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Fabrication and invitro evaluation of electrospun GTR membrane composed of polyvinyl alcohol, chondroitin sulphate, CissusQuadrangularis and extracellular matrix

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ABSTRACT:

Introduction: Guided tissue regeneration (GTR) treatment of periodontitis is a surgical procedure to implant a regeneration membrane that specifically aims to restore the periodontal tissues supporting the teeth. Electrospinning was applied to fabricate the Polyvinyl alcohol membrane and to graft chondroitin sulphate on its surface. Chondroitin sulfate is a major component of many connective tissues, including cartilage, bone, skin, ligaments and tendons. The Cissusquadrangularis plant has been used to treat a variety of ailments. Few studies suggest that it may have medicinal properties like maintenance of bone health. The current study aims to prepare a GTR membrane composed of polyvinyl alcohol, chondroitin sulphate, cissusquadrangularis and extracellular matrix and to check the properties of the novel membrane.

Materials and methods:

Cissusquadrangularis leaves were gathered and dried from a herbal garden. The leaves were dried, and then they were crushed with a mortar and pestle into a fine powder. Chondroitin sulphate was involved by adding 2 g of Chondroitin sulphate powder to 50 mL of water and stirring until a uniform, viscous chondroitin sulphate solution was produced in combination with cissusquadrangularis, polyvinyl alcohol and Extracellular matrix . The resultant membrane was subjected to SEM Analysis, Contact angle, Swelling and degradation analysis and MTT Assay.

Results: SEM analysis of the membrane suggest that dispersed, scattered morphology of fibers. Contact angle is inversely proportional to wettability. Contact angle of the test group is 48.2 and the control group is 61.8 . Test group had a higher percentage of swelling than control group. In contrast, percentage degradation was comparatively lesser in the test group than in the control group. Control group had an increased viability rate than test group in MTT assay.

Conclusion: In the present investigation Polyvinyl alcohol was chosen in combination with chondroitin sulphate as a facilitator layer for use as a GTR membrane to provide mechanical support and prevent rapid epithelial attachment or promote target cell proliferation. In this study, a novel material consisting of polyvinyl alcohol, chondroitin sulphate and cissusquadrangularis and Extracellular matrix membrane had a good mechanical properties and a good cell viability rate.

Keywords: Polyvinyl alcohol, chondroitin sulphate, Cissusquadrangularis, extracellular matrix, guided tissue regeneration membrane.

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INTRODUCTION:

Up to 90% of the world's population may be afflicted with periodontal diseases, which are very common. One of the periodontal illnesses, periodontitis causes the loss of bone and connective tissue, which is a primary factor in adult tooth loss. Severe periodontitis is typically thought to have a significant impact on several systemic disorders, including diabetes and coronary artery disease. Traditional periodontal therapy has concentrated on therapies that promote periodontal regeneration, including bone grafts, guided tissue regeneration (GTR), and stem cell therapy. Of these, GTR has emerged as the most promising therapy and has been used extensively in clinical treatment due to its practicality and efficacy. (1,2)

A regeneration membrane is surgically implanted as part of the GTR periodontitis therapy in order to specifically rebuild the periodontal tissues supporting the teeth.(3) The ideal regeneration membrane is essential to the success of this treatment because it serves as a barrier to stop epithelial and gingival connective cells from growing into periodontal defects and as a favorable niche to maximize the migration and proliferation of periodontal ligament (PDL) cells, cementoblasts, and osteoblasts within the periodontal defects to support the reconstruction of the supporting tissue. The regeneration membrane should be simple to handle during surgery, biocompatible to integrate with the tissue, allow for space maintenance, and sustain the barrier function for however long is necessary.(4)(5)

Non-resorbable substances, such as expanded-polytetrafluoroethylene (e-PTFE), were the first to be applied in clinical settings in the development of the GTR membrane. However, materials like e-PTFE require sutures to hold them in place because they are typically challenging to work with.(6) As a result, removing them needed a second surgical procedure, and various problems were noted, particularly early exposure that caused inflammation. Resorbable materials were explored so that they wouldn't need to be removed after implantation in order to avoid the unpleasant side effects of the second surgical procedure. Although collagen and other materials from nature are typically biocompatible, they tend to break down too quickly before the bone defect and other periodontal tissues can fully repair. In order to meet the need of the regeneration period, which is typically at least 4 months, composite membranes are therefore preferable candidates because they have strong biocompatibility and regulated degradation times. However, a negative side effect of strong biocompatibility is that it accelerates the rate of overall proliferation of periodontal, gingival, and epithelial connective cells. (6,7)

