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Combination of gelatine -alginate hydrogel composite as a dual membrane for extra oral and Intraoral tissue repair.

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ABSTRACT

Background: Tissue repair and regeneration are critical aspects of healthcare, particularly in the fields of maxillofacial surgery and dentistry. Hydrogel-based materials have emerged as promising candidates for tissue engineering due to their biocompatibility and tunable properties. The repair of extraoral and intraoral tissues poses unique challenges, as they are exposed to different physiological environments and have distinct functional requirements. Gelatine and alginate are widely used biomaterials known for their biocompatibility, bioactivity, and biodegradability. By combining these materials into a hydrogel composite, it is possible to develop a dual membrane that can be applied to various tissue repair scenarios.

Aim: The present study focused on the synthesis of Combination of gelatine -alginate hydrogel composite as a dual membrane for extra oral and Intraoral tissue repair.

Materials and Methods: Gelatine preparation: Gelatine is extracted from collagen-rich sources such as porcine or bovine skin through a controlled heating and enzymatic process.; Alginate preparation: Alginate is derived from brown algae and is typically obtained as a powder. Composite formation: The gelatine and alginate are mixed at appropriate ratios to form a homogenous hydrogel composite. The crosslinking mechanism can be achieved through ionic gelation or covalent crosslinking, depending on the desired properties. Characterization: The physical, mechanical, and biological properties of the gelatine-alginate hydrogel composite are evaluated using techniques such as rheology, scanning electron microscopy (SEM), and cell culture studies.

Results: In FUNCTIONAL GROUP: The functional groups present in the network are carboxylate group, amide, C=O, NH3; HAEMOLYSIS: Haemolysis percentage is less than 1% which indicates high bio compatibility.; CONTACT ANGLE: The hydrogel exhibited an average contact angle of 31.02°, indicating high surface hydrophilicity.; SEM ANALYSIS: Scanning electron microscopy revealed a uniform and interconnected porous network structure with pores ranging from 50–200 µm, supporting cell adhesion and nutrient diffusion.

Conclusion: The combination of gelatine and alginate in a hydrogel composite offers a promising dual membrane system for extraoral and intraoral tissue repair. The composite's biocompatibility, mechanical strength, and dual functionality make it a suitable candidate for a wide range of applications in maxillofacial surgery and dentistry. Further research and optimization are required to fully exploit the potential of this innovative hydrogel composite for tissue engineering and regenerative medicine.

Keywords: Gelatine, Alginate, dual membrane, tissue repair.

INTRODUCTION

Enticing biomaterials called hydrogels are made of cross-linked polymeric networks that are three-dimensional (3D) and hydrophilic. These networks allow hydrogels to absorb and hold a lot of biological fluids [1] . Because they are degradable matrices, they are ideal for neo-tissue growth and can facilitate minimally invasive cell transplantation into the human body through injection [2] . Because hydrogels can have their properties customized through cross-linking techniques, they can be stimulated by changes in pH, temperature, metabolite concentration, osmotic pressure, and specific molecules like glucose or antigens [3] . Because of these remarkable properties, hydrogels are used in a wide range of applications, such as food additives, superabsorbents, wound dressings, biomedical implants, TE scaffolds, diagnostic components, drug delivery carriers, and biosensors [4] .

Significant structural and functional properties of proteins, such as their biocompatibility, biodegradability, abundance, and decreased capacity to elicit tissue inflammatory responses, make them ideal for the production of hydrogels [5] . Furthermore, proteins with inherent advantages for the development of hydrogels can be hydrogelized through the use of physical, chemical, and enzymatic treatments. All proteins can, in fact, form crosslinks, especially when amine and carboxylic acid functional groups are present [6] . Physical cross-linking, or the main regulating mechanism for the gelation process of proteins, is the aggregation of proteins into a gel network [7] . The resulting network keeps water inside its structure and can be stabilized by hydrogen bonds and non-covalent cross-links like hydrophobic, van der Waals, and electrostatic interactions [8] .

