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ORIGINAL ARTICALE

FABRICATION AND CHARACTERIZATION OF VITAMIN C CONJUGATED CISSUS QUADRANGULARIS MEMBRANE FOR GUIDED TISSUE REGENERATION - AN IN VITRO STUDY

Sneha Harshini[1], Jayalakshmi Somasundaram[2], Balaji Ganesh Subramanian[3], Gurumoorthy Kaarthikeyan[4]

[1]Undergraduate, Department of Periodontics,Saveetha Dental College and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India Email id: snehaharshini@gmail.com
[2]Reader,White lab - Materials Research centre, Saveetha Dental College and hospitals,Saveetha Institute of Medical and Technical Sciences (SIMATS),Saveetha University,Chennai -77, Tamilnadu, India. Email id: jayalakshmisomasundaram@saveetha.com
[3]Reader, Department of Periodontics,Saveetha Dental College and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India Email id: balajiganeshs.sdc@saveetha.com
[4]Professor and Head of Department, Department of Periodontics, Saveetha Dental College and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India Email id: drkarthik79@yahoo.co.in
Corresponding author Jayalakshmi Somasundaram Reader, White lab - Materials Research centre, Saveetha Dental College and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai -77, Tamilnadu, India. Email id: jayalakshmisomasundaram@saveetha.com

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ABSTRACT

Background: Cissus quadrangularis has gained attention for its rich content of bioactive compounds, including flavonoids, carotenoids, and polyphenols, notably Vitamin C.These compounds are recognized for their antioxidant, anti-inflammatory, and tissue reparative properties, may provide the membrane more bioactivity, which would encourage cell division and proliferation.This main aim of this study is the production of vitamin C membrane impregnated with Cissus quadrangularis for soft tissue regeneration.

Materials and Methods: To prepare the vitamin C membrane with the impregnation of Cissus quadrangularis (CQ) , 1 gram of CQ was dissolved in 10 mL of distilled water and heated at 80°C for 24 hours. Following membrane formation, FTIR (Fourier Transform InfraRed Spectroscopy), contact angle, tensile strength testing and antimicrobial activity was compared between the CAR control group membrane and the test group membrane which contains CAR with the impregnation of vitamin C.

Results: The tensile strength of the test group was better than the control group. The test group showed the tensile strength of 23.86 N whereas the control group showed 27.99 N. CAR membrane had a contact angle of 81.88 degrees. This implies lower wettability. Whereas the cissus quadrangularis and vitamin C incorporated membrane had a contact angle of 49.92 degrees. The test group showed better wettability, tensile strength and anti microbial activity as compared to the control group.

Conclusions: The present study showed that CAR membranes with the impregnation of Cissus quadrangularis showed increase in hydrophilicity. The mechanical property was increased and the antimicrobial activity showed an enhanced zone of inhibition. The membrane can serve multipurpose dental applications and can be incorporated in various periodontal procedures for guided tissue regeneration.

Keywords: vitamin C, Cissus quadrangularis, soft tissue regeneration, Periodontal regeneration

INTRODUCTION

Gingivitis and periodontitis are two of the periodontal disorders, which are arguably the most prevalent illnesses in the world. Since periodontitis is the leading cause of tooth loss in adults worldwide, these people are susceptible to edentulism, masticatory dysfunction, and multiple tooth loss, which can negatively impact their nutrition, quality of life, and self-esteem in addition to having significant socioeconomic and medical costs. It is possible to prevent, diagnose, treat, and control periodontitis with the help of proper medical care and long-term secondary prevention.[1]While periodontal flap surgeries and conventional nonsurgical therapy are effective in slowing the progression of periodontal disease. Guided bone/tissue regeneration (GBR/GTR) techniques are surgical techniques used to regenerate lost bone/tissue and are accepted treatment modalities for alveolar bone augmentation and the reconstruction of lost periodontal structures in periodontal defects.[2] Barrier membranes are used in these techniques to impede the ingrowth of soft tissue into defect sites and to promote periodontal tissue or bone regeneration. Various biomaterials are used in guided tissue regeneration. Nowadays naturally occurring plant-based chemicals with bioactive and regenerative qualities are used as strategy to treat periodontal defects.[3]

