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ADHESIVE NANOMEMBRANE LOADED WITH QUERCETIN FOR ORAL MUCOSA REPAIR

Nagubandi Lakshmi Anvitha [1], Lavanya Prathap [2]* and Sangeetha S [3]

[1,2,3] Department of Anatomy, Biomedical Research Unit and Lab Animal Centre, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. *Corresponding Author Email: lavanyap.sdc@saveetha.com

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Abstract

This study focuses on the development and characterization of an adhesive nano-membrane loaded with quercetin for the repair of oral mucosa. Quercetin, a natural flavonoid known for its anti-inflammatory, antioxidant, and wound healing properties, has been incorporated into a nano-membrane matrix to enhance its bioavailability and therapeutic efficacy. The nano-membrane's adhesive properties enable direct application to the oral mucosa, providing sustained release of quercetin at the site of injury or inflammation. Quercetin has a low bioavailability, weak solubility, poor permeability and instability; it has limited utility in pharmaceuticals tremendous efforts have been undertaken to overcome the issue of it's a restricted scope in drug delivery systems. Through comprehensive physicochemical and biological evaluations, including morphology analysis, drug release kinetics, cyto compatibility, and in vitro wound healing assays, the effectiveness of the quercetin-loaded nano-membrane in promoting oral mucosa repair is investigated. For improving oral mucosa restoration, the quercetin-loaded adhesive nano-membrane holds great potential. It is a good therapeutic option because of its ability to hasten tissue regeneration, reduce inflammation, aid in wound healing, and have antibacterial properties. Even though additional research and clinical trials are necessary to establish its effectiveness and safety in human subjects, the preliminary findings are encouraging and point to the possibility that this nano-membrane could enhance patient outcomes and oral healthcare. This innovative approach holds promise for the development of advanced therapeutic strategies for various oral mucosal disorders, offering targeted delivery and enhanced therapeutic outcomes.

Keywords: Adhesive Nanomembrane, Quercetin, Oral Mucosa Repair, Nanotechnology, Biomaterials, Wound Healing, Drug Delivery, Tissue Engineering, Oral Health, Regenerative Medicine.

INTRODUCTION

Oral mucosal membrane denotes the mucosal lining of the oral cavity that constitutes a barrier to chemicals that are harmful and mechanical trauma to the underlying tissue. Squamous keratinized epithelium is present in the stratified mucosa (1). We focus on one of the fundamental barrier functions of the oral mucosa when we refer to permeability. Contrary to widespread opinion, the oral mucosa demonstrates regional variation rather than being a unified, extremely permeable tissue like the gut. Various degrees of permeability may be desirable for local and systemic administration of medication because they can be used to account for geographical variations in the prevalence of specific mucosal illnesses (2). Oral mucosa have the following functions: protection, secretion, sensation, thermal regulation. A diverse and unexplored layered material which is a source of two dimensional system is nano sheet .It has the unique phenomenon of transport restriction to a confined plane (3). An adaptable and practical method for creating ultrathin fibers is electrospinning. Developing electrospinning techniques and creating electros pun nanofibers to suit or enable numerous applications has advanced remarkably (4). The electros pun nanofiber nonwoven materials have an exceptional pore-interconnectivity and an extraordinarily high surface-to-mass (or volume) ratio. These attributes give the nanofibers the desirable qualities for a variety of cutting-edge applications, in addition to the functions and