Alternative methods for the formation of PBHs include hydrogels with single network wrapping or multiple network superposition, as single-matrix hydrogels frequently display poor mechanical properties [9] . By using this technique, Tang and colleagues produced a polymer as the second matrix and a protein as the first using a protein misfolding strategy, in which heat was applied to the protein to cause its denaturation, aggregation, and gelation [10] .
The matrices were then superimposed to create a double-matrix PBH with greatly improved adhesion properties and mechanical features. The creation of the 3D network structure PBH through the creation of strong, resilient covalent bonds within its network is the primary objective of the chemical cross-linking process [11] . Several chemical cross-linking techniques have been reported, including chemical coupling [12] , click reactions [13] , and covalent cross-linking of specific chains and target residues on proteins [14] . This cross-linking technique yields improved mechanical properties under physiological conditions, enhanced stability, and a controllable degradation rate [15] . In addition, the source of covalent bonds may affect the structure of the hydrogels that are created. When polymers are present, proteins form covalent connections that result in the creation of hydrogels with novel properties [16] .
The combination of gelatine-alginate hydrogel composite as a dual membrane offers a promising solution for repairing both extra oral and intraoral tissues. Hydrogel biomaterials have shown immense potential in regenerative medicine and tissue engineering applications [17] . Gelatine, alginate, and fibrinogen, three natural polymers extensively used in regenerative medicine, are combined to form a gel-like membrane that closely mimics the extracellular matrix of native tissues [18] . One of the advantages of this dual membrane is its ability to support cell-based applications in cancellous bone [19] .
This combination is achieved through the co-precipitation of gelatine and alginate molecules, ensuring the structural integrity and shape fidelity of the printed hydrogel [20] . Furthermore, the addition of alginate to the gelatine hydrogel enhances its mechanical properties, making it more suitable for load-bearing musculoskeletal tissues [18] .
In addition, the gelatine-alginate hydrogel composite has been shown to promote stem cell proliferation and osteoblast differentiation, making it an ideal candidate for bone tissue regeneration and defect repair [21] . The incorporation of nano-attapulgite into the gelatine-alginate hydrogel composite further enhances its mineralization effect, promoting bone regeneration [22] . Overall, the combination of gelatine and alginate in a hydrogel composite offers great potential for dual membrane applications in extra oral and intraoral tissue repair, providing a versatile and effective solution in the field of regenerative medicine. By combining gelatine and alginate in a hydrogel composite, a dual membrane is created that closely mimics the extracellular matrix of native tissues, making it an ideal material for tissue repair and regeneration. The gelatine-alginate hydrogel composite serves as a dual membrane for both extra oral and intraoral tissue repair, offering unique advantages in terms of biocompatibility, structural integrity, mechanical properties, and mineralization effects.
This combination of gelatine and alginate in a hydrogel composite provides a versatile solution for tissue repair and regeneration in both extra oral and intraoral settings. The gelatine-alginate hydrogel composite offers a dual membrane for extra oral and intraoral tissue repair due to its biocompatibility, structural integrity, mechanical properties, and mineralization effects.

MATERIALS AND METHODS

Preparation of Gelatine Solution:

Gelatine was prepared from collagen-rich sources and dissolved in distilled water to obtain a 6% (w/v) solution. The solution was heated under controlled conditions with continuous stirring to ensure complete dissolution and homogeneity.

Preparation of Alginate Solution:

Sodium alginate, derived from brown algae, was prepared as a 6% (w/v) solution in distilled water. The mixture was stirred thoroughly until a uniform, lump-free solution was obtained.

Formation of Gelatine--Alginate Hydrogel Composite:

Equal volumes (50 mL each) of the prepared 6% gelatine and 6% alginate solutions were combined under constant stirring to form a homogeneous hydrogel mixture. The blending was carried out under controlled conditions to ensure uniform distribution and optimal interaction between polymer chains. Crosslinking was achieved through ionic interactions using 0.1 M calcium chloride ($CaCl_2$) solution, which was added dropwise to the mixture under continuous stirring. The crosslinked hydrogel was allowed to stabilize at 4°C for 24 hours to complete the gelation process.

Freeze-Drying Process:

The prepared hydrogel samples were subjected to freeze-drying at -80°C for 4 hours followed by lyophilization for 24 hours to obtain a porous and stable membrane structure suitable for tissue engineering applications.