Microfibers, nanofibers, and mesh-like membranes comprised of these fibers can be produced using the widely used technology of electrospinning. A few of the optimal characteristics for the regeneration membrane of periodontal-guided tissues are superior mechanical qualities and a high specific surface area, which are both present in the fibrous structures made by this method.. Additionally, by enhancing the fiber diameters and electrospinning conditions, the surface topology and pore size are modifiable. To create a biomimetic interface, many different types of biomaterial, including chondroitin sulphate, were grafted onto polyvinyl alcohol. chondroitin sulphate and glycosaminoglycan (GAG), which has been shown to be one of the primary elements of extracellular matrix (Extracellular matrix), share a similar structural makeup.(8) Previous research has demonstrated the superior biocompatibility and advantages of chondroitin sulphate-based scaffolds for wound healing. Due to chondroitin sulphate's high bioactivity, an improvement in biocompatibility can be anticipated when chondroitin sulphate was modified on a polyvinyl alcohol scaffold.(9)

A vine called Cissusquadrangularis can be found in Africa and some regions of Asia. Its plant material is often dried and ground into a powder for medicinal use.(10) The herb Cissusquadrangularis may have anti-inflammatory, pain-relieving, and antioxidant properties. It might also encourage the growth of bones. There are numerous ailments for which people utilize Cissusquadrangularis, although the majority of these uses lack solid scientific basis, including obesity, fractures, joint discomfort, poor bone mass, and many others.(11)

In this study, polyvinyl alcohol was electrospun to create a new composite membrane for the regeneration of periodontal-guided tissues. To improve both biocompatibility and breakdown, the membrane was aminolyzed with chondroitin sulphate. In order to achieve a faster healing at site of tissue regeneration cissusquadrangularis is also added to it. With the help of this design, the regeneration membrane can exhibit properties common to both natural and synthetic membranes, such as strong biocompatibility and regulated degradation rate. A fibroblast barrier is another distinctive characteristic of modern electrospinning, which has also proven to have tremendous therapeutic potential in the treatment of periodontitis.(12) The current study aims to prepare a GTR membrane composed of polyvinyl alcohol, chondroitin sulphate, cissusquadrangularis and extracellular matrix and to check the properties of the novel membrane.

MATERIALS AND METHODS:

Sample preparation:

CissusQuadrangularis leaves were gathered and dried from a herbal garden. The leaves were dried, and then they were crushed with a mortar and pestle into a fine powder. In order to extract the secondary metabolites from CissusQuadrangularis , 500 mL of ethanol and 100 g of powdered CissusQuadrangularis were combined, and the mixture was shaken for 24 hours at 240 rpm. After letting the mixture settle for a full day, the supernatant was removed and the CissusQuadrangularis extract was extracted using rotary evaporation. The following phase was fabricating chondroitin sulphate which involved adding 2 g of Chondroitin sulphate powder to 50 mL of water and stirring until a uniform, viscous chondroitin sulphate solution was produced.

After being cut from the carcass, the ovine tendon (TENDON) was smashed into tiny pieces and placed in 40 milliliters of 20% phosphate-buffered saline (PBS). The decellularization fluid was made with one gram of sodium dodecyl sulfate (SDS), 200 µL of Triton-X, and 100 mL of distilled water. The tendon sample was then combined with 25 mL of the decellularization fluid and shaken at 37 °C until foam formed. Until there was no longer any froth development, the froth was removed from the decellularization fluid. The extracellular matrix of the polyvinyl alcohol, chondroitin sulphate and the natural substanceCissusQuadrangularis were combined in the following manner to create the hydrogel samples, which were then solidified in sample wells.

SEM Analysis:

After freeze drying, scanning electron microscopy (SEM, JEOL, Tokyo, Japan) was used to examine the morphological traits of the scaffolds. Platinum was sputtered onto the cross-sections of freeze-dried samples while they were being coated at room temperature. All scaffolds were photographed using a 100X microscope.

Contact Angle:

The water contact angles of the scaffolds were determined using goniometer (Ossila) to ascertain their hydrophilicity. The scaffolds were divided into 1 cm 1 cm square specimens for the measurements, and they were then set on the testing plate. 50 L of distilled water was then gently poured upon the ready specimens. By snapping images as soon as water droplets made contact

with the scaffolds (within two seconds), the contact angles between the droplets and the scaffolds were measured. Three measurements were taken on each scaffold at various locations.

