biomaterial-based scaffolds capable of supporting cellular adhesion, proliferation, and differentiation are essential for effective tissue repair. Carbon nanotubes (CNTs) possess remarkable mechanical strength, electrical conductivity, and nanoscale architecture, making them attractive candidates for tissue engineering applications. Hydroxyapatite (HAp), a calcium phosphate compound similar to the mineral component of bone, is widely used due to its excellent bioactivity and biocompatibility.

In the present study, a lyophilized composite membrane composed of CNT and HAp was formulated using sodium alginate as a polymer matrix. The CNTs were acid-treated and dispersed within the alginate solution followed by incorporation of HAp to form a composite scaffold. The resulting membrane was subjected to lyophilization to obtain a porous structure suitable for tissue regeneration.

Morphological characterization was performed using scanning electron microscopy (SEM), which revealed a porous and interconnected microstructure favorable for cell attachment. ATR-FTIR spectroscopy confirmed the presence of functional groups associated with CNT, alginate, and HAp within the composite membrane. Cell viability assays demonstrated approximately 96% cell viability, indicating good cytocompatibility of the scaffold.

These results suggest that the CNT/HAp lyophilized membrane possesses promising structural and biological properties for soft tissue regeneration and tissue engineering applications..

INTRODUCTION

Soft tissue regeneration remains a major challenge in periodontal and oral reconstructive procedures due to the limited regenerative capacity of damaged tissues and the need for biomaterials that can effectively support cell proliferation and tissue healing. Recent advances in tissue engineering have focused on the development of scaffold-based regenerative materials that mimic the extracellular matrix and provide a favorable microenvironment for cellular attachment, growth, and differentiation [1,2].

Carbon nanotubes (CNTs) have attracted considerable attention in biomedical applications owing to their exceptional mechanical strength, high surface area, biocompatibility, and ability to enhance cellular interactions. CNT-based scaffolds have demonstrated promising results in tissue engineering by promoting cell adhesion and proliferation while improving the mechanical stability of regenerative constructs [3,4]. However, the use of CNTs alone may be insufficient to provide the bioactive cues required for optimal tissue regeneration.

Hydroxyapatite (HAP), a naturally occurring calcium phosphate mineral, is widely used in regenerative medicine because of its excellent biocompatibility, bioactivity, and osteoconductive properties. HAP has been extensively investigated for its ability to support tissue integration and facilitate cellular responses necessary for tissue repair and regeneration [5,6]. The incorporation of hydroxyapatite into composite scaffolds has been reported to enhance biological performance and improve the regenerative potential of biomaterials..

The combination of CNTs and HAP has emerged as a promising strategy for the development of multifunctional scaffolds with superior mechanical and biological characteristics. CNT/HAP composites provide a synergistic effect by combining the mechanical reinforcement offered by CNTs with the bioactive properties of hydroxyapatite, thereby creating an environment conducive to tissue regeneration [7,8].

Lyophilization is a widely employed fabrication technique in tissue engineering that produces highly porous three-dimensional scaffolds with interconnected pore structures. Such porous architectures facilitate nutrient diffusion, cellular infiltration, and vascularization, all of which are essential for successful tissue regeneration [9]. Therefore, the formulation of a lyophilized CNT/HAP membrane may represent a novel and effective approach for soft tissue regeneration.

The present study aimed to formulate and characterize a lyophilized membrane composed of carbon nanotubes and hydroxyapatite and to evaluate its potential application as a scaffold for soft tissue regeneration through morphological, physicochemical, and biological assessments [10].

2. Aim

To formulate and characterize a lyophilized membrane composed of carbon nanotubes and hydroxyapatite for soft tissue regeneration applications.

Materials and Methods :

2.1 Materials

Multi-walled carbon nanotubes (CNTs), hydroxyapatite (HAp) powder, and sodium alginate were used for the preparation of the composite membrane. All reagents used in this study were of analytical grade and used without further purification. Deionized water was used as the solvent for preparing all solutions.

2.2 Preparation of CNT and HAp Dispersion

To ensure uniform distribution within the polymer matrix, CNT and hydroxyapatite particles were first dispersed in the selected solvent. The CNTs were subjected to dispersion using ultrasonication, which helps break down nanotube agglomerates and improves their distribution in solution.

Hydroxyapatite powder was then added to the CNT dispersion under continuous stirring. The mixture was further sonicated to obtain a homogeneous suspension, ensuring proper interaction between CNT and HAp particles. Achieving uniform dispersion is critical for enhancing the structural and functional properties of the final composite membrane.