Characterization Techniques:

The synthesized hydrogel composite was characterized using multiple analytical methods:
Scanning Electron Microscopy (SEM): to evaluate surface morphology and internal microstructure. Samples were sputter-coated with gold and imaged at an accelerating voltage of 10 kV.

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Fourier Transform Infrared Spectroscopy (FTIR): to identify functional groups and confirm interactions between gelatine and alginate. FTIR spectra were recorded in the range of 4000–400 cm^{-1} using a Bruker spectrometer.
Hemolysis Assay: to assess blood compatibility. Fresh human blood was diluted with saline and incubated with hydrogel samples at 37°C for 1 hour. Distilled water served as the positive control and saline as the negative control. After centrifugation, absorbance was measured at 540 nm to calculate the hemolysis percentage.
Contact Angle Measurement: to determine surface wettability and hydrophilicity of the membrane. A 5 µL droplet of deionized water was placed on the membrane surface, and the contact angle was measured using a goniometer (n=5).

RESULTS
The developed gelatine–alginate hydrogel composite demonstrated favorable structural, biological, and surface properties, supporting its potential application as a dual membrane for extraoral and intraoral tissue repair.

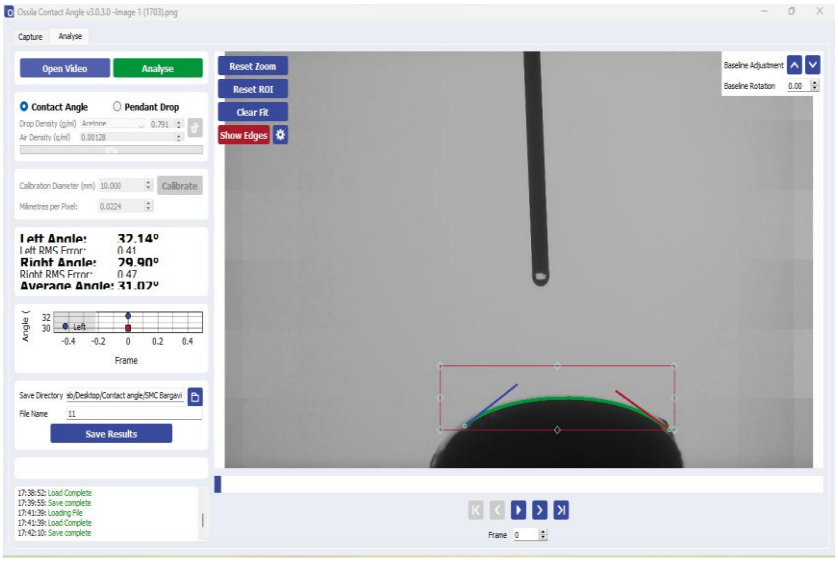

Figure 1: Contact Angle Measurement:

The hydrogel exhibited an average contact angle of 31.02°, indicating high surface hydrophilicity. This property is favorable for cell attachment and proliferation in tissue engineering applications.

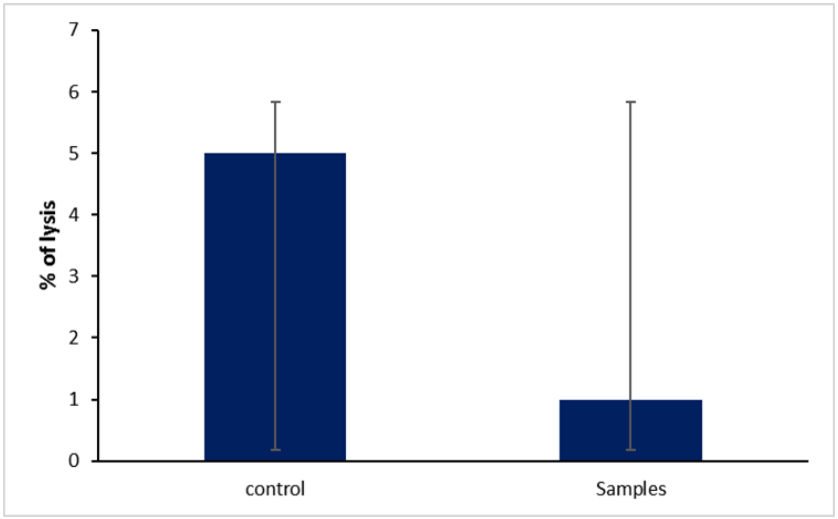

Figure2: Hemolysis Analysis:

The hemolysis percentage of the gelatine–alginate hydrogel composite was found to be less than 1%, indicating excellent hemocompatibility and suitability for biomedical applications which was even less than that of the control group.