surface chemistry of the polymer itself (5). The multilayer polymer coating of the nanofibers permits constant outflow which includes a moiety which is active. The nanofibers can be further personalized to transport numerous pharmaceuticals. In order to transport medicines like antibiotics and anti-cancer, DNA, RNA, proteins, and growth factors, electros pun nanofibers made of polymers are implemented (6). To elicit the desired therapeutic effects, nanofiber-based drug-delivery devices are broadly applicable for precise drug release based on target location and timing. On average there could be an impact from the same delivery system on the release of kinetics if there is a change in nano formulation in a particular drug (7). Under optimal conditions, electrospinning is a simple, reasonably priced, and fascinating fiber-forming method that requires little effort (8) innovations in electrospinning-based nanomaterials for biomedical uses, including tissue engineering, mats with antimicrobial property, patches for quick hemostasis and wound dressings, and using drug delivery systems (9). Nanofibers have been produced by electrospinning for use in a variety of biomedical and dental applications, including the regeneration of teeth, the therapy of wounds, and the prevention of dental caries.In order to create scaffolds for the regeneration of dental tissues such dentin, periodontium, oral mucosa, bone, and cartilage, a variety of polymers and composite materials have been electrospun. In-depth investigation into the manufacturing process, characteristics, and functionality of electrospun nanofibers has already produced favorable findings for the expected biomedical applications (10). A Flavonoid which is widely found in foods is quercetin which exhibit many properties like antibacterial, anti-inflammatory and anti-cancer. Prevents oral mucosal lesions. The antioxidant quercetin prevented radiation-induced oral mucositis. By inhibiting numerous pathogenic processes by targeting Oral mucositis brought on by radiation quercetin targeting BMI -1.quercetin has the potential to be used as a preventative measure as well as a treatment (11). Minor mouth ulcers are cured by quercetin cream on the skin completely healed and the pain was eased. Patients expressed appreciation for the topical quercetin's ease of use and expressed no dislike for its consistency or flavour. Quercetin is a promising novel supplementary therapy for treating common aphthous ulcers that is safe, well-tolerated, and extremely effective (12). Different plant extracts have been tested for their ability to promote wound healing through both in vitro and in vivo research. Traditional medicinal herbs have long been utilized in the treatment of wounds. Their phytochemical components, primarily quercetin, contribute to their capacity for healing in the treatment of wounds. Quercetin has significant biological implications that can speed up the healing of lesions (13). Due to its multifunctionality, which includes antioxidant, antibacterial, anti-inflammatory, and antineoplastic effects, quercetin has found increasing acceptance as an oral therapy. In addition, because quercetin has a low bioavailability, tremendous efforts have been undertaken to overcome the issue of its restricted scope in drug delivery systems (14). Due to its limited permeability, instability, less bioavailability, and decreased solubility, the pharmaceutical sector can only use QC to a limited extent. Numerous methods have been used to increase the bioavailability of QC, including theusing drug delivery methods that appear to offer higher solubility and bioavailability, such as inclusion complexes, liposomes, nanoparticles, or micelles (15). Quercetin may have positive effects on dental health, according to in vitro and animal studies, but larger clinical trials are required to determine whether it is safe and effective for use in people. There is not enough clinical data to support certain uses of quercetin in the context of oral mucosa. Quercetin showed many allergic reactions. Oral mucosal membrane should be repaired after an

abscess or any surgical procedure to prevent accumulation of bacteria which could lead to infection. It was done by using antibiotics, anti-inflammatory drugs, B cell therapy, but had symptoms like eye pain, fever, and chills so, we are developing an adhesive membrane of quercetin for oral mucosal repair. By developing an adhesive nanomembrane of quercetin which has antioxidant and anti-inflammatory properties and it is a wound healer, it helps in fast healing of wounds. Aim of this study is to synthesize the nanomembrane of quercetin for oral mucosa repair (16, 17).

MATERIALS AND METHODS

Gelatin and PVA were combined in an 80:20 ratio with the addition of quercetin and gently mixed overnight. To formulate the polymer solution for nanofiber synthesis, 12% w/v gelatin was dissolved in a 50% acetic acid solution and stirred for 12 hours (18). Bioactive quercetin was incorporated at a concentration of 5% weight/volume and stirred for one hour. The fabrication of nanofibers was carried out using Holmarc electrospinning equipment. The horizontal collecting plate was positioned 10 cm away from the spinneret tips. The spinneret was linked to a high voltage source, while the collection plate was connected to a ground electrode. Electrospinning parameters for the polymer solution were optimized based on preliminary testing, set to 14 kV voltage and a flow rate of 0.5 ml/h (19).