The medicinal plant Cissus quadrangularis (CQ), which has long been used in herbal therapy, has shown great promise in accelerating the repair of soft tissues and bones. According to reports, it possesses anabolic, analgesic, and anti-inflammatory properties. It can also improve tissue healing and collagen formation. It has been demonstrated that CQ's active ingredients, which include flavonoids, polyphenols, and triterpenoids, contribute to its regenerative qualities, which makes it a desirable option for soft tissue repair applications. Its bioavailability and the requirement for improved delivery systems that may more precisely target and promote tissue regeneration, however, frequently restrict its clinical use.[4] Vitamin C (ascorbic acid), a well-known antioxidant and cofactor in collagen synthesis, plays a crucial role in the formation of collagen fibers during tissue repair and regeneration. It is essential for stabilizing collagen and promoting cellular functions that contribute to wound healing. Given its pivotal role in tissue regeneration, the conjugation of CQ with Vitamin C offers a novel approach to improving the regenerative potential of CQ, enhancing its efficacy in soft tissue repair.[5]

This research aims to explore the development of a Vitamin C conjugated Cissus quadrangularis membrane as a bioactive material for soft tissue regeneration. By harnessing the synergistic effects of CQ and Vitamin C, we seek to create a membrane that not only supports cellular attachment and proliferation but also stimulates the regenerative processes necessary for the healing of soft tissues.[6] The conjugation of Vitamin C to CQ is expected to improve the stability and bioactivity of the composite, while also enhancing the therapeutic potential for faster and more effective tissue regeneration. The main aim of this study is to fabricate and characterize Vitamin C conjugated Cissus quadrangularis membrane for guided tissue regeneration.

MATERIALS AND METHODS

MEMBRANE PREPARATION

To prepare the carrageenan membrane with the impregnation of Cissus quadrangularis (CQ) and vitamin C, 1 gram of CQ was dissolved in 10 mL of distilled water and heated at 80°C for 24 hours. This process facilitated the extraction of bioactive compounds from the plant material. After heating, the solution was filtered through Whatman filter paper to obtain a clear CQ extract, which was set aside for later incorporation into the CAR matrix. Simultaneously, a CAR solution was prepared by dissolving 5 grams of carrageenan in 100 mL of distilled water, and this mixture was kept overnight to ensure complete dissolution. Once fully dissolved, the carrageenan solution was divided into two equal parts. The first 50 mL served as the base membrane, consisting of pure carrageenan. To the second 50 mL, 50 mg of vitamin C was added, and the mixture was stirred for 5 hours to achieve a uniform dispersion of vitamin C. This vitamin C impregnated carrageenan solution was then poured into a petri dish and placed in a deep freezer at -20°C for 24 hours to promote solidification. After freezing, the membranes were lyophilized to remove any residual moisture, yielding stable CAR membranes ready for guided tissue regeneration applications. The materials were divided into two groups: The control group consists of carrageenan(CAR) and the test group consists of the addition of Cissus quadrangularis and vitamin C to carrageenan (CAR+vitamin C).

CHARACTERISATION OF THE MEMBRANES

The membranes were cut into smaller samples and tested using different parameters. FTIR (Fourier Transform InfraRed Spectroscopy), contact angle, tensile strength testing and antimicrobial activity was compared between the CAR control group membrane and the test group membrane which contains CAR with the impregnation of cissus quadrangularis and vitamin C.

RESULTS

Fourier transform infrared spectroscopy (FT-IR) was measured with the help of Perkin Elmer. This FT-IR comparison shows spectra for a CAR (carrageenan) control group sample and a CAR+ vitamin C test group membrane. When Vitamin C is present, the CAR+ vitamin C test group (blue) spectrum exhibits more peaks or shifts than the CAR (yellow) spectrum. These variations most likely show that vitamin C has been added to the CAR matrix. The addition of vitamin C is confirmed by the unique peaks between 1000 and 800 cm that are present in the CAR+ vitamin C spectrum but lacking in the CAR spectrum. These peaks are probably caused by the Amide and carboxyl groups in vitamin C indicates the formation of material. Amide and carboxyl groups in CAR revealed that the amide I and amide Il bands, which are typically located at cm-' and cm, respectively, are typical FT-IR peaks for CAR. Interactions between Hydroxyl Groups revealed that possible hydrogen bonding interactions between CAR and vitamin C may be indicated by shifts or variations in intensity around the 3200–3600 cm-1 area (hydroxyl stretching) (Figure 1).