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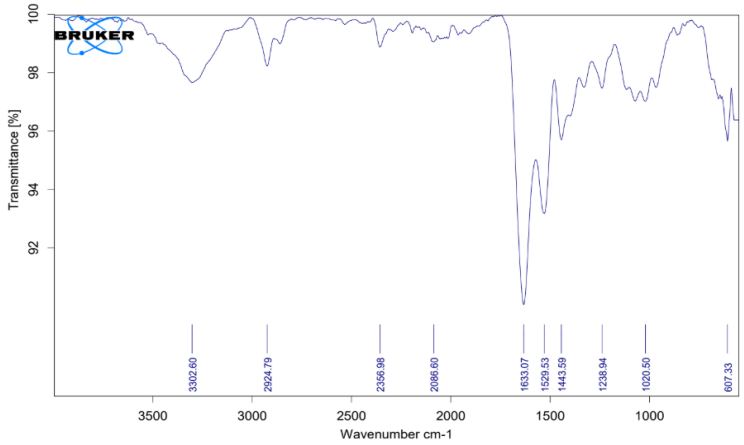

Figure3: Functional Group Analysis (FTIR):

FTIR spectra confirmed the presence of characteristic functional groups such as carboxyl ($–COO^-$), amide (C=O), and amino (NH_3) groups. These findings suggest successful interaction and integration between gelatine and alginate within the composite matrix.

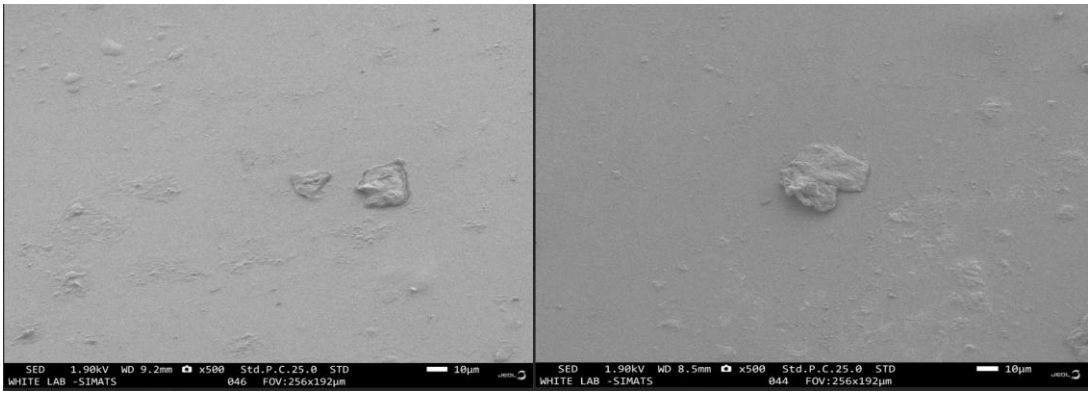

Figure 4&5: Surface Morphology (SEM):

SEM analysis revealed a uniform and interconnected porous network structure. This morphology supports enhanced cell adhesion, nutrient diffusion, and overall tissue integration.

DISCUSSION

The matrices were then superimposed to create a double-matrix PBH with greatly improved adhesion properties and mechanical features. The creation of the 3D network structure PBH through the creation of strong, resilient covalent bonds within its network is the primary objective of the chemical cross-linking process [11]. Several chemical cross-linking techniques have been reported, including chemical coupling [12], click reactions [13], and covalent cross-linking of specific chains and target residues on proteins [14]. This cross-linking technique yields improved mechanical properties under physiological conditions, enhanced stability, and a controllable degradation rate [15]. In addition, the source of covalent bonds may affect the structure of the hydrogels that are created. When polymers are present, proteins form covalent connections that result in the creation of hydrogels with novel properties [16].

