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Fabrication of electrospun scaffold using bone powder incorporated poly vinyl alcohol and Cissusquadrangularis - An invitro study

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ABSTRACT:

INTRODUCTION:

Guided tissue regeneration (GTR) prevents connective tissue from entering the bone reformation site and interference in osteogenesis. It also creates a space under the surgical flap that acts as a scaffold for the growth of cells and blood vessels. This study aims to develop a novel electrospun scaffold by incorporating bone powder into Cissusquadrangularis extract and PVA, harnessing the synergistic properties of these constituents for enhanced tissue regeneration. The membrane's design aims to achieve optimal porosity, mechanical strength, and bioactivity to foster guided tissue regeneration in clinical applications.

MATERIALS AND METHODS:

The experimental process involved the preparation of various solutions, including bone powder incorporated PVA/Cissusquadrangularis,. These were combined to create a homologous mixture of bone powder incorporating PVA/Cissusquadrangularis. Electrospinning was then used for fabrication, resulting in fibrous particles and a GTR membrane. Two groups were included: a test group (bone powder incorporated PVA/Cissusquadrangularis) and a control group (PVA). The effectiveness of the membrane on the basis of swelling, degradation and biocompatibility, contact angle was tested.

RESULTS:

The test membrane composed of bone powder incorporated PVA/Cissusquadrangularis showed a higher degradation rate, a good swelling ratio, and a slightly lower cell viability compared to the control membrane.

CONCLUSION:

Biodegradable electrospun bone powder incorporated PVA/Cissusquadrangularis membranes have many opportunities to be used as GTR membranes for the treatment of periodontitis and have properties that serve a better choice for patients.

KEYWORDS: Electrospinning, Guided tissue regeneration, Cissusquadrangularis, Extracellular matrix, Bone regeneration

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INTRODUCTION:

Guided tissue regeneration (GTR) techniques have revolutionized the field of regenerative medicine, particularly in dentistry by facilitating the repair and regeneration of damaged tissues in periodontitis patients(1). An essential component of GTR is the use of membranes that act as barriers, selectively allowing cell migration while preventing the infiltration of undesired cell types during tissue regeneration processes.(2,3) Specialized dental procedure aimed at facilitating the regrowth of tissues around teeth that have been lost due to periodontal disease or other factors. The primary objective of GTR is to regenerate lost periodontal structures, including alveolar bone, periodontal ligament, and cementum.The process of GTR involves placing a barrier membrane between the soft tissue and the bone.(4) This membrane, which can be made from materials such as expanded polytetrafluoroethylene (e-PTFE) or resorbable polymers, serves to block the rapid in-growth of epithelial and connective tissues into the wound area. By doing so, it allows the slower-growing bone and periodontal ligament cells to repopulate the area without competition, thus promoting more effective tissue regeneration.

Polymeric membranes, especially those fabricated through electrospinning techniques, have gained significant attention due to their tunable properties and ability to mimic the extracellular matrix, fostering favorable conditions for tissue regeneration. Polyvinyl Alcohol (PVA), a biocompatible and water-soluble polymer, stands out as a promising material for membrane fabrication due to its mechanical strength and versatility in blending with various additives. Incorporating natural substances into electrospun membranes has emerged as a strategy to enhance their biological properties.(5,6) PVA can be fabricated into porous structures that facilitate cell migration and proliferation. This porosity is crucial for supporting new tissue ingrowth, particularly bone and periodontal ligament cells, PVA's hydrophilic nature supports cell adhesion and nutrient transport, creating an optimal environment for tissue regeneration. PVA membranes can be designed to have sufficient strength and flexibility to cover and protect the defect area effectively, ensuring that the regenerative process is not disrupted by external forces. PVA can be engineered to degrade at a controlled rate, matching the tissue healing timeline. This ensures that the membrane provides adequate support during the critical phases of

tissue regeneration and then gradually resorbs without the need for surgical removal. PVA membranes are well-tolerated by the body's tissues, minimizing the risk of adverse reactions.