Swelling & degradation analysis:

After dry weighing a portion of the membrane samples, they were submerged in 10 mL of a 20% PBS solution at 37°C. The samples were taken out of the PBS after an hour, and any extra fluid on the surface was wiped off. The degree of swelling and fluid absorption was then measured using wet weight on the samples. 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:

1 mL of complete culture media is placed in each well of the six-well plate. The bottom well was then filled with 0.5 mg/mL MTT. After that, the plate was incubated for 4 hours at 37C. The formed formazan crystals were then solubilized by adding 100 l of DMSO solution to each well after the growth media had been aspirated from the insert and well during incubation. For two minutes, the cell types were gently shaken to evenly distribute the blue reaction product and solvent. In order to measure the cell viability, 100 l of the colored DMSO were transferred from each insert and well to a fresh 96-well plate. A microplate reader was used to measure the absorbance at 450 nm.

RESULTS:

The molecular structure or the morphology of the prepared GTR membrane was observed using scanning electron microscopy (SEM, JEOL, Tokyo, Japan). It shows an uniform morphology of fibers in control group.

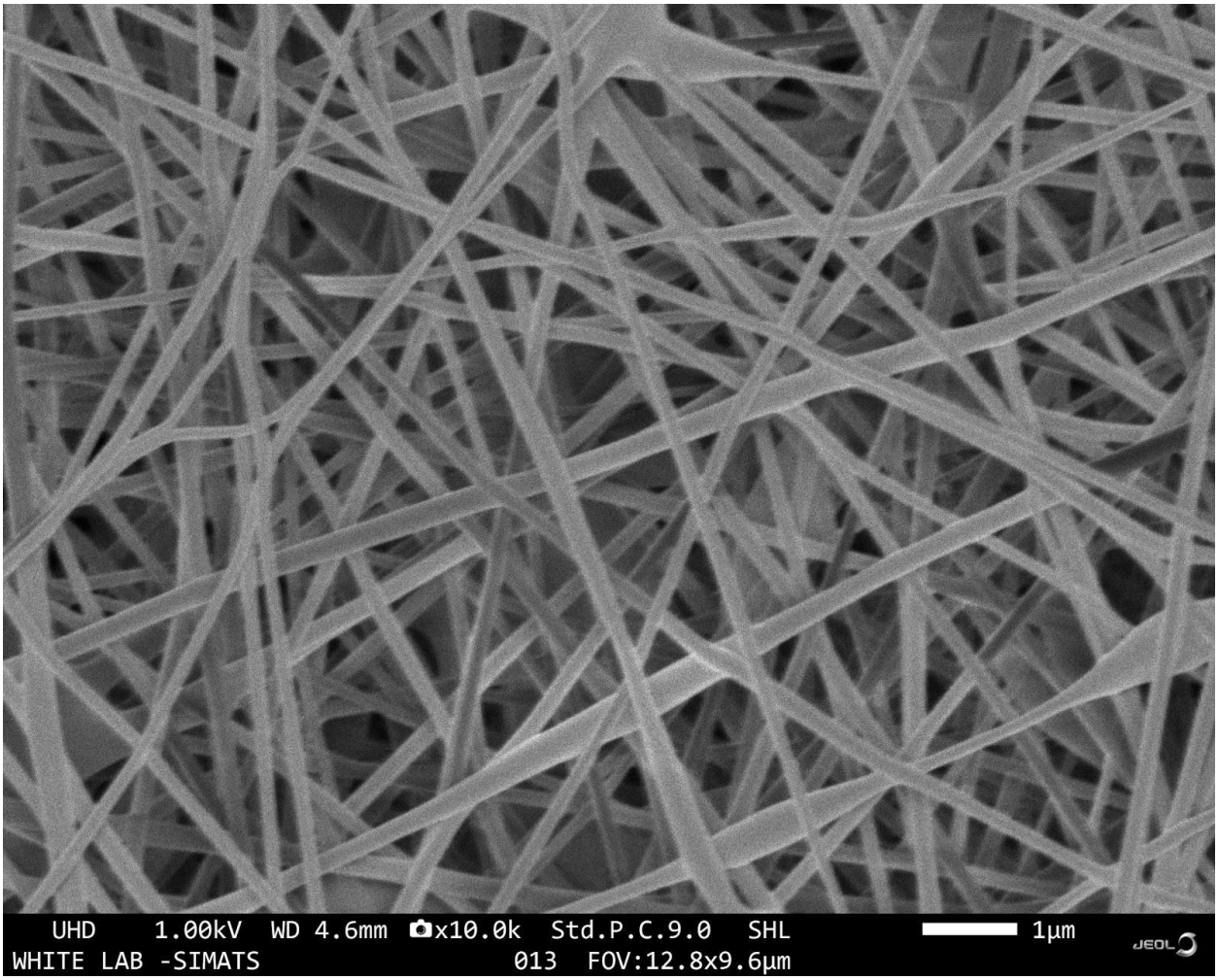

Figure 1: SEM image of control group

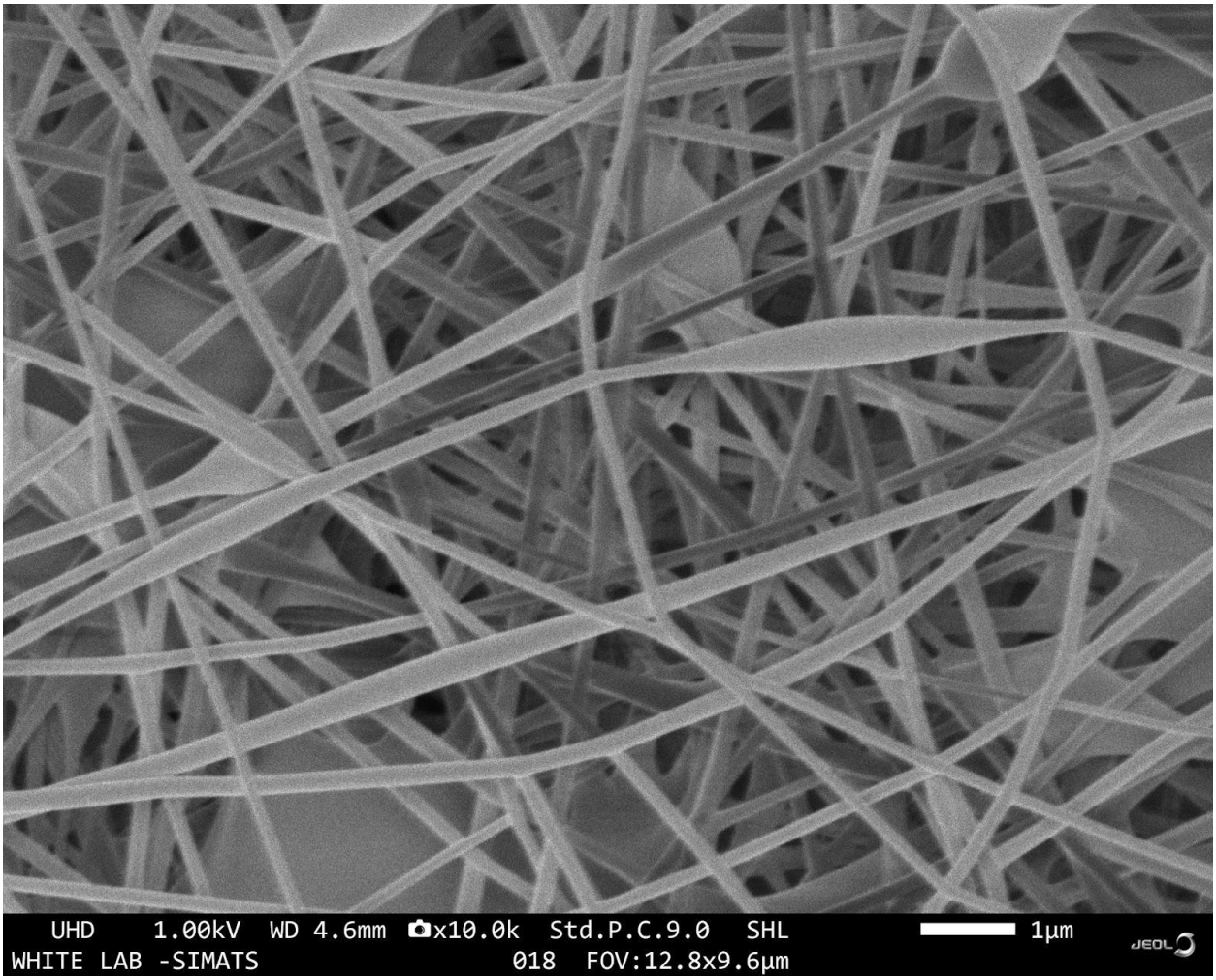

Figure 2 : SEM image of test group. The test group shows a dispersed, scattered morphology of fibers in the test group.