The contact angle of 31.02° observed in this study confirms the high hydrophilicity of the gelatin-alginate composite. This is consistent with previous reports where gelatin and alginate-based hydrogels exhibited contact angles below 50°, indicating favorable surface properties for cell adhesion [23, 24]. A hydrophilic surface promotes protein adsorption and subsequent cellular interactions, which are critical for tissue integration [25].

The hemolysis percentage of less than 1% demonstrates excellent blood compatibility of the developed membrane. According to ASTM F756-17 standards, materials with hemolysis below 2% are considered non-hemolytic [26, 33]. This finding suggests that the gelatin-alginate composite is safe for applications involving contact with blood or vascularized tissues, which is particularly relevant for extraoral repair scenarios [27].

FTIR analysis confirmed the presence of amide bands (I, II, and III) characteristic of gelatin, along with carboxylate groups from alginate. The absence of new peaks indicating chemical crosslinking suggests that the interaction between gelatin and alginate is primarily physical and ionic in nature, which is desirable for maintaining biodegradability while ensuring structural stability [28].

The porous and interconnected microstructure observed via SEM is crucial for tissue engineering applications. Pore sizes in the range of 50–200 μm are considered optimal for cell infiltration, vascularization, and nutrient exchange [29] . The freeze-drying technique employed in this study effectively preserved this architecture, which mimics the native extracellular matrix and supports tissue regeneration [30] .

Collectively, these results indicate that the gelatin-alginate hydrogel composite possesses the essential physicochemical and biological properties required for a dual membrane in extraoral and intraoral tissue repair [31]. The combination of hydrophilicity, hemocompatibility, and suitable porous architecture positions this material as a promising candidate for further in vitro and in vivo evaluation.

The combination of gelatine-alginate hydrogel composite as a dual membrane offers a promising solution for repairing both extra oral and intraoral tissues. Hydrogel biomaterials have shown immense potential in regenerative medicine and tissue engineering applications [17]. Gelatine, alginate, and fibrinogen, three natural polymers extensively used in regenerative medicine, are combined to form a gel-like membrane that closely mimics the extracellular matrix of native tissues [18]. One of the advantages of this dual membrane is its ability to support cell-based applications in cancellous bone [19].

This combination is achieved through the co-precipitation of gelatine and alginate molecules, ensuring the structural integrity and shape fidelity of the printed hydrogel [20]. Furthermore, the addition of alginate to the gelatine hydrogel enhances its mechanical properties, making it more suitable for load-bearing musculoskeletal tissues [18].

In addition, the gelatine-alginate hydrogel composite has been shown to promote stem cell proliferation and osteoblast differentiation, making it an ideal candidate for bone tissue regeneration and defect repair [21]. The incorporation of nano-attapulgite into the gelatine-alginate hydrogel composite further enhances its mineralization effect, promoting bone regeneration [22]. Overall, the combination of gelatine and alginate in a hydrogel composite offers great potential for dual membrane applications in extra oral and intraoral tissue repair, providing a versatile and effective solution in the field of regenerative medicine [32]. By combining gelatine and alginate in a hydrogel composite, a dual membrane is created that closely mimics the extracellular matrix of native tissues, making it an ideal material for tissue repair and regeneration. The gelatine-alginate hydrogel composite serves as a dual membrane for both extra oral and intraoral tissue repair, offering unique advantages in terms of biocompatibility, structural integrity, mechanical properties, and mineralization effects.

CONCLUSION

The combination of gelatine and alginate in a hydrogel composite offers a promising dual membrane system for extraoral and intraoral tissue repair. The composite's biocompatibility, mechanical strength, and dual functionality make it a suitable candidate for a wide range of applications in maxillofacial surgery and dentistry. Further research and optimization are required to fully exploit the potential of this innovative hydrogel composite for tissue engineering and regenerative medicine.

Future studies should focus on evaluating the mechanical properties, degradation behavior, and cytocompatibility of this hydrogel composite using relevant cell lines. In vivo studies in appropriate animal models will also be necessary to assess the regenerative potential of this dual membrane in both extraoral and intraoral defect scenarios.

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