Fabrication and Evaluation of Antibacterial Analysis of a Seaweed Biopolymer Based Hydrogel for Periodontal Therapeutic Applications

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Abstract
AIM : The aim of the study is to estimate the biological and mechanical properties of hydrogel fabricated from the mixture of sea weeds. Introduction: Hydrogel is a promising material for local antimicrobial application. Hydrogel refers to a kind of biomaterial synthesized by either synthetic or biopolymer. Carrageenan is used for coughs, bronchitis, tuberculosis, and intestinal problems. The ability to combine agar with chitosan and carrageenan with inorganic substances such as tricalcium phosphates and hydroxyapatite makes these matrices also useful for oral delivery, burn wound healing and bone TE. Chitosan has potent, much adhesion, homeostatic action, film-forming ability, biodegradability and anti-microbial action. Materials and methods: In a flask 0.1g of chitosan is added to the distilled water and heated for an hour. Meanwhile the solubility is checked. After checking the solubility of 0.1g of Agar, Rutin and 0.2g of carrageenan added to 100 ml of chitosan. Mixed solution is kept Ina magnetisms stirrer for 1 hour at 100 degree Celsius. Anti-microbial activity : Agar well diffusion and Disc diffusion method is used. Then incubated for 37 degrees Celsius for 48 hours. The zone of inhibition is recorded. Mechanical properties: (FTIR) Fourier transform infrared and contact angle were measured Results: The results showed that the hydrogel formed from the mixture of seaweed has potential antimicrobial activity against streptococcus mutans as the concentration increases and it also showed that the physical properties of the hydrogel derived from seaweed had more promising characteristics as the concentration increases. Conclusion: Seaweed will provide striking bioactives, which can be used as a promising medicine for the treatment of human diseases, orelse it can be used as new antimicrobial agents to be the replacement for synthetic antibacterial agents used in various applications. Hydrogel prepared from the mixture of seaweeds can be used in the application of drug delivery; it had shown that it had promising antimicrobial activity and physical properties.
Keywords: Antimicrobial properties, Hydrogel, seaweed extract.

1. Introduction

Periodontitis is one the most common oral diseases affecting millions of individuals worldwide. According to WHO global burden of oral disease survey 2019, severe periodontal diseases are estimated to affect around 14% of the global adult population, representing more than one billion cases worldwide(1,2). It is now well accepted that periodontal disease is strongly associated with systemic diseases such as diabetes, cardiovascular disease, Alzheimer's disease, and other inflammatory comorbidities(3,4). This greatly affects the masticatory efficiency and esthetics which has an impact on the health and quality of life of the patient(5). This highlights that early diagnosis and prompt treatment is the need of the hour(6).

With dental plaque being the primary etiologic factor for the initiation and progression of the inflammatory process, elimination of dental plaque becomes imperative(7). Though mechanical debridement with scaling and root planing is the gold standard nonsurgical treatment, severe cases warrant adjuvant modalities. With some bacteria like porphyromonas gingivalis, actinobacillus actinomycetemcomitans

doi.org/10.31838/hiv23.03.44 Received: 20.10.22, Revised: 22.11.22, Accepted: 10.12.22.

having the ability for intra-epithelial colonization, chemotherapeutics in the form of systemic antibiotics, local drug delivery agents and mouth washes are necessary(8). Due to abuse of systemic antibiotics leading to emergence of bacterial resistance and associated systemic complications, the search for alternative antimicrobials is on.

Recently, the use of hydrogels for dental applications has drawn great attention. Hydrogels are three-dimensional (3D) networks formed by molecular chains embedded in a water-rich environment(9,10). With properties like antimicrobial, antioxidant, anti-inflammatory and biocompatibility, they are ideal for drug carrier applications in periodontal therapy. They are obtained from both natural and synthetic sources. Marine seaweeds are a rich source of natural polysaccharides and proteins with excellent hydrophilicity and biocompatibility that are ideal for hydrogel fabrications. They are rich sources of chitosan, collagen, gelatin, fucoidan, alginates that are used for the preparation of hydrogels(11). They have physical properties like high porosity three-dimensional structure with excellent hydrophilic ability and adjustable viscoelastic behavior. They mimic the extracellular matrix such that it is favorable for cellular attachment, proliferation and differentiation(12). Hydrogels can be customised according to requirements by addition of drugs and growth factors. With inclusion of cells of interest and growth factors they can also be utilised for periodontal tissue engineering applications(13). In this study, we prepared a hydrogel with fucoidan, gelation, chitosan, guar gum and analysed its antimicrobial and mechanical properties for periodontal applications. Our team has extensive knowledge and research experience that has translate into high quality publications(14–23)

2. Materials and Methods

The study was conducted at the Department of Biomaterials at Saveetha Dental College, Chennai. The hydrogel was prepared using fucoidan, gelatin, chitosan and guar gum as the components.

Source Of Biomaterials

Gelatin, chitosan and fucoidan were obtained from Himedia Laboratories Pvt Ltd , Mumbai, India. Chitosan from shrimp cells with molecular weight 3800 - 20000 Daltons and 75 % degree of deacetylation was used. Guar gum powder (Nature's harvest) was obtained from PC Industries.

Fabrication Of Hydrogel

The injectable hydrogel was prepared with fucoidan(0.1%), gelatin(0.1%), chitosan(0.1%) and guar gum (0.1%) as components. The chitosan extracted from the shrimp shells with a high molecular weight of 3800-20,000 daltons was used. Chitosan was dissolved in 0.1% acetic acid. Distilled water was added to the mixture to obtain the required concentration. The mixture was then stirred at 65 degrees and 500 rpm for 20 minutes. Fucoidan, guar gum were then added to this mixture, stirred and allowed to mix thoroughly for 2 hours. Gelatin was added to the entire components to obtain a hydrogel consistency. The prepared hydrogel was then subjected to antimicrobial analysis.

Antimicrobial Testing

Zone of inhibition (ZOI) measurement

Antibacterial activity of the seaweed derived hydrogel were assessed against Gram-positive bacteria streptococcus mutans and staphylococcus aureus using Mueller–Hinton agar (MHA; Himedia, Mumbai, India) plate by agar well-diffusion technique. The MHA was prepared in double distilled water (pH 7.0) and sterilized in autoclave at 121 ˚C for 15 min. Then, the sterilized MHA was poured into the perti plate and allowed to solidify at room temperature in laminar flow. Inoculum containing 106 cfu/mL of the freshly prepared bacterial culture was spread onto the MHA plates with a sterile cotton swab moistened with the suspension of the respective microbial culture. Then, three wells (9 mm in diameter) were punched into the MHA medium. 2 wells were filled with different concentrations (100 µg, and 25 µg) of the prepared hydrogel with the help of micropipette and kept at room temperature for 4 h to allow diffuse compound into the medium. The third well was filled with erythromycin 5µg for streptococcus mutans and cephalexin 5µg for staphylococcus aureus as control drug. Then, the culture plates were incubated at 37 °C for 24 h. After incubation, the diameter (mm) of the zone of inhibition was recorded in each plate. The results were expressed as mean value with standard deviation (SD) of triplicate experiment.

3. Results

Antibacterial Testing

(i) Zone of inhibition (ZOI) and minimum inhibitory concentration (MIC)

The antibacterial activity of the prepared hydrogel was assessed against Gram-positive organisms streptococcus mutans and Staphylococcus aureus at two different concentrations (100 µg, and 25µg). The agar disc diffusion method was used. As shown in Fig.1, the result evidently indicates that the hydrogel at both concentrations showed a slightly positive zone of inhibition against both bacteria. The zone of inhibition increased in corelation to the concentration of the drug though it was lower than the control drugs used.

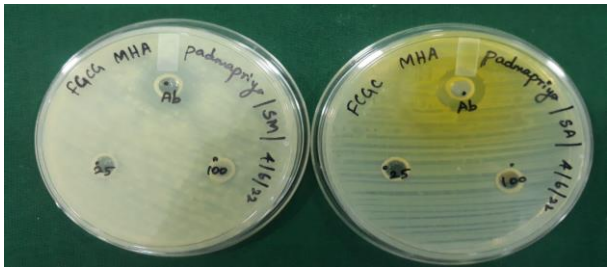

Fig1: The hydrogel poured petridishes were placed with streptococcus mutans and staphylococcus aureus, and the zone of inhibition is seen.

4. Discussion

Periodontal disease is a polymicrobial infection affecting the supporting tissues of the teeth, resulting in pocket formation, gingival recession, severe bone loss ultimately leading to tooth loss(24)(25). Though mechanical debridement removes the primary etiology, severe cases require additional therapy with systemic and local antibacterial agents to eradicate the intracellular bacteria(26)(27). Intracellular bacterial colonisation with porphyromonas gingivalis, actinobacterium actinomycetemcomitans and fusobacterium nucleatum act as a constant source of reinfection. Though systemic antimicrobials are effective in the treatment, they are associated with side effects and emergence of bacterial resistance(28). To overcome these challenges hydrogels have been developed to act as local drug carriers.

In addition to being used as water or drip adsorption pads in the food packaging industry, hydrogels have a long history of use in agriculture and biomedical fields such as tissue engineering, wound dressing, drug delivery, biosensors, and sorbents for the removal of dyes and heavy metals. Recent studies have drawn attention towards marine seaweed biopolymers-based hydrogels due to their excellent biocompatibility, biodegradability, safety, antimicrobial, antioxidant, anti-inflammatory properties. In the present study a hydrogel was prepared using focidan, chitosan, gelatin, guar gum and its antimicrobial efficacy was analyzed(29).

Chitosan is an abundant linear polysaccharide and a cationic polyelectrolyte present in nature. It is present abundantly in the exoskeleton of marine organisms and crustaceans. The interesting characteristics of chitosan rely on its cationic nature and high charge density in solution, which confers pH responsiveness and mucoadhesive properties(30). It has intrinsic antimicrobial properties and has shown to inhibit the growth of a fairly diverse range of bacteria. Similarly, fucoidan refers to a family of sulfated polysaccharides isolated from several brown algae and marine invertebrates(31). Fucoidans exhibit some newsworthy pharmacological effects such as antithrombotic, antitumoral, antiviral, immunomodulatory, antioxidant, and anti-inflammatory activity. These properties have been attributed to various factors such as high sulfate content, molecular weight and structure, type of sugar content etc(32). In reality, these matrices are especially helpful for oral delivery and burn wound healing due to the ability to combine fucoidan with other polymers like chitosan and gelatin as well as inorganic components like tricalcium phosphate and hydroxyapatite (HAp) (eg, micro/NPs, fibres, and films). Guar gum (GG), a non-ionic polysaccharide, is a promising chemical because of the many uses it may be put to. It provides stabilisation and thickness in liquid pharmaceutical formulations as well as acting as a binder and disintegrating agent in solid pharmaceutical formulations. Gelatin is made from collagen that has been taken from the skin, bones, and connective tissues of animals including domesticated cattle, fowl, pigs, and fish. Collagen is then partially hydrolyzed to generate a collection of peptides and proteins called gelatin. Some of the bonds between and between the constituent proteins are disrupted during hydrolysis. Its chemical make-up closely resembles that of its parent collagen in many ways. Gelatin used in photographic and pharmaceutical products is typically made from pig skin and cattle bones. In order to create hydrogel with the proper consistency, gelatin is used because it has good hydrophilicity and gelation properties.

In the present study, the hydrogel showed good antibacterial activity against common oral bacteria staphylococcus aureus and streptococcus mutans in comparison with the control drug. The zone of inhibition increased in correlation with the concentration of the hydrogel. This may be attributed to the antimicrobial properties of the component polysachharides chitosan, fucoidan and guar gum. The high sulfate content in the polysachharides may have played a role in the antibacterial activity. There are many examples of hydrogels based on fucoidan and chitosan in the literature that were created primarily as a solution for wound healing. Fucoidan and chitosan scaffolds have also been proven to be effective biomaterials for bone regeneration. Studies have proven the advantageous effects of seaweed biopolymers-based hydrogels in periodontal therapeutic and tissue engineering applications. More research should be conducted to further explore the role of seaweed biopolymers hydrogels in the treatment of periodontal conditions.

5. Conclusion

In the present study, the hydrogel prepared from the seaweed has potential antimicrobial activity against oral pathogens, so it can be used in local drug delivery system and further studies have to be done in large sample sizes for better results. Further invitro and invivo studies has to be done to explore biological interactions and drug delivery and tissue engineering.

6. Acknowledgement

The authors would like to acknowledge the help rendered by Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai.

Conflict Of Interest

No conflict of interest

Source of Funding

The present project is funded by

1. Saveetha Institute of Medical and Technical Sciences
2. Saveetha Dental College and Hospitals
3. Saveetha University

4. Lakshmi Farm Seeds, Virudhunagar.

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