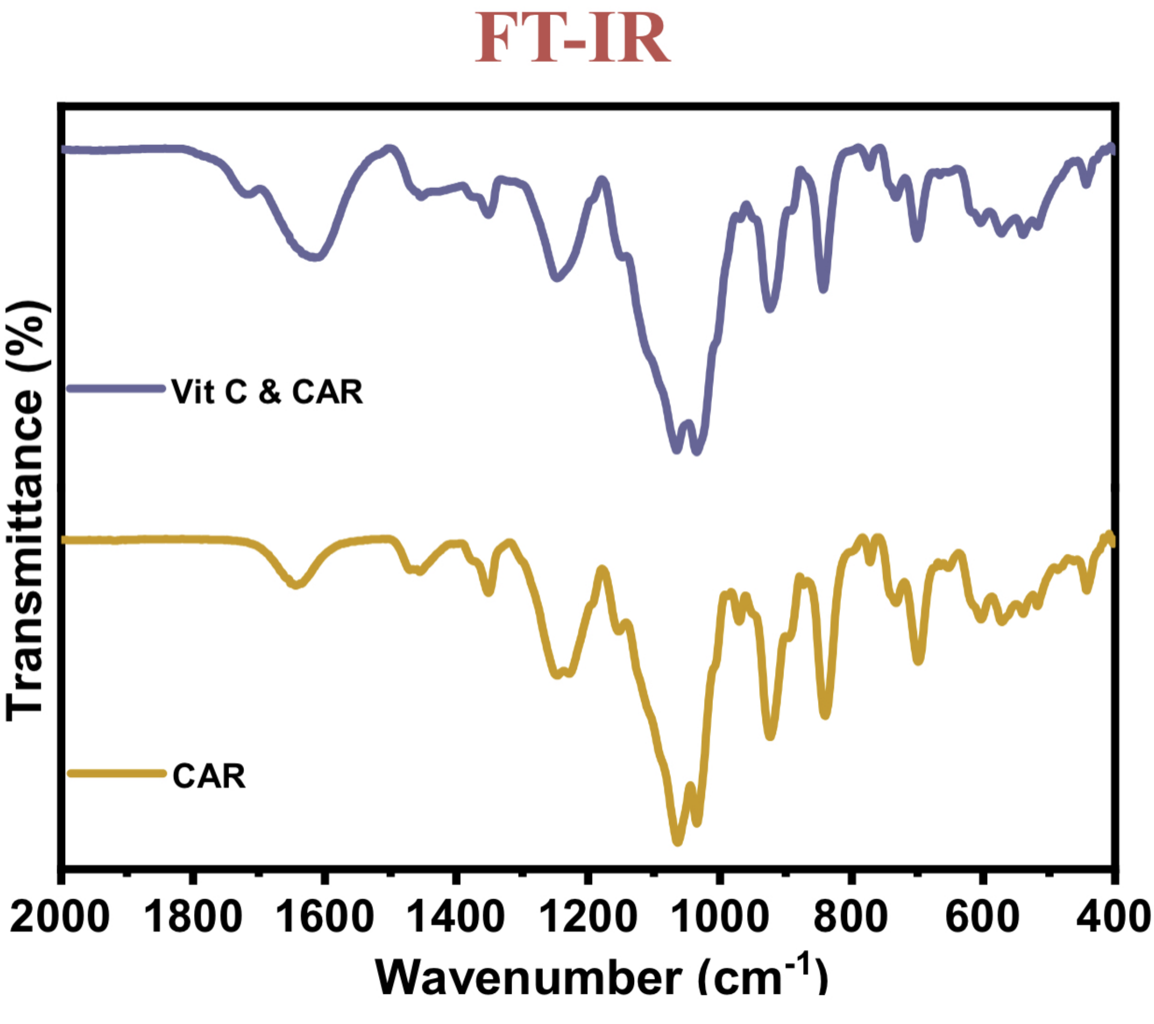
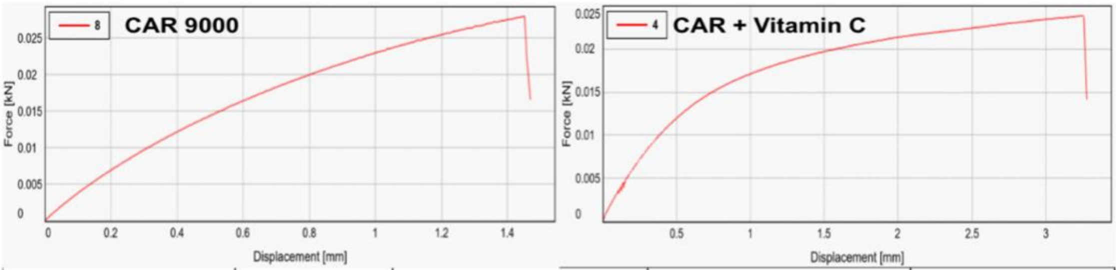