Cissusquadrangularis, a medicinal plant known for its osteogenic and anti-inflammatory properties, has shown promising potential in tissue engineering applications. Additionally, bone powder, rich in osteoconductive elements, serves as an attractive additive to promote bone regeneration. Calcium, anabolic steroidal compounds, vitamin C, and vitamin A are all present in significant concentrations in Cissusquadrangularis. (7,8)The steroidal compounds derived from Cissusquadrangularis demonstrated a significant impact on the early regeneration of all connective tissue originating from mesenchymal cells, thereby enhancing the process of bone repair.

This study aims to develop a novel electrospun scaffold by incorporating bone powder into Cissusquadrangularis extract and PVA, harnessing the synergistic properties of these constituents for enhanced tissue regeneration. The membrane's design aims to achieve optimal porosity, mechanical strength, and bioactivity to foster guided tissue regeneration in clinical applications.The integration of bone powder into Cissusquadrangularis extract and the PVA matrix is hypothesized to not only enhance the mechanical and structural properties of the membrane but also to provide a conducive microenvironment for cell adhesion, proliferation, and differentiation, ultimately promoting efficient tissue regeneration.

MATERIALS AND METHODS:

Fabrication of Scaffold:

Preparation of Polymer Solutions:A 10% w/v solution of PVA was prepared by stirring in a solvent mixture of (1:1, v/v) at 500 rpm for 2 hours. HA was dissolved in (1:1, v/v) by stirring the mixture at 500 rpm for 3 hours, resulting in a 5% w/v gelatin solution. A 3% w/v Rutin solution was prepared at 500 rpm for 2 hours. All polymeric solutions were prepared at room temperature (37 ± 1 °C).

Incorporation of Extracellular matrix (ECM): Ovine tendon derivedECM (0.5%, w/v) was added to the final polymeric solution while stirring slowly with a magnetic stirrer for 24 hours.

Fabrication of Nanofibrous scaffold: Electrospinning (ES) was employed to fabricate nanofibrous substrates from the polymer blend (PVA/C.quadrangularis+ECM) with different ratios (0%, 1%, 3%, and 5%). The polymer blend was loaded into a 5 mL plastic syringe fitted with a 22×32 mm needle. A syringe pump was used to inject the polymer solution at a flow rate of 0.9 mL/h. A high voltage of 10 kV was applied at the tip of the needle, maintaining a distance of 10 cm between the needle and the collector. The electrospinning process was conducted at a temperature of 25 ± 1 °C and a humidity of 45%. Substrates were collected on a flat aluminum plate.The prepared scaffold was subjected to the following tests. Control group was the conventional GTR scaffold.

SEM Analysis:The morphological characteristics of scaffolds were observed using scanning electron microscopy (SEM, JEOL, Tokyo, Japan) after freeze drying. The cross-sections of freeze-dried samples were coated with platinum via a sputter-coater at ambient temperature. Micrographs of all scaffolds were taken.

Contact Angle:

The water contact angle of the scaffolds was determined using a goniometer (Ossila) to test their hydrophilicity.The scaffolds were divided into 1 cm and 1 cm square samples for measurement and placed on a test plate.Then 50 L of distilled water was gently poured onto the prepared samples.By taking pictures as soon as the water droplets contacted the scaffolds (within two seconds), the contact angle between the water droplets and the scaffolds was measured.Three measurements were made on each scaffold at different locations.

Swelling & degradation analysis:

After partially dry weighing the membrane samples, they were immersed in 10 ml of 20% PBS solution at 37°C. Samples were removed from PBS after one hour and any additional liquid on the surface was wiped off. The degree of swelling and liquid absorption is then measured by the wet weight of the sample. Using dry weight (W0) and wet weight (Ww), the swelling ratio (SR) was computed as follows: SR = ((Ww - W0)/W0) x 100%. The information was displayed as mean ± standard deviation, with n = 3. The degradation ratio (DR) was calculated by the comparison of weight of material at day 0 to day 7 and datas were presented as mean ± standard deviation, where n = 3.

