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GELATIN HYDROXYAPPATITE 3D POROUS SCAFFOLD FOR REGENERATIVE DENTISTRY

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Abstract

Three-dimensional (3D) printed scaffolds have emerged as a promising alternative to traditional bone transplants for the regeneration of alveolar bone lesions. This scoping study aims to explore the effects of 3D-printed scaffolds utilizing hydroxyapatite (HA) and calcium phosphates on alveolar bone regeneration in animal models. Biomaterials such as hydroxyapatite and tricalcium phosphates have been extensively employed in the creation of these scaffolds. The study highlights the urgent need for scaffolds that combine high particle content and ductility for effective bone tissue engineering. To address this, a gradient 3D porous gelatin scaffold with hydroxyapatite is fabricated, with the ultimate goal of its application in regenerative dentistry. The physiochemical properties of the scaffold are also thoroughly characterized.

Keywords: Three-Dimensional Printed Scaffolds, Alveolar Bone Regeneration, Hydroxyapatite, Calcium Phosphates, Biomaterials, Bone Tissue Engineering, Gelatin Scaffold, Regenerative Dentistry, Physiochemical Properties.

INTRODUCTION

Hydroxyapatite (HA), a calcium phosphate ceramic, has garnered significant attention in the field of bone research due to its similarity to bones and teeth in terms of structure, function, and chemical makeup(Lin and Chang 2015). In fact, up to 70% of the inorganic component of human bone is comprised of HA, with 25% organic matter and 5% water(Du, Chen et al. 2021). Studies have shown that hydroxyapatite exhibits strong cell affinity, promoting the adhesion, proliferation, and integration of osteoblasts for direct bone formation. The use of natural bone for repairing bone defects presents numerous challenges, leading researchers to explore suitable artificial bone materials(Vallet-Regi and González-Calbet 2004). These artificial bone substitutes are designed to closely mimic the biological properties of natural bone. Various synthetic materials, including organic polymers, metals, and glass ceramics, have been developed as potential alternatives(Balhuc, Campian et al. 2021). Nanomaterials, characterized by their dimensions being less than 100 nm in at least one direction, have demonstrated superior qualities compared to conventional materials in orthopedic-related research. It has been suggested that nanoparticles may enhance osseointegration, which is crucial for the long-term effectiveness of implants(Kareem, Bulut et al. 2024). The treatment of impaired bones resulting from diseases, trauma, or tumor resection poses a significant challenge for clinicians and researchers. Traditional approaches involve using autografts for critical size bone defects, but they come with risks such as donor site morbidity and limitations due to donor shortage. Bone tissue engineering has emerged as a promising field for managing critical size bone defects by utilizing biocompatible materials and exploring novel stem cell sources(Ibrahim, Youness et al. 2024).

Anomalies in the maxillofacial bone, caused by factors such as trauma, tumors, inflammation, and congenital deformities, can significantly impact the functionality and aesthetics of the maxillofacial region(Iyer, Hariharan et al. 2021). Achieving perfect bone regeneration in this region is particularly challenging due to its irregular shape, intricate structure, and unique biological activities(Jimson 2021). Although biomaterial scaffolds have shown success in clinical applications, there is a need for scaffolds that cater specifically to the diverse requirements of maxillofacial bone regeneration(RAILEAN, GUDUMAC et al. 2023). Addressing these requirements is essential for maximizing bone regeneration capacity and improving patient care. Advancements in 3D printing technologies have allowed for the creation of scaffold structures that have the potential to promote endogenous tissue regeneration(Ghantous, Nashef et al. 2020). However, the specific variables that contribute to tissue regeneration through 3D printed scaffolds are still not fully understood. Researchers have been working on developing computer tools to optimize scaffold designs, aiming to unlock the full potential of 3D printing in tissue regeneration(Gundu, Varshney et al. 2022). The widespread application of 3D printing technology in bone defect repair, diagnosis, and rehabilitation has opened up new markets for promising businesses. With the integration of materials science, tissue engineering, and digital medicine, 3D printing technology has facilitated the creation of products with stable mechanical properties, good osteoinductive potential, and excellent biocompatibility(Borrelli, Hu et al. 2020). The combination of different biological materials and 3D printing techniques has led to the development of patient-specific bioactive scaffolds for the clinical application of bone defect healing. The microstructure of these 3D printed scaffolds plays a crucial role in meeting the intricate requirements of bone defect repair and individualized patient care(Stefanelli, Mundada et al. 2018).

Nano-hydroxyapatite (nHAp) has recently garnered significant interest due to its wide range of therapeutic, regenerative, and preventive applications(Pirttiniemi, Peltomäki et al. 2009). HA, a widely used mineral in remineralization, tissue engineering, dentinal hypersensitivity treatment, and periodontal bone regeneration, has proven to be effective(Gholamalizadeh, Nazifkerdar et al. 2023). Synthetic hydroxyapatite (HAP), with its fixed calcium to phosphorus ratio (Ca:P) of 1.67, is the most commonly used calcium phosphate bioceramic in biomedical applications such as bone scaffolds, bone fillers, implant coatings, and drug delivery systems(Velasquillo, Madrazo-Ibarra et al. 2020). HAP can be produced through various techniques, including dry, wet, and thermal fabrication methods, or extracted from natural sources such as corals or animal bones, or synthesized from chemical precursors. The varied crystalline phases, sizes, and morphologies of calcium phosphates formed through these processes provide distinct properties to HAP, such as bioactivity, bioabsorbability, and biocompatibility. In the development of bone substitutes, it is crucial to ensure that their compositions closely resemble those of the original bone. Natural bone consists of both organic and mineral elements, such as type I collagen and hydroxyapatite. The triple helix structure of collagen fibrils serves as a nucleation site for HA deposition. However, the limited solubility of traditional HA grafts hinders their degradation and replacement with new bone. By reducing HA particles to nanosize, nano-hydroxyapatite (nHA) agglomerates can be readily absorbed and resorbed by cells. Nanoparticles exhibit higher surface activities and a greater specific surface area

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compared to bulk materials. Natural polymers like collagen, cellulose, alginate, and gelatin, known for their proven biocompatibility, are commonly used in various applications. Synthetic polymers, on the other hand, are preferred for their minimal immunological impact(Khojasteh and Hosseinpour 2020). Gelatin, despite being inexpensive and promoting cell growth and differentiation, has limited mechanical strength, hindering its widespread use in the regeneration of hard tissues. Efforts have been made to enhance the mechanical properties of gelatin by blending it with other synthetic polymers or bioceramics. In conclusion, hydroxyapatite and its nanoscale counterparts, along with the development of artificial bone substitutes, tissue engineering, 3D printing technologies, and the application of nano-hydroxyapatite, have revolutionized the field of bone regeneration. With ongoing research and advancements, the potential for improving bone defect repair and patient care continues to expand(Hernandez, Sánchez et al. 2020).

MATERIALS ANS METHODS

In this method, the formulation of Nanohydroxyapatite (nHAP) particles began by adding specific quantities of various chemicals(Damiri, Fatimi et al. 2024). Sodium chloride (NaCl) was added at a concentration of 6.547 g/L, followed by sodium bicarbonate (NaHCO3) at 2.268 g/L, potassium chloride (KCl) at 0.373 g/L, disodium hydrogen phosphate (Na2HPO3) at 0.141 g/L, magnesium chloride (MgCl2.6H2O) at 0.305 g/L, and 15 ml of hydrogen chloride (HCl). Additionally, calcium chloride (CaCl2) was added at a concentration of 0.277 g/L, sodium sulfate (Na2SO4) at 0.071 g/L, and Tris(hydroxymethyl)aminomethane (CH2OH)3 CNH2 at 6.057 g/L. Once all the chemicals were added, they were thoroughly mixed to prepare the solution. Following this, 920 ml of calcium chloride solution was added. The gas foaming technique was employed to induce porosity in the 3D scaffolds. This technique allowed for the creation of a gradient of mineral content and porosity. After the nanoparticles were formulated, they underwent a thorough washing process, which involved being washed six times. The particles were then filtered using filter paper and incubated for three hours(Pinchuk, Sobierajska et al. 2024). To complete the drying process, the filtered particles were placed in an oven set at 80 degrees Celsius.

The methodology employed in this study aimed to achieve a gradient of mineral content and porosity in the 3D scaffolds. It was crucial to confirm the presence of the nano-sized particles in the scaffold. In order to validate this, the researchers conducted

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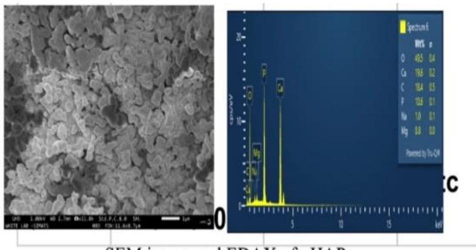
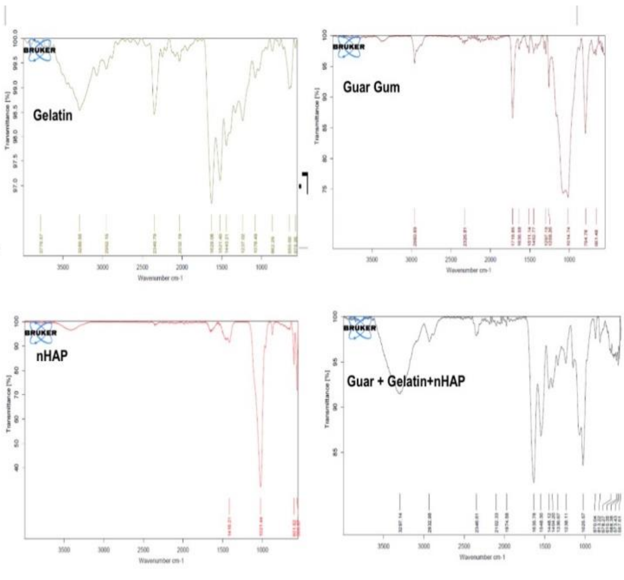
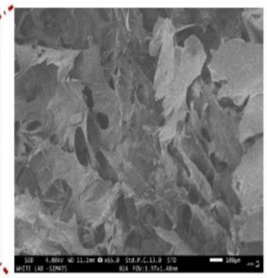
an analysis using infrared (IR) spectroscopy. The IR spectra provided evidence of the presence of nanohydroxyapatite (nHAP) in the developed 3D gradient scaffold. This methodology allowed for the controlled formulation of nanohydroxyapatite particles and the subsequent development of a 3D gradient scaffold with desired mineral content and porosity. The confirmation of the presence of nHAP in the scaffold through IR spectroscopy demonstrated the success of the fabrication process.

RESULTS


SEM image and EDAX of nHAP




Porous scaffold with nHAP


SEM image of nanoparticle incorporated porous scaffold

IR Spectra of A) Gelatin; B) Guar Gum; C) nHAP and D) 3D porous Hydrogel

The porous scaffold showed the porous densities, where it was indicated that where the porous scaffolds are high is where there is an increase in porosity and where the low-density scaffold region showed more mineral content present in it. The SEM image and the EDAX of the hydroxyapatite nano particles were taken and analyzed. The IR spectra of Gelatin, Guar gum, nHAP and a combination of them were analyzed.

DISCUSSION

In recent years, the field of additive manufacturing (AM) has played a crucial role in fabricating bone scaffolds. These scaffolds are essential for various medical applications, such as the repair and reconstruction of the upper or lower jaw(Ambika, Manojkumar et al. 2019, Chockalingam, Sasanka et al. 2020). The success of these fabrication methods heavily relies on the acquisition and processing of digital data representing the target tissue(Yuvaraj, Sangeetha et al. 2020). Researchers have recognized the significance of scaffold internal microstructures, leading to a growing focus on studying and optimizing the data collection and processing techniques. Several techniques have been explored to create porous bone scaffolds, including chemical/gas foaming, solvent casting, particle/salt leaching, freeze drying, thermally induced phase separation, and foam-gel methods(Balaji, Bhuvaneswari et al. 2022). However, these techniques have limitations in controlling pore size, shape, and interconnectivity(Akshaya and Ganesh 2022). Moreover, they often lack the ability to build scaffolds with customized porosity to address specific flaws. Additive manufacturing techniques, such as rapid prototyping (RP), solid freeform fabrication

(SFF), and 3D printing (3DP), have emerged as promising solutions. These techniques enable the direct manufacture of complex scaffold geometries from computer-aided design (CAD) files, offering greater control and customization options(Velumani, Arasu et al. 2023).

To evaluate the effectiveness of bone scaffolds, clinical studies involving patients who underwent microsurgical repair of the upper or lower jaw following resection were conducted. The study included thirty eligible patients with conditions such as osteomyelitis, alveolar atrophy, or malignant tumors(Velumani, Arasu et al. 2023). The focus of the study was on radiologic follow-up and clinical assessments to identify implant-related problems. These assessments involved gathering and analyzing both clinical and radiologic data to gain insights into the long-term performance of the scaffold implants. In one specific case, a 66-year-old male patient underwent mandibular reconstruction using osteotomized FFF (freeform fabrication) to remove a tumor. Prior to the surgery, a CT-based 3D model of the patient's jaw was used to pre-bend a universal titanium fixation plate. This plate, along with eleven titanium screws, was then used to fix the monocortical. Follow-up CT scans were performed at 4, 16, and 28 months after the surgery to monitor the progress and evaluate the effectiveness of the reconstruction.

In order to examine the morphology of collagen sponges treated with 80 weight percent of HA (hyaluronic acid), scanning electron microscopy (SEM) was utilized(Anil, Ibraheem et al. 2022). The freeze-drying process was employed, and the samples were analyzed using SEM at an accelerating voltage of 15–20 kV. This analysis provided valuable insights into the structural properties of the collagen sponges and the effects of the HA treatment. In a different approach, scales from tilapia fish (*Oreochromis mossambicus)* were used as a potential source for scaffold fabrication. The scales were carefully collected and rinsed to remove any adhered particles(Seyedsajadi and Fathi 2019). To eliminate exterior hyaluronic acid and proteins, the scales underwent treatment with a NaOH solution. After thorough cleaning and baking, the scales were calcined in air(Divya Sri, Vishnu Priya et al. 2020, Lakrat, Jabri et al. 2022). This approach highlights the exploration of alternative materials for scaffold fabrication. Overall, these discussions shed light on the importance of acquiring and processing digital data for fabricating bone scaffolds using additive manufacturing techniques. The limitations of existing methods and the potential of AM techniques in achieving customized scaffolds have been highlighted. The clinical studies and radiologic assessments provide valuable insights into the long-term performance of the scaffold implants. Additionally, the utilization of SEM analysis and the exploration of alternative materials demonstrate the ongoing research efforts to enhance scaffold fabrication and improve patient outcomes.

CONCLUSION

In conclusion, the nano hydroxyapatite (HAP) gradient 3D porous scaffold represents a promising advancement in the field of dentistry. By integrating the remarkable properties of gelatin and hydroxyapatite, this scaffold demonstrates great potential for various applications within the dental industry. Firstly, the biocompatibility of gelatin ensures that the scaffold is well-tolerated by the human body, reducing the risk of adverse reactions or complications. Additionally, gelatin possesses regenerative properties, allowing for tissue repair and regeneration, which is crucial in dental procedures such as bone grafting and implant placement.Furthermore, the incorporation of hydroxyapatite, the primary mineral found in natural bone tissue, enhances the scaffold's ability to mimic the structure and composition of the surrounding bone. This not only facilitates the integration of the scaffold with the host tissue but also promotes bone growth and osseointegration, leading to long-term stability and improved clinical outcomes. Overall, the nano hydroxyapatite (HAP) gradient 3D porous scaffold presents a promising solution for dental applications, offering a combination of biocompatibility, regenerative properties, and biomimetic characteristics. As research and development in this field continue to advance, it is expected that this scaffold will play a vital role in transforming the field of dentistry, revolutionizing treatment options, and improving patient outcomes.

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Conflict of Interest:

None to declare.

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