Figure 1. shows the Fourier transform infrared spectroscopy

Tensile strength was measured in newtons and it was tested using the INSTRON 3000 universal testing machine. The tensile stress for 17.02 MPa with 8.15% tensile strain at break and 17.01 MPa tensile stress at break whereas the addition of Cissus quadrangularis to vitamin C (test group) showed a tensile stress of was 19.99 MPa, with 3.63% tensile strain at break and 19.99 MPa tensile stress at break. The tensile strength of CAR (control group)was 23.86 N whereas the CAR + vitamin C (test group) showed 27.99 N. This shows an enhanced property of tensile strength in the membrane that was incorporated with vitamin C and cissus quadrangularis (Figure 2).

Tensile Strength

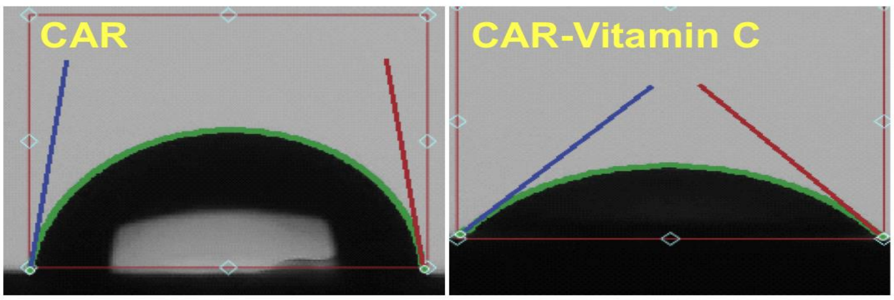


Materials Name	Maximum Force [N]	Tensile stress at Tensile strength [MPa]	Tensile strain (Displacement) at Break (Standard) [%]	Tensile stress at Break (Standard) [MPa]
Vitamin C	27.99	19.99	3.63	19.99
CAR	23.86	17.02	8.15	17.01

Figure 2. represents the Tensile strength

Contact angle was measured using an ossila goniometer. Control CAR membrane had a contact angle of 81.88 degrees. This implies lower wettability. Whereas the cissus quadrangularis and vitamin C incorporated membrane had a contact angle of 49.92 degrees. This suggests higher wettability of the test group membrane. Thus the test group membrane had a good wettability property (Figure 3).

Contact Angle



CAR		CAR-Vitamin C	
Left Angle:	**81.98°**	**Left Angle:**	**48.91°**
Left RMS Error:	0.39	Left RMS Error:	0.74
Right Angle:	**81.78°**	**Right Angle:**	**50.93°**
Right RMS Error:	0.35	Right RMS Error:	0.53
Average Angle:	**81.88°**	**Average Angle:**	**49.92°**

Figure 3. shows the difference in contact angle

The zone of diameter of S. mutans when incorporated with vitamin C and cissus quadrangularis was 13 mm whereas in the control group with CAR was 4 mm. This shows that better antimicrobial activity was found in the test group membrane than the control group membrane (Figure 4).

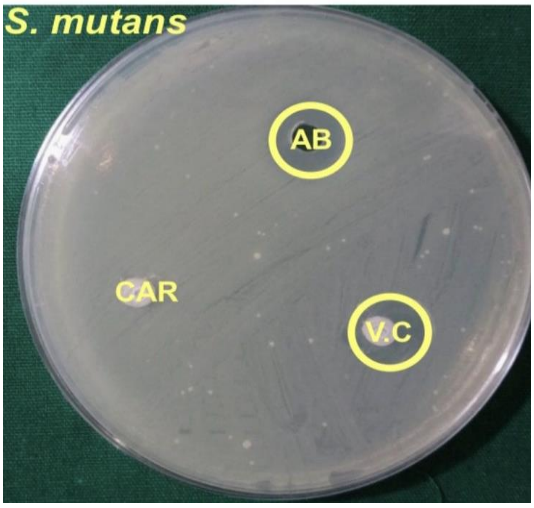


Figure 4. Shows antibacterial activity

DISCUSSION

In this study, we have successfully fabricated and characterized a novel vitamin C conjugated Cissus quadrangularis membrane (Vitamin C-CQ membrane) designed for guided tissue regeneration (GTR), with a specific focus on its in vitro performance.[7] The purpose of this research was to explore the potential benefits of combining the bioactive properties of Cissus quadrangularis, a well-known plant with healing properties, with vitamin C, an essential nutrient known for its antioxidant and collagen synthesis-promoting effects. The resultant membrane was assessed for its physical, chemical, and biological properties to evaluate its suitability for application in tissue regeneration, particularly in bone and soft tissue healing**.**