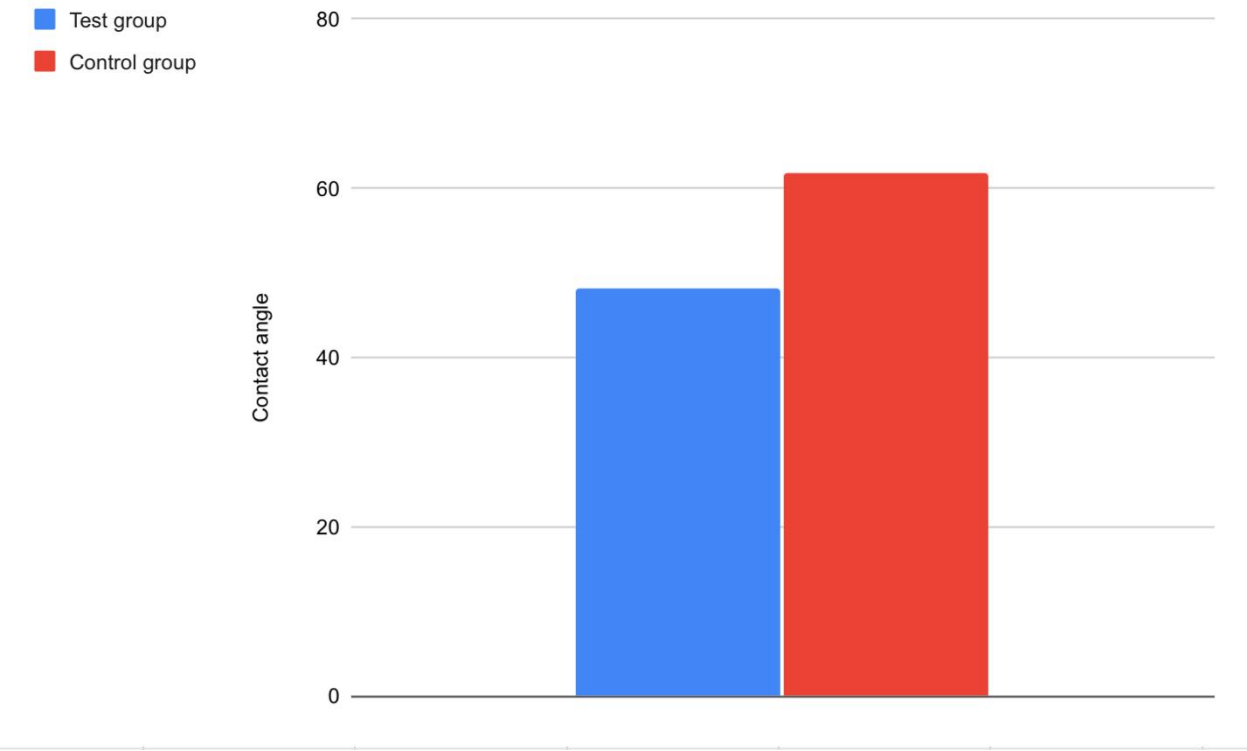

Figure 3: Comparison of contact angle between test group and control group

Contact angle of the test group is 48.2 and the control group is 61.8 . Contact angle is inversely proportional to wettability. Higher the contact angle, lower will be the wettability. In our study, test group has a lower contact angle and so higher wettability will be seen.