2.3 Preparation of Polymer–Nanocomposite Mixture

A sodium alginate solution was prepared separately by dissolving sodium alginate in deionized water under continuous magnetic stirring until a clear and viscous polymer solution was obtained.

The previously prepared CNT/HAp dispersion was gradually added to the sodium alginate solution. The mixture was stirred continuously to ensure homogeneous distribution of nanoparticles within the polymer matrix. Mechanical stirring and sonication were used to prevent particle aggregation and maintain uniformity in the composite solution.

The resulting mixture formed a CNT/HAp–alginate nanocomposite solution suitable for membrane fabrication.

2.4 Membrane Casting

The homogeneous nanocomposite solution was cast into molds of the desired shape and dimensions. In this study, petri dishes were used as casting molds to obtain thin membrane sheets.

The solution was carefully poured into the molds to ensure uniform thickness across the membrane surface. The casting step allowed the polymer–nanocomposite mixture to spread evenly and form a continuous film prior to the freezing process.

2.5 Freezing Process

After casting, the samples were subjected to controlled freezing to solidify the structure. The membranes were placed in a low-temperature environment to allow the formation of ice crystals within the polymer matrix.

The freezing process plays a crucial role in determining the internal structure of the scaffold. The formation of ice crystals creates interconnected pores, which later become permanent pores after solvent removal. These pores are essential for facilitating cell infiltration and nutrient diffusion within the scaffold.

2.6 Lyophilization (Freeze-Drying)

The frozen membranes were subjected to lyophilization (freeze-drying) to remove the solvent through sublimation under reduced pressure. During this process, the frozen solvent transitions directly from the solid phase to the vapor phase without passing through the liquid state.

Lyophilization preserves the porous architecture generated during freezing and results in a lightweight, highly porous membrane structure. This method is widely used in tissue engineering scaffold fabrication because it produces interconnected pores that enhance biological performance.

2.7 Morphological Characterization

The surface morphology and microstructural characteristics of the lyophilized membrane were examined using Scanning Electron Microscopy (SEM). Before imaging, the samples were coated with a thin conductive layer to prevent charging during electron beam exposure.

SEM images were recorded to evaluate pore distribution, surface topography, and structural integrity of the composite membrane.

2.8 ATR-FTIR Spectroscopic Analysis

The chemical composition and functional groups present in the composite membrane were analyzed using Attenuated Total Reflectance–Fourier Transform Infrared (ATR-FTIR) spectroscopy.

Spectra were recorded in the range of 4000–500 cm^{-1} to identify characteristic peaks corresponding to hydroxyl groups, phosphate groups, and carbon-based functional groups. This analysis helps confirm the successful incorporation of CNTs and hydroxyapatite within the polymer matrix.

2.9 Biocompatibility Assessment

The biocompatibility of the fabricated membrane was evaluated through in vitro cell culture studies. Cells were seeded onto the surface of the CNT/HAp membrane and cultured under standard conditions.

Cell adhesion, spreading, and proliferation were observed using microscopic imaging. The cytocompatibility of the scaffold was further assessed by performing a cell viability assay, and the results were compared with a control group.

This evaluation helps determine whether the composite membrane provides a favorable environment for cellular growth and tissue regeneration.

2.10 Statistical Analysis

All experiments were performed in triplicate. The obtained data were analyzed and expressed as mean ± standard deviation. Statistical comparisons between control and experimental groups were conducted where applicable.

Results :

3.1 Morphological Characterization of Lyophilized CNT/HAp Membrane (SEM)

The surface morphology and structural characteristics of the fabricated lyophilized CNT/HAp membrane were analyzed using scanning electron microscopy (SEM). The SEM micrographs revealed a porous and interconnected microstructure formed during the lyophilization process. Such porous architecture is desirable for tissue engineering scaffolds because it facilitates nutrient transport, cellular infiltration, and extracellular matrix deposition.

The CNT/HAp composite membrane exhibited a rough surface with distributed micro-pores, which can enhance cellular attachment and proliferation. The presence of CNTs within the matrix may contribute to improved structural stability and reinforcement of the scaffold. Additionally, the interconnected porous network created during freeze-drying provides an appropriate microenvironment for tissue regeneration.