The healing of musculoskeletal tissues, such as bone, tendons, and ligaments, is dependent on the capacity of collagen synthesis and cross-linking.[8] Poorly developed extracellular matrices derived from collagen can lead to inadequate tissue structures and biomechanical strength, which can result in unsatisfactory outcomes and an increased risk for reinjuries.[9] Basic science investigations on the biochemical pathways after a musculoskeletal injury have suggested that vitamin C, also known as ascorbic acid, may enhance collagen synthesis and soft tissue healing. Another study ascertained the innate early biomineralization proficiency and sufficient mechanical stability complemented with controlled degradation of the chitosan–collagen scaffold; hence, could be proposed as a good candidate for regenerative bone tissue engineering. Studies have shown that Vitamin C plays a crucial role in collagen synthesis, a fundamental process in tissue repair. In a previous study , it was concluded that doping HA-based hydrogel scaffold with the natural product CQ has increased the tenogenic potential. When the tenogenesis capacity is increased, it also plays a significant role in orienting the collagen fibers, thereby inducing more tensile strength.[10] By facilitating collagen production and deposition, Vitamin C-conjugated membranes promote cellular proliferation and extracellular matrix formation, essential for restoring tissue integrity. In this study, Vitamin C incorporated carrageenan membrane showed increased tensile strength ensuring better regenerative properties of guided tissue regenerated (GTR) membrane.[11]

Ensuring the biocompatibility and long-term stability of the membranes is crucial for their clinical translation.The surface functionalization of Cissus quadrangularis with vitamin C was confirmed through Fourier-transform infrared (FTIR) spectroscopy, which showed characteristic peaks corresponding to both vitamin C and Cissus quadrangularis.[12] This supports the notion that vitamin C was successfully incorporated into the membrane and could potentially impart additional benefits, such as antioxidants and collagen biosynthesis, critical for tissue repair processes. Hydrophilic properties have a water contact angle (WCA) of less than 90°. From this study , the sample with vitamin C can be said to have the better hydrophilicity with the lower contact angle of 49.92°.[13] The higher contact angle value would reflect a better surface solid hydrophobicity. In a previous study by Mat Yassin et al. (2022) produced a carrageenan-based biofilm with surface hydrophobicity ranging from 81 to 107°. The films were blended with lipid which was extracted from *cholera vulgaris.* In this work, the hydrophobicity reduced with the addition of vitamin C. This is probably due to vitamin C being highly soluble in water.[14]

The traditional medicinal practice of Ayurveda has long utilized CQ to address a spectrum of diseases and ailments.[15] Its recognized osteogenic properties prompted an exploration into its potential application for periodontitis.[16] Our findings confirm the antibacterial properties of CQ, shedding light on its potential therapeutic role in combating periodontal infections.In a previous study , results demonstrated significant antibacterial properties of CQ aqueous and ethanolic extracts against *Porphyromonas gingivalis*. This study shows S. mutans when incorporated with vitamin C and cissus quadrangularis had better antimicrobial activity when compared to CAR.

A more thorough assessment of the vitamin C-carrageenan membrane antibacterial efficacy would come from testing against other bacteria, particularly those that are frequently linked to periodontal illnesses or pertinent to the intended use (e.g. P. gingivalis, Actinobacillus). Because the study was conducted in a controlled in vitro setting, it might not accurately reflect situations in vivo.It will be necessary to test the material's regenerative capabilities using animal models. These limitations should be addressed in future studies to enhance the results and provide a more solid foundation for potential periodontal regenerative applications.

CONCLUSION

From this study we can conclude that, Cissus quadragularis conjugated vitamin C membrane shows high hydrophilicity and antibacterial activity along with high tensile strength when compared to pure carrageenan membrane. Therefore it can be used for guided tissue regeneration material in managing periodontitis patients .

DECLARATIONS

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No

Conflict of Interest

No

Support

No

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