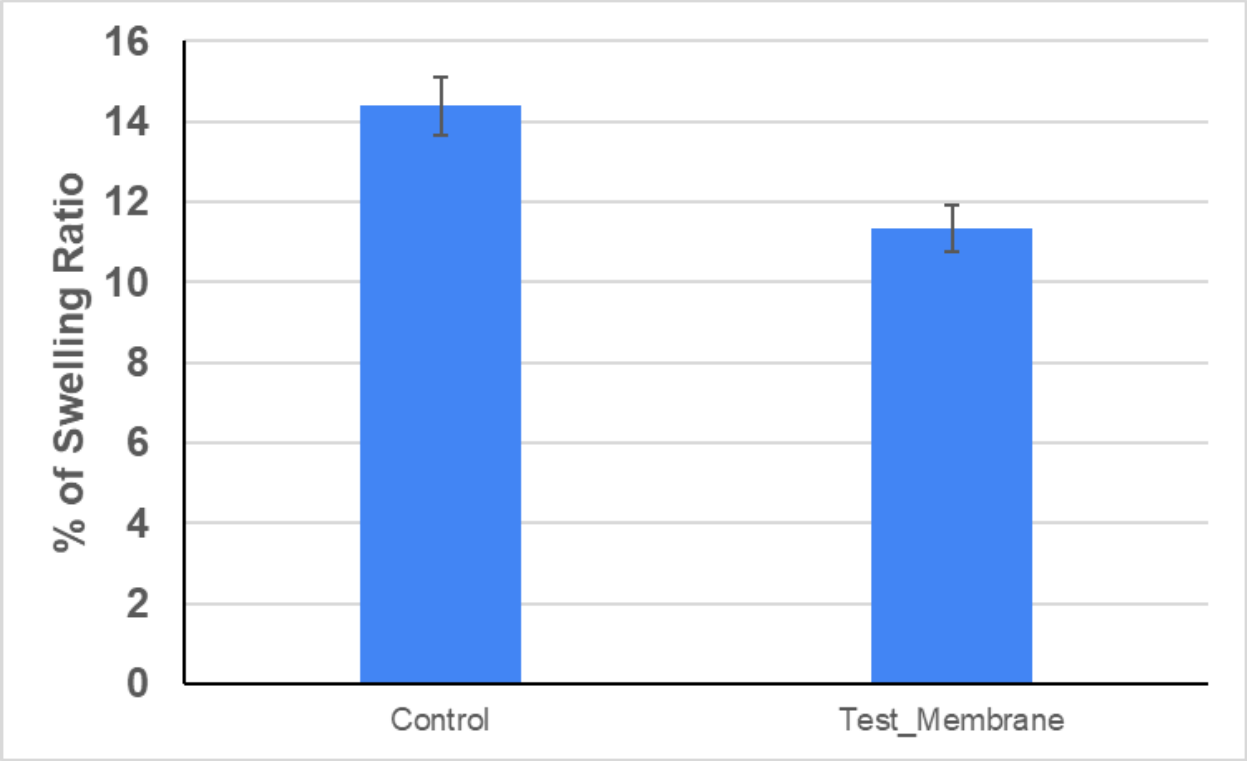

Figure 4: Percentage of swelling ratio in control group and our test membrane

As seen in Figure 4, the volume of fluid ingested or absorbed by each sample was computed, and the results were compared between test group and control group. Test group had a percentage swelling of 11.7 % and control group had a percentage swelling of 14.2%