MTT assay for biocompatibility

In the 6-well plate with 1 mL of complete culture medium per well. Next, 0.5 mg/mL MTT was added to the bottom well. The plate was then incubated at 37 C for 4 hours. After incubation, the culture medium was aspirated from the insert and well, and the resulting formazan crystals were solubilized by adding 100µlof DMSO solution per well. The cell types were gently shaken for 2 minutes to mix the blue reaction product uniformly with the solvent. Finally, 100µl of the colored DMSO was transferred from each insert and each well to a new 96-well plate for the quantification of cell viability. Absorbance at 450 nm was measured using a microplate reader.

RESULTS:

SEM analysis is a powerful analytical technique to perform analysis on a wide range of materials, at high magnifications, and to produce high resolution images. The fibers were uniformly dispersed in the SEM images of the test group (Figure 1). The volume of fluid released and degradation of membrane by each sample was computed, and the results were compared between the test group and control group for 7 days. Test group had a percentage degradation of 26.3% on day 1, 39% on day 5 and 53.4% on day 7 and the control group had a percentage degradation of 37.1% on day 1, 49% on day 5 and 59.8% on day 7, Degradation was comparatively lesser in test group than in control group. The volume of fluid ingested or absorbed by each sample was computed, and the results were compared between the test group and control group. Test group had a percentage swelling of 11.5% and control group had a percentage swelling of 14.4% The viability rates of the membranes in both the groups were analyzed by the MTT compatibility assay and the values were compared , Control group had a viability of 98.3% and test group had a viability rate of 90.7%. Contact angle values showed 43.94 degree in the control group and 38.74 degree in the test group , which shows values of the test group were lower than control group (Figure 2 and Figure 3). Therefore it indicates that bone powder incorporated Poly vinyl alcohol and cissusquadrangularis has higher wettability than the control group and can be used as a component in guided tissue regeneration.

