Development of Mucoadhesive Bandages for Oral Drug Delivery Applications

A Dissertation Submitted to IIT Madras and SCTIMST in Partial Fulfilment of the Requirement for the Degree of

Master of Technology in Clinical Engineering

Jointly offered by

Indian Institute of Technology, Madras

Christian Medical College, Vellore

Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum

Research accomplished by: **Ankita Modi**

Under the supervision of

Dr. Manju S.

Scientist E

Dept. Biomaterial Science and Technology, Biomedical Technology Wing

Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum India-695012

June 2023

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, तिरुवनन्तपुरम- 11, केरल
Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram- 11, Kerala
(भारत सरकार क□□ धीन राष्ट्रीय महत्व का संस्था)
(An Institution of National Importance under Government of India)
दूरभाष सं./Telephone No. 0471-2443152 फाक्स/Fax 0471-24464332550728
ई-मल/E-mail :sct@sctimst.ac.in वबसाइट/ Website : www.sctimst.ac.in

Dr. Manju. S
Scientist E
Division of Dental Products
Dept. Biomaterial Science and Technology
Biomedical Technology Wing
SCTIMST, Trivandrum-695012
Phone: +914712520341; Mobile: +919539263017
Email: manjus@sctimst.ac.in

14th June 2023

CERTIFICATE

This is to certify that dissertation entitled **"Development of Mucoadhesive Bandages for Oral Drug Delivery Application"** is an authentic record of the work carried out by **Ms. Ankita Modi,** under my supervision in partial fulfilment for the degree of Master of Technology in Clinical Engineering and no part of this dissertation has been presented before for any other degree.

Trivandrum

Dr. Manju.S
M.Tech Project Guide

श्री चित्रा तिरुनाल आयुर्विज्ञान और प्रौद्योगिकी संस्थान, तिरुवनन्तपुरम- 11, केरल
Sree Chitra Tirunal Institute for Medical Sciences and Technology,
Thiruvananthapuram- 11, Kerala
(भारत सरकार क□□ धीन राष्ट्रीय महत्व का संस्था)
(An Institution of National Importance under Government of India)
दूरभाष सं./Telephone No. 0471-2443152 फाक्स/Fax 0471-24464332550728
ई-मल/E-mail :sct@sctimst.ac.in वबसाइट/ Website : www.sctimst.ac.in

DECLARATION

I hereby declare that the project work entitled **"Development of Mucoadhesive Bandages for Oral Drug Delivery Applications**" is submitted to SCTIMST in partial