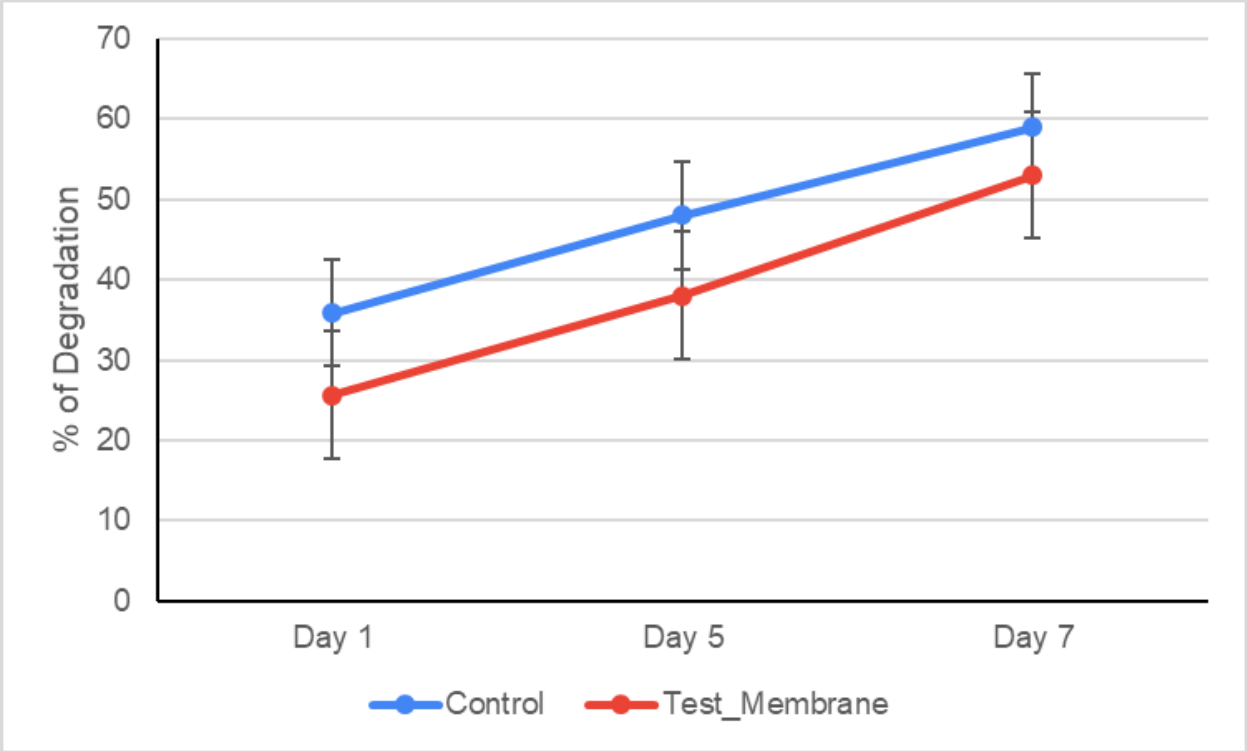

Figure 5: Percentage of degradation of control group and our test membrane

As seen in Figure 5. 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 27.3% on day 1 , 39.7% on day 5 and 53.3% on day 7 and the control group had a percentage degradation of 37.2% on day 1, 49.3% on day 5, 59.7% on day 7. Degradation was comparatively lesser in test group than in control group.

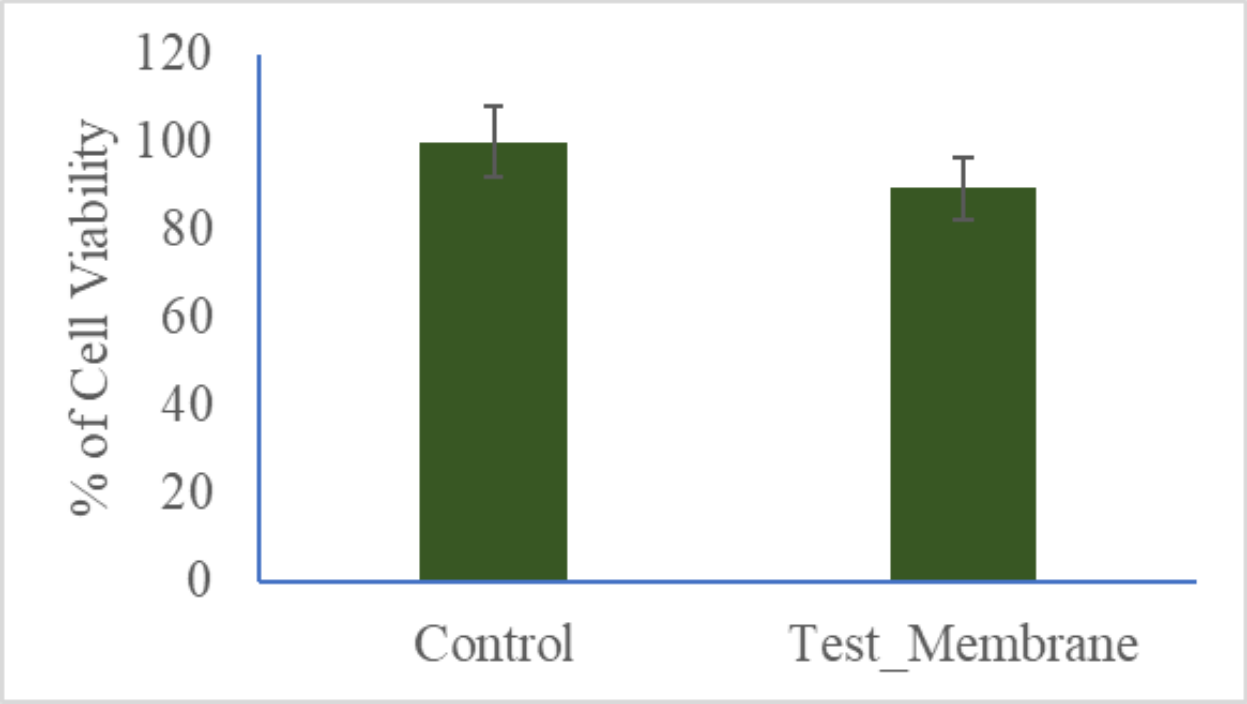

Figure 6: Percentage of cell viability of control group and test membrane

The viability rates of the membranes in both the groups were analyzed by the MTT compatibility assay and the values were compared, as shown in Figure 6. Control group had a viability of 98.3% and test group had a viability rate of 90.7%