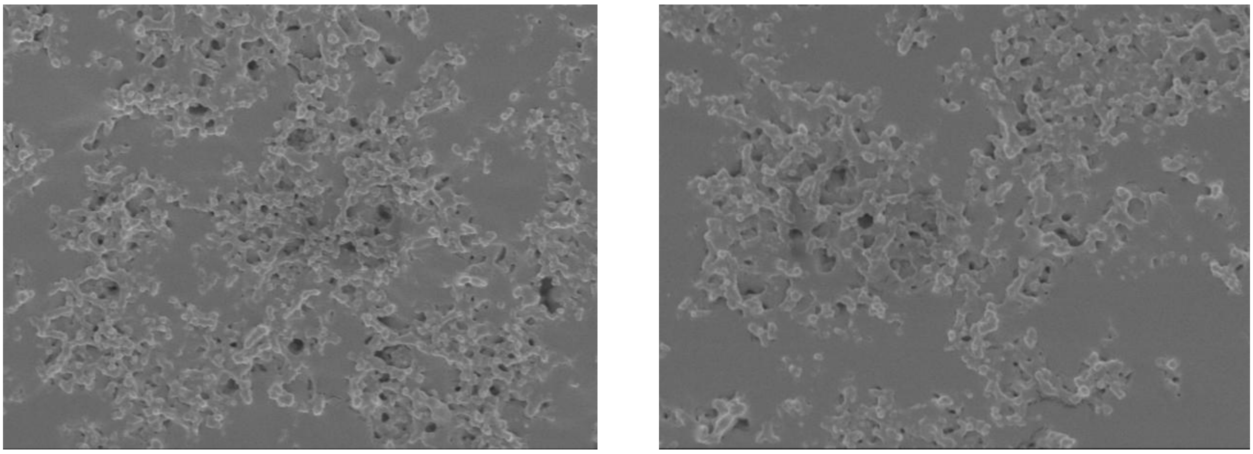

Figure 1. Scanning electron microscopy (SEM) images showing the morpohological and topographical characterization of the lyophilized CNT/HAp membrane at a scale of 0.5 µm. The micrographs demonstrate a porous and interconnected structure formed during the lyophilization process, which is beneficial for cell attachment, nutrient diffusion, and tissue regeneration.

3.2 ATR-FTIR Analysis

ATR-FTIR spectroscopy was performed to identify the functional groups present in the CNT/HAp composite membrane and to confirm the successful incorporation of its components. The FTIR spectrum showed characteristic peaks corresponding to hydroxyl (–OH), phosphate (PO_4^{3-}), and carbonate groups, which are typically associated with hydroxyapatite. Peaks corresponding to carbon-based functional groups were also observed, confirming the presence of carbon nanotubes within the composite matrix.

The broad absorption band observed around **3200–3500 cm^{-1}** corresponds to hydroxyl groups, which may arise from both alginate and hydroxyapatite components. The peaks near **1000–1100 cm^{-1}** are attributed to phosphate stretching vibrations of hydroxyapatite. Additional peaks observed in the spectrum indicate interactions between CNTs, hydroxyapatite, and the polymeric matrix, suggesting successful composite formation.

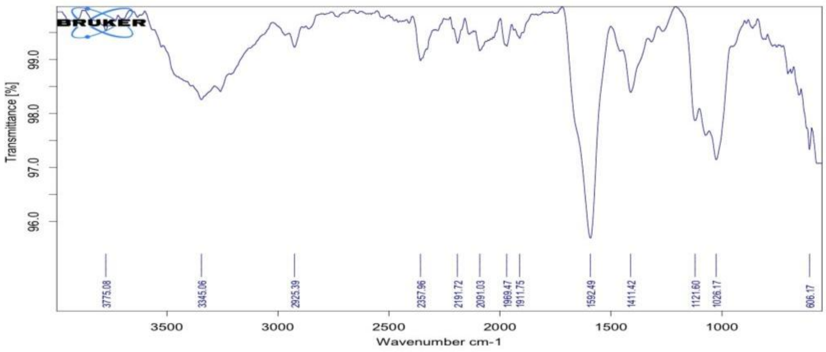

Figure 2. ATR-FTIR spectrum of the lyophilized CNT/HAp membrane showing characteristic functional groups associated with carbon nanotubes, hydroxyapatite, and the alginate matrix. The presence of hydroxyl and phosphate groups confirms the successful formation of the composite scaffold.

3.3 Cell Attachment and Viability

The cytocompatibility of the fabricated CNT/HAp membrane was evaluated by observing cell morphology and measuring cell viability compared with the control group. Microscopic observation showed that cells were able to attach and spread on the surface of the composite scaffold, indicating that the material provides a suitable environment for cellular growth.

Cells cultured on the CNT/HAp membrane exhibited elongated morphology and clear attachment patterns similar to those observed in the control group. This suggests that the composite scaffold does not exhibit cytotoxic effects and supports normal cellular behavior.