The nanomembrane was characterized using SEM, FTIR, Water Contact Angle, and Oral delivery. SEM image taken using JEOL JSM IT 800 after 30s Platinum coating. FTIR using Bruker Alpha II model – from 500 to 400 cm-1 at a resolution of 4 cm-1 within the range of 32 scans.

Wettability Test

The sessile drop method was used to assess the wettability of the electrospun fiber mats utilizing a contact angle measuring equipment (ossila goniometer). Cut the nanofiber membranes into uniform pieces or use appropriate substrates of desired dimensions. Clean the substrates thoroughly with a suitable solvent (ethanol) and allow them to dry completely. Set up a contact angle goniometer equipped with a high-resolution camera and image analysis software. Ensure proper calibration of the equipment according to manufacturer instructions. Place the dried nanofiber membrane or substrate on a flat and level surface. Ensure that the sample surface is free from any visible defects or debris. Dispense a small droplet (e.g., 1-5 µL) of a selected test liquid (water, glycerol) onto the surface of the nanofiber membrane using a micropipette. Ensure that the droplet is deposited gently and centrally on the surface to minimize any disturbances. Use the goniometer's camera to capture a clear and focused image of the droplet on the surface of the nanofiber membrane. Calculate the average contact angle and standard deviation from multiple measurements to represent the wettability of the nanofiber membrane. Compare the obtained contact angles with those of control samples or different experimental conditions to assess the effect of surface modifications or treatments on wettability (20, 21).

Oral delivery

Oral delivery of encapsulated bioactive cells observed in artificial saliva and absorbance was measured using a spectrophotometer. Cultivate the bioactive cells of interest using appropriate cell culture techniques and media. Prepare the encapsulation material, such as alginate, agarose, or other biocompatible polymers, according to established protocols. Encapsulate the bioactive cells within the polymer

matrix using methods such as droplet-based encapsulation or microfluidic techniques. Wash the encapsulated cells to remove excess polymer and culture medium and ensure uniform encapsulation. Prepare artificial saliva solution mimicking the composition of natural saliva, including electrolytes (Na+, K+, and Cl-), buffers (phosphate), mucins, and enzymes (amylase). Adjust the pH and osmolarity of the artificial saliva solution to physiological levels to mimic oral conditions accurately. Place the encapsulated bioactive cells in a suitable container or device that mimics the oral cavity environment. Introduce the artificial saliva solution into the container to simulate oral fluid conditions. Incubate the encapsulated cells in artificial saliva at physiological temperature (37°C) for a predetermined period to simulate oral transit and exposure (22).

Sampling and Absorbance Measurement

At specified time intervals, carefully collect samples from the container containing encapsulated bioactive cells and artificial saliva. Centrifuge the collected samples to separate the encapsulated cells from the saliva solution. Measure the absorbance of the supernatant using a spectrophotometer at an appropriate wavelength specific to the bioactive cells or encapsulation material. Use a blank containing only artificial saliva to calibrate the spectrophotometer and account for background absorbance. Record absorbance values for each sample and time point (23).

DPPH radical scavenging capacity:

According to Jayan et al. (2019), the antioxidant activity of quercetin in both its un-encapsulated and encapsulated forms was assessed using the DPPH radical scavenging assay at equal concentrations (both at 25 ug/ml). Nanofibers with comparable levels of bioactive substances were put through the DPPH assay. Initially, 70% methanol was used to dissolve the encapsulates. Each sample received approximately 3 ml of DPPH stock solution (100 uM produced in methanol) before being incubated at 30+2 °C for 30 min in the dark. With the aid of a UV-Vis spectrophotometer, the solution's maximum absorbance at 517 nm was discovered. The absorbance of the DPPH solution was taken to be 100% active, and the percentage of inhibition was calculated using the equation below.

$$\text{Inhibition of DPPH (\%)} = (A_{\text{CONTROL}} - A_{\text{SAMPLE}}) / (A_{\text{CONTROL}}) \times 100$$

Where the absorbance values of the control DPPH solution and the sample, A CONTROL and A SAMPLE, respectively (24).

RESULTS AND DISCUSSION

From an electrospun machine non-woven submicron level nanofibers were synthesized. Uniform bead free nanofibers encapsulating quercetin was fabricated which is an effective adhesive drug delivery system.

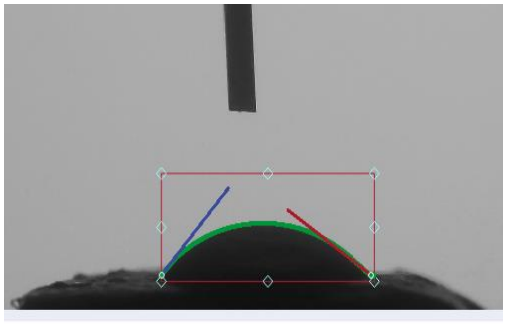

Fig 1: Water contact angle of nanofibers

Table 1: Water contact angle of nanofibers

Frame Number	0
Time (s)	0
Left Angle (°)	48.12
Right Angle (°)	33.76
Average Angle (°)	40.94
Left Contact Point (Pixel)	832.2
Right Contact Point (Pixel)	1251.5
Droplet Width (Pixels)	419.3

Low water contact angle of 48% % of quercetin loaded Gel-PVA nanofiber (Table 1) is an effective adhesive drug delivery system that confirms the effective loading of bioactive nanofibers system.

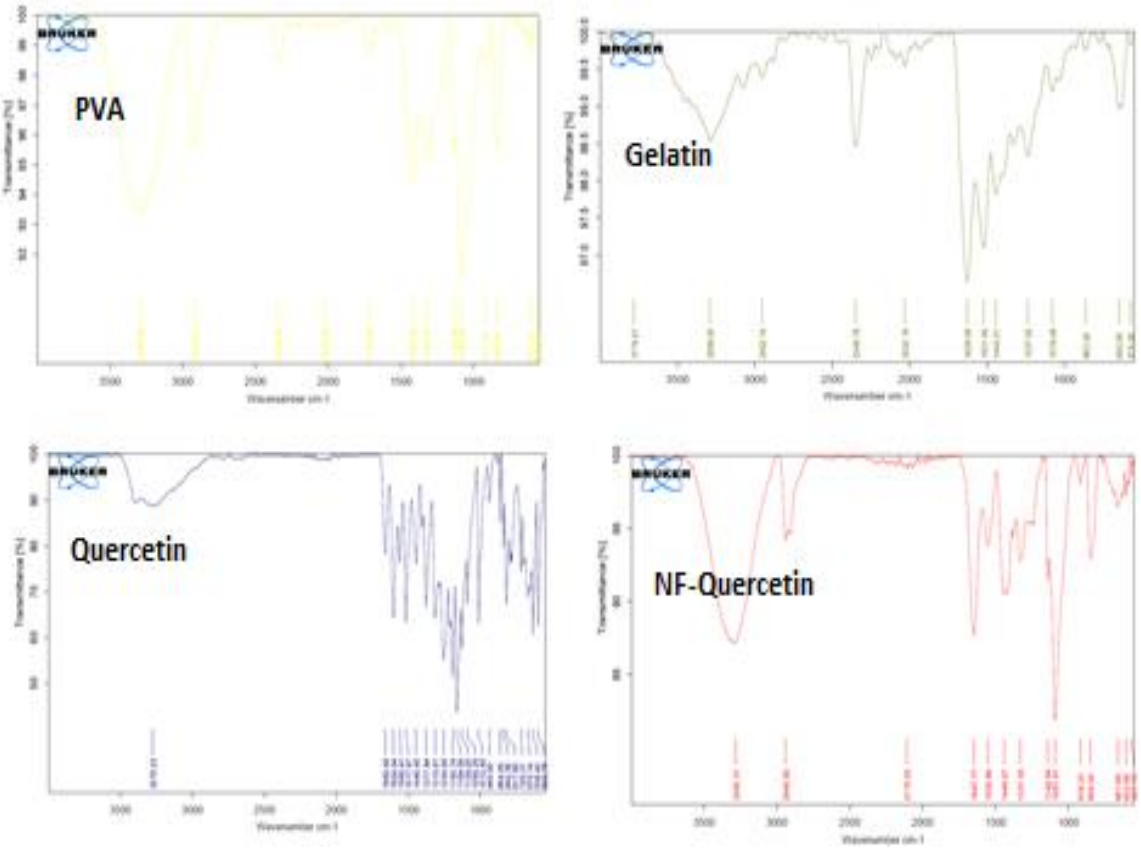

Fig 2: IR spectra A) PVA; B) Gelatin;C) Quercetin (Que); D) Nanofiber with Quercetin

FTIR peaks of native PVA, Gelatin and quercetin and quercetin encapsulated in nanofiber are given in Fig 2. Effective encapsulation of quercetin in nanofiber confirmed

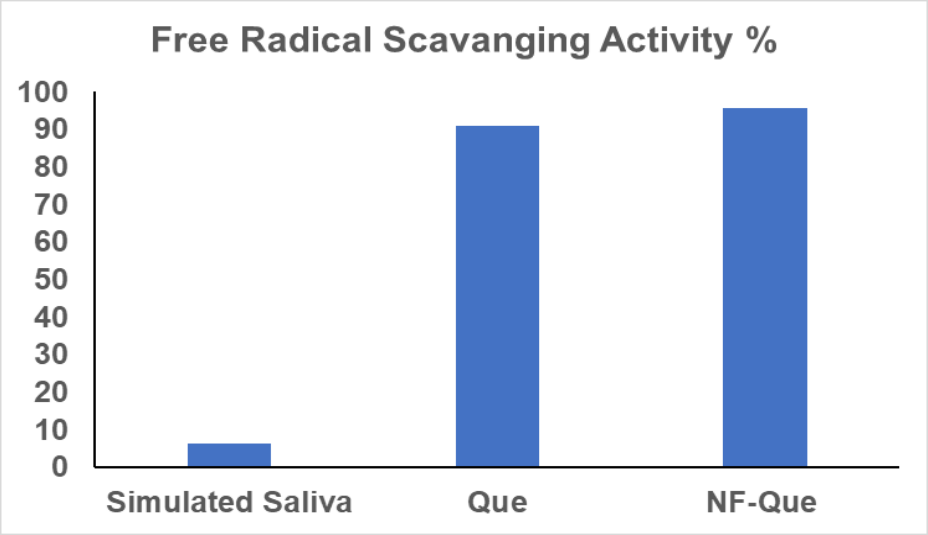

Fig 3: Anti-oxidant activity of unencapsulated and encapsulated bioactive in simulated saliva

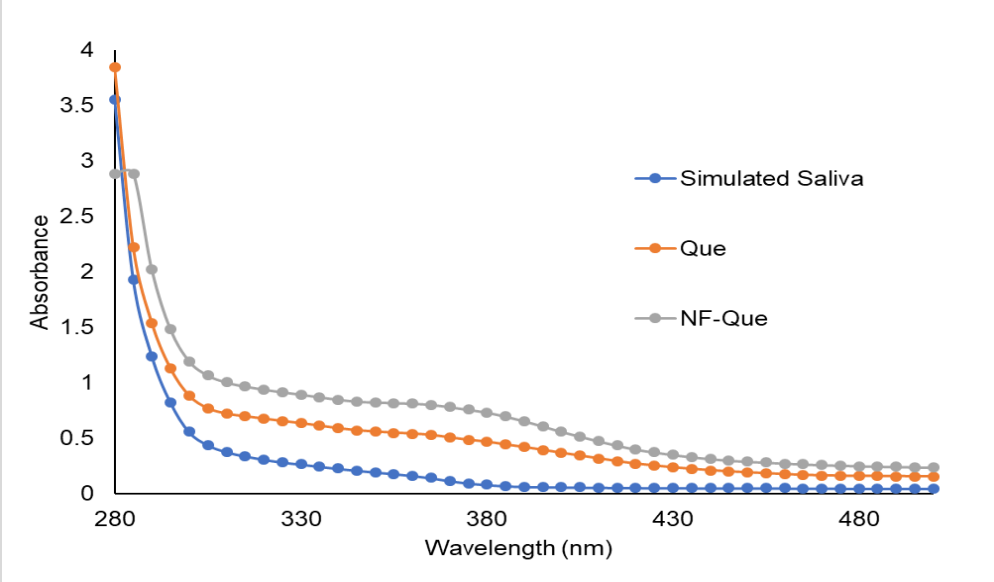

Fig 4: Absorbance of unencapsulated and encapsulated bioactive in simulated saliva

Uniform fibers were formed and it was subjected to oral salivary phase which showed enhanced solubility of the quercetin.we observed increased solubility of the quercetin and upon evaluation for antioxidant activity the encapsulated molecules showed greater antioxidant activity. Clinical research and testing is needed to confirm the adhesive nanomembrane's effectiveness and safety in people. Drug delivery system advancements may help manage and improve the release profile of quercetin from the nanomembrane, improving the patient experience.

The quercetin-loaded adhesive nanomembrane has enormous promise for enhancing oral mucosa restoration. It is an effective therapeutic choice due to its capacity to

speed up wound healing, lower inflammation, assist tissue regeneration, and have antimicrobial qualities. Although more testing and clinical trials are required to confirm its efficacy and safety in human subjects, the early results are encouraging and suggest that this nanomembrane could improve patient outcomes and oral healthcare

DISCUSSION

The discussion should begin by summarizing the effectiveness of the developed adhesive nano-membrane loaded with quercetin for oral mucosa repair. This may include a recap of the experimental findings related to wound healing, anti-inflammatory effects, and tissue regeneration observed in in vitro and possibly in vivo studies (16,31). Highlight any significant differences observed between the quercetin-loaded nano-membrane and control groups or existing treatment modalities. Discuss the potential mechanisms underlying the enhanced therapeutic effects of quercetin when delivered via the nano-membrane platform. Assess the biocompatibility of the quercetin-loaded nano-membrane by discussing results from cytotoxicity assays and any other relevant biocompatibility studies conducted. Address safety concerns related to long-term exposure to quercetin and the nano-membrane in the oral cavity. Discuss strategies employed to minimize potential adverse effects while maximizing therapeutic benefits (17, 18). Discuss opportunities for optimizing the properties of the nano-membrane, including its adhesive strength, flexibility, and drug release kinetics. Consider factors such as polymer composition, fabrication techniques, and crosslinking methods that could be further optimized to enhance performance (25,30). Explore the potential for incorporating additional bioactive agents or synergistic compounds to further augment the therapeutic efficacy of the nano-membrane for oral mucosa repair. Evaluate the translational potential of the quercetin-loaded nano-membrane for clinical use in treating oral mucosal disorders. Discuss challenges and considerations related to scaling up production, regulatory approval, and commercialization (20,28)

Propose potential clinical scenarios where the nano-membrane could be applied, such as the treatment of oral ulcers, mucositis, or post-operative wound healing, and discuss the expected benefits and limitations in each context. Compare the quercetin-loaded nano-membrane with existing therapies or conventional treatment modalities for oral mucosa repair, such as topical gels, mouthwashes, or systemic medications (26,29). Highlight the advantages of the nano-membrane approach, such as targeted drug delivery, sustained release kinetics, and enhanced tissue penetration, compared to traditional treatments. Identify potential areas for future research and development to further advance the field of oral mucosa repair using nanotechnology-based approaches. Suggest directions for mechanistic studies to elucidate the underlying biological pathways involved in the therapeutic effects of quercetin-loaded nanomembranes. Discuss opportunities for interdisciplinary collaboration and integration of emerging technologies, such as tissue engineering and 3D printing, to create more advanced and personalized oral mucosa repair solutions (27).

CONCLUSION

Summarize the key findings and insights gained from the discussion, emphasizing the potential of the quercetin-loaded nano-membrane as a promising therapeutic approach for oral mucosa repair. Reiterate the significance of the study's findings in advancing the field of oral medicine and highlight the importance of continued research

and innovation in developing effective and safe treatment modalities for oral mucosal disorders.

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