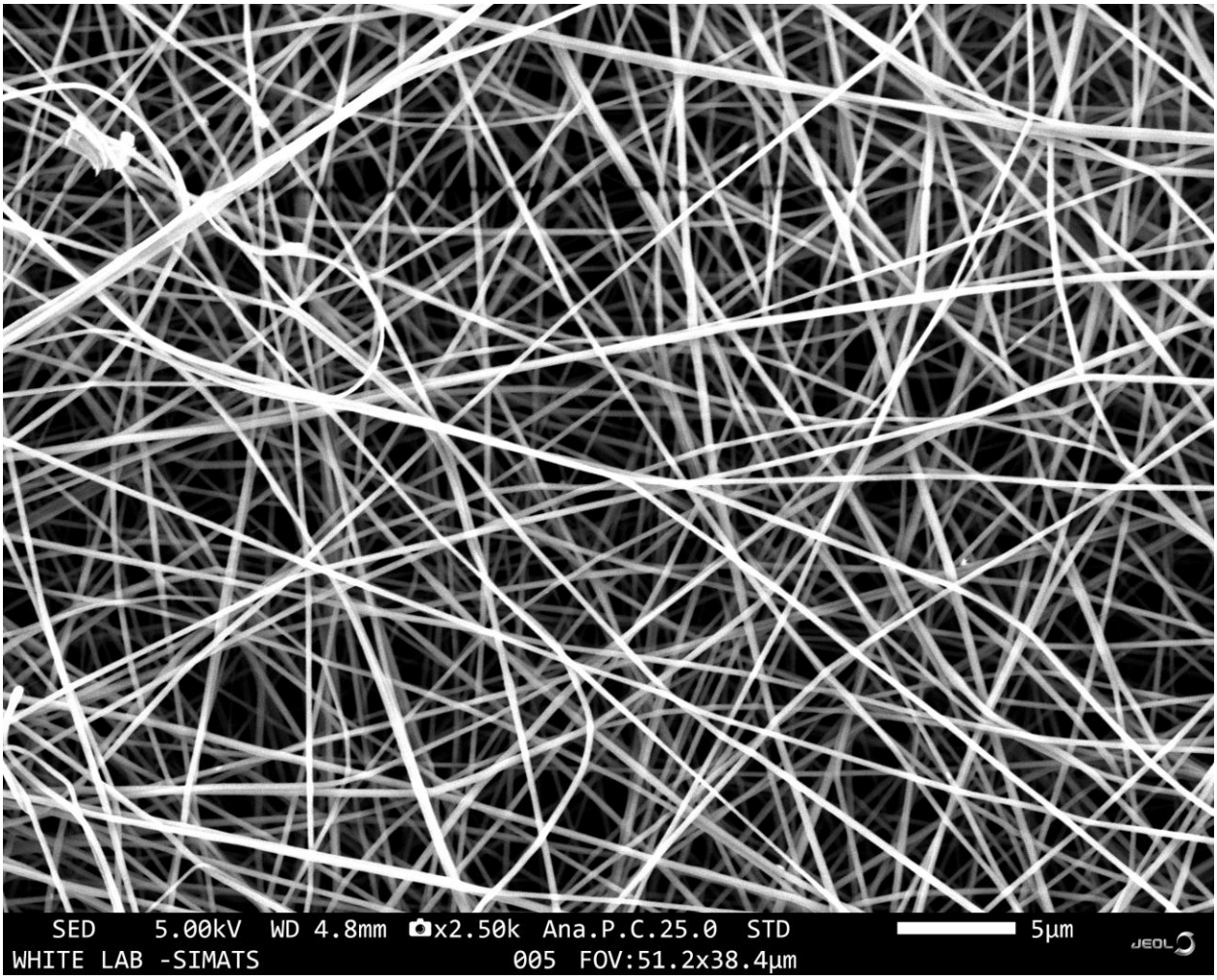

Figure 1: The surface morphology of The test group:PVA/Cissusquadrangularis scaffold

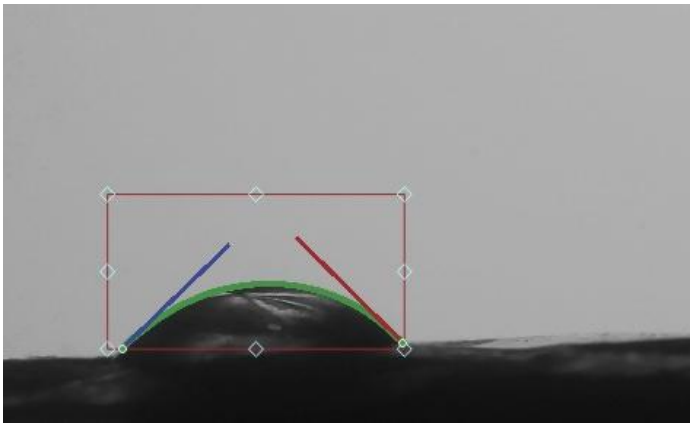

Figure 2: Test group Contact angle testing

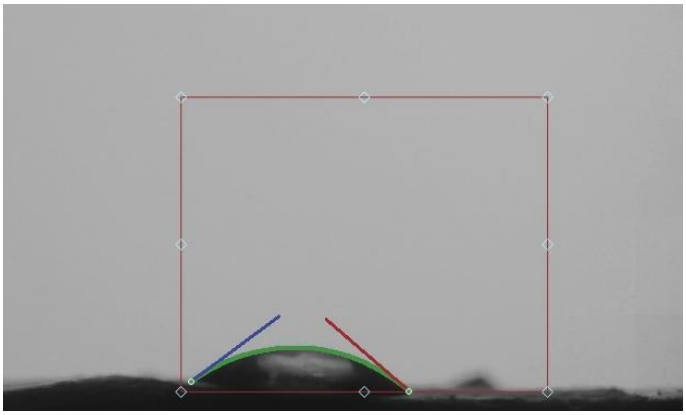

Figure 3: Control group for Contact angle testing

DISCUSSION:

An important component is the electrospinning method used to make the PVA/Cissusquadrangularis composite membrane. Precision control over fiber diameter, porosity, and overall membrane structure is possible with electrospinning(9–12). This method is especially useful for modifying the membrane's mechanical and physical characteristics to fit the requirements of GTR applications.(13)Characterizing the fiber morphology and structure of the membrane is one of the study's major findings. The electrospinning procedure enables the development of nanofibrous structures that, in terms of scale, resemble the extracellular matrix (ECM) in nature(14–15). For effective tissue regeneration, this nanostructure is crucial for fostering cell adhesion, proliferation, and differentiation. A GTR scaffold success depends on its capacity to satisfy a number of requirements, including its performance in terms of swelling, degradation, and biocompatibility. This investigation thoroughly assesses these variables to determine how well the membrane performs in clinical settings.

A key component of the membrane's functionality is how it behaves as it swells. The patient may experience discomfort and probable problems as a result of excessive edema. On the other hand, inadequate swelling might prevent tissue regrowth. A balance is intended to be struck by the electrospun composite membrane, which permits controlled swelling that maintains tissue

hydration without producing discomfort.Determining the membrane's longevity and suitability for long-term tissue regeneration requires an understanding of the dynamics of its deterioration. If membranes disintegrate too slowly, tissue maturation may be hampered, while membranes that break down too quickly may not offer enough support. To make sure it fits the planned regenerative timetable, the degradation profile of the composite membrane is evaluated.Any medical product or material must meet the unavoidable condition of being biocompatible(16). The GTR scaffold or membrane must not cause cytotoxicity or unfavorable immunological reactions in order to function properly(17). To ensure the safety of the PVA/Cissusquadrangularis composite membrane in clinical settings, rigorous biocompatibility testing is carried out.(18).

This membrane or scaffold has a wide range of practical applications in numerous clinical settings. Bone regeneration, which is essential in dental implantology and the treatment of periodontal disorders, can be accomplished with it. Its importance in periodontal regeneration cannot be emphasized since it makes gingival and periodontal ligament healing easier(19). Additionally, the membrane's biocompatibility and controlled swelling make it a significant tool for reducing patients' post-operative discomfort(20).By enabling less intrusive treatments, the deployment of this composite GTR membrane may also improve treatment outcomes overall by lowering patient morbidity. Surgical procedures might be less intrusive using a membrane that encourages regulated tissue regeneration, enhancing patient comfort and hastening recovery anti-inflammatory properties of C.quadrangularis helps in reducing inflammation at the site of application, creating a favorable environment for tissue healing and regeneration(21)(22). A study concluded that the GTR-based therapy results in a more effective healing when compared with non selective procedures, where a poorly or unorganized collagenous scar tissue is observed, characterized by epithelial down growth along the root surface which prevents the formation of periodontal attachment(23).The adventure doesn't end here; more investigation and improvement are required to unlock the full potential of this composite membrane.

Our research conclusions corroborate with prior studies, indicating that the control group exhibited higher degradation rates, lower swelling percentages, and superior cell viability compared to the test membrane over the assessed time periods. Further studies can be conducted to investigate the properties and interactions of GAG (Glycosaminoglycans), collagen, and DNA within the electrospun GTR membrane.Future research can refine the features of the membrane

for particular therapeutic applications by examining variations in C.quadrangularis concentration, various ECM components, and electrospinning parameters.

CONCLUSION:

From the results obtained, we can conclude that the tested membrane scaffold composed of PVA/Cissusquadrangularis has better biocompatibility, better contact angle and promotes cell proliferation compared to pure PVA. In addition, it can also prevent fibroblast invasion. Therefore, biodegradable electrospun PVA/CS membranes have many opportunities to be used as guided regeneration membranes for the treatment of periodontitis. The selected PVA combined with bone powder as the support layer was used as a GTR membrane to provide mechanical support and prevent rapid epithelial attachment or promote proliferation of target cells. These results suggest that the composition and properties of the test membrane are a better choice for patients.

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