Quantitative analysis of cell viability demonstrated approximately **96% viability compared with the control**, indicating good cytocompatibility of the scaffold. The high viability suggests that the CNT/HAp membrane can support cellular proliferation and may serve as a promising scaffold for soft tissue regeneration

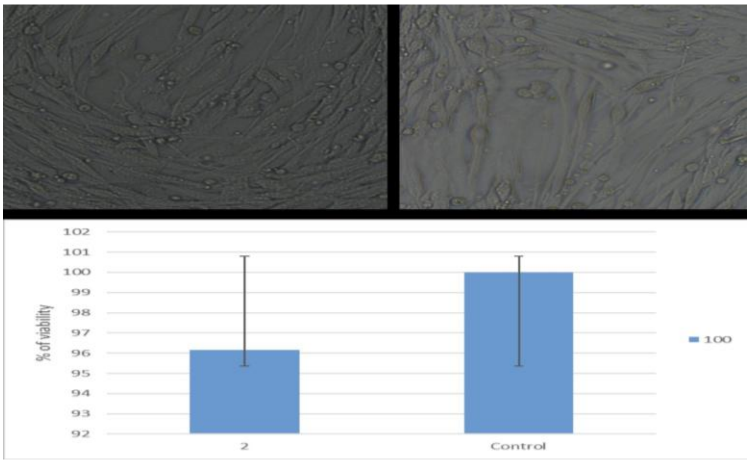

Figure 3. Cell attachment and viability analysis of the CNT/HAp membrane. (A) Microscopic image of cells in the control group. (B) Cells cultured on the CNT/HAp composite membrane showing good attachment and spreading.

(C) Quantitative analysis of cell viability indicating approximately 96% viability compared with the control group.

Discussion :

The present study successfully formulated a lyophilized membrane incorporating carbon nanotubes and hydroxyapatite for potential application in soft tissue regeneration. The combination of CNTs and HAP resulted in a composite scaffold exhibiting desirable structural and biological characteristics, supporting the concept that nanocomposite biomaterials can provide an effective platform for tissue engineering applications [11,12].

SEM analysis demonstrated a porous surface morphology within the lyophilized membrane. Scaffold porosity is a critical parameter in tissue engineering because it facilitates cell attachment, migration, nutrient transport, and waste removal. Previous studies have reported that highly porous lyophilized scaffolds promote enhanced cellular infiltration and tissue integration, thereby improving regenerative outcomes [13,14]. The porous architecture observed in the present study suggests that the CNT/HAP membrane may provide a favorable microenvironment for soft tissue regeneration.

The FTIR analysis confirmed the presence of characteristic functional groups associated with both carbon nanotubes and hydroxyapatite, indicating successful incorporation of the components within the composite scaffold. Similar observations have been reported in previous investigations where FTIR spectroscopy was used to verify the formation of CNT/HAP nanocomposites and evaluate interactions between constituent materials [15]. The preservation of these functional groups is important for maintaining the biological and physicochemical properties of the scaffold.

Carbon nanotubes are known to enhance the mechanical performance of biomaterials through their high tensile strength and elastic modulus. The incorporation of CNTs within scaffold matrices has been shown to improve structural integrity and resistance to deformation while simultaneously supporting cellular adhesion and proliferation [16,17]. In the present study, CNTs likely contributed to the stability and reinforcement of the lyophilized membrane, making it suitable for regenerative applications.

Hydroxyapatite plays a significant role in enhancing the biological performance of scaffolds by providing bioactive surfaces that promote cell attachment and tissue integration. Previous studies have demonstrated that HAP-containing composites exhibit improved cellular responses and regenerative potential compared with non-bioactive materials [18]. The incorporation of hydroxyapatite within the CNT matrix may therefore have contributed to the favorable cell-material interactions observed in this study.

Cell viability assessment demonstrated approximately 96% viability in cells attached to the CNT/HAP membrane, indicating excellent biocompatibility of the fabricated scaffold. High cell viability is an essential prerequisite for successful tissue engineering applications, as it reflects the ability of the material to support cellular survival and proliferation without inducing cytotoxic effects. Comparable findings have been reported for CNT-based and HAP-based composite scaffolds used in regenerative medicine, where high levels of cellular compatibility were observed following scaffold implantation or in vitro testing [19,20].

The synergistic interaction between CNTs and hydroxyapatite may explain the favorable biological performance observed in the present study. CNTs provide mechanical reinforcement and increased surface area for cell attachment, whereas hydroxyapatite contributes bioactivity and cellular signaling cues. Together, these characteristics create a multifunctional scaffold capable of supporting soft tissue regeneration and tissue engineering applications [21].

Despite the promising findings, the present investigation was limited to in vitro characterization and biological evaluation. Further studies involving animal models and clinical trials are necessary to assess long-term biocompatibility, degradation behavior, regenerative efficacy, and clinical applicability. Additionally, future research may focus on incorporating stem cells, growth factors, or therapeutic agents into the CNT/HAP membrane to further enhance its regenerative potential [22].

Conclusion :

The present study successfully formulated a lyophilized CNT/HAp composite membrane using sodium alginate as a polymer matrix. SEM analysis revealed a porous microstructure suitable for cellular infiltration, while ATR-FTIR spectroscopy confirmed the presence of functional groups corresponding to the composite components. The membrane demonstrated high cytocompatibility with approximately 96% cell viability, indicating its potential for biomedical applications.

These findings suggest that the CNT/HAp lyophilized membrane may serve as a promising scaffold for soft tissue regeneration, tissue engineering, and regenerative medicine applications.

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REFERENCES

1. Sumio Iijima (1991). Helical microtubules of graphitic carbon. Nature.
2. Robert Langer and Joseph Vacanti (1993). Tissue engineering. Science.
3. Benjamin S. Harrison and Anthony Atala (2007). Carbon nanotube applications for tissue engineering. Biomaterials.
4. Dorozhkin, S. V. (2010). Bioceramics of calcium orthophosphates. Biomaterials.
5. O'Brien, F. J. (2011). Biomaterials and scaffolds for tissue engineering. Materials Today.
6. Wang, H., Chu, C., Cai, R., et al. (2015). Synthesis and bioactivity of gelatin/MWCNT/hydroxyapatite nanofibrous scaffolds for bone tissue engineering. RSC Advances.
7. Rajesh, R., et al. (2015). Development of CNT–alginate–hydroxyapatite composite scaffold using freeze-drying technique. Journal of Biomedical Materials Research.
8. Kaur, G., et al. (2014). Carbon nanotube reinforced hydroxyapatite nanocomposites for bone tissue engineering. Journal of Biomedical Nanotechnology.
9. Li, X., et al. (2019). Nanomaterials for tissue engineering scaffolds. Journal of Biomedical Materials Research.
10. Zhang, Y., et al. (2017). Carbon nanotube reinforced collagen/hydroxyapatite scaffolds for bone regeneration. Biomaterials.
11. Wang, W., et al. (2019). Nanocomposite biomaterials for regenerative medicine applications. Advanced Healthcare Materials.
12. Gupta, A., et al. (2016). Nanocomposite scaffolds in bone tissue engineering. Journal of Tissue Engineering.
13. Li, M., et al. (2018). Hydroxyapatite-based nanomaterials for biomedical applications. Acta Biomaterialia.
14. Zhang, L., et al. (2020). Carbon nanotube reinforced polymer scaffolds for regenerative medicine. Materials Science and Engineering C.
15. Chen, Q., et al. (2018). Nanocomposite biomaterials for bone tissue regeneration. Progress in Polymer Science.
16. Gaharwar, A. K., et al. (2014). Nanocomposite hydrogels for tissue engineering. Biotechnology Advances.
17. Kim, H. W., et al. (2005). Hydroxyapatite/polymer composite scaffolds for tissue engineering. Journal of Biomedical Materials Research.
18. Bose, S., Roy, M., & Bandyopadhyay, A. (2012). Recent advances in bone tissue engineering scaffolds. Trends in Biotechnology.
19. Liu, X., et al. (2013). Nanostructured biomaterials for tissue engineering. Biomaterials.
20. Wang, X., et al. (2023). Additive manufacturing of PLA/CNT/HAp scaffolds for cell proliferation. Scientific Reports.
21. Singh, R., et al. (2018). Freeze-dried scaffolds for tissue regeneration. Journal of Biomaterials Science.
22. Ma, P. X. (2004). Scaffolds for tissue fabrication. Materials Today.
23. Hollister, S. J. (2005). Porous scaffold design for tissue engineering. Nature Materials.
24. Liu, H., & Webster, T. J. (2007). Nanomaterials for tissue engineering applications. Biomaterials.
25. Vallet-Regí, M., et al. (2015). Nanostructured hydroxyapatite biomaterials for bone regeneration. Chemical Society Reviews.
26. Khang, D., et al. (2008). Carbon nanotube based scaffolds for tissue regeneration. Nano Letters.
27. Liu, Y., et al. (2019). Carbon nanomaterial-based hydrogels for tissue engineering. International Journal of Nanomedicine.
28. Rahaman, M. N., et al. (2011). Bioactive glass in tissue engineering. Acta Biomaterialia..