DISCUSSION:

Various synthetic membranes are currently available and in use for regenerative purposes, but the use of natural materials in the fabrication of GTR membranes offers higher biocompatibility and lower cytotoxicity, hence it is preferred. Although there are many studies being conducted and reported on the use of GTR membranes or periodontal regeneration, studies are scarce on the use of natural products and Extracellular matrix of the tendon in chondroitin sulphate membranes.(13) This study was conducted to find a suitable natural product that can be incorporated into polyvinyl alcohol and chondroitin sulphate, to increase the regenerative potential of the GTR membrane. We chose the traditional medicinal plant *Cissusquadrangularis*the natural product and the results were very promising.

The purpose of the scanning electron micrographs of the samples was to analyze and contrast the morphological changes that result from the combination of raw materials and components. The SEM image in figure 1 has a highly rough morphology with a high degree of cresting and troughing, whereas the SEM image in figure 2 i.e test membrane has a fibrous morphology with a high density of interconnected fibers. By combining all the ingredients, a highly irregular structure is produced that increases the membranes' surface area and, as a result, improves the scaffold's interaction with the surrounding tissues at the implantation site. As the damaged or destroyed tendon tissue regenerates, the greater contact will give the scaffold more mechanical stability, preventing further injury while the healing process is underway.(14)

The contact angle is considered as a tendency that exists in a liquid to spread on to the entire solid surface. When the contact angle is higher than 90^o, the substance is considered non-wetting, however, in cases with a contact angle of $<90^o$, the substance is considered as wetting the substrate. Complete wetting is represented by the contact angle of zero. A better interaction between solid and liquid surfaces is considered when the contact angle is low. From figure 3 we can conclude that the test group has a lower contact angle and higher wettability.

To determine how much the produced membrane will expand upon absorbing the tissue fluid surrounding the location of its application, swelling ratio analysis is carried out. In order to prevent the membrane sample from expanding in tissue fluid and harming nearby tissues or interfering with the proper functioning of nearby or underlying tissue structures, it is best to have a low swelling ratio. Using Figure 4 Test group had a percentage swelling of 11.7 % and the control group had a percentage swelling of 14.2% .So test group has a better property in terms of swelling.

To determine how much the produced membrane will degrade after its function of regeneration of tissue. It is necessary to find out the percentage of degradation of the synthesized membrane. Once after the function of the membrane is over the material has to be degraded. In order to prevent the membrane sample from causing further damage in nearby tissues it is best to have a higher degradation ratio. From figure 5 we can conclude that degradation was comparatively lesser in the test group than in the control group.

The efficacy, appropriateness, and biocompatibility of the membrane for tissue regeneration in the human body are evaluated by measuring the cell viability of the membrane samples using the

MTT compatibility assay. Figure 6 illustrates that the control group exhibits the maximum degree of cell viability according to the MTT testing.

It is necessary to look into whether the active ingredients in Cissusquadrangularis have influenced the outcomes of the tests carried out for this study.(15) Other natural or synthetic materials that can be employed in addition to Cissusquadrangularis and extracellular matrix in chondroitin sulfate require more investigation.(16)

Previous studies done by (17) suggest that cissusquadrangularis has potentially useful flavonoids which decreases the inflammatory process and enhances the regeneration of tissues. Therefore in accordance with our study we can state that cissusquadrangularis and its extracts are potentially useful plant species in the stream of periodontal health.

To further optimize the swelling rates and mechanical tolerance as well as boost the membranes' cell survival and biocompatibility, other combinations ought to be looked at. Our study proposes a method for the preparation of GTR membranes with good cytocompatibility and degradation resistance. Further studies should be done in animal models.

CONCLUSION:

In this study, a novel material consisting of polyvinyl alcohol, chondroitin sulphate and cissusquadrangularis and Extracellular matrix membrane had a good mechanical properties and a good cell viability rate. Our results indicate that the created membrane in this study offers better osteoconductive properties and significant responses making it reasonable to suggest this novel Polyvinyl alcohol, Chondroitin sulphate, Cissusquadrangularis and Extracellular matrix based GTR membrane as a potential novel periodontal biomaterial for guided tissue regeneration surgery.

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CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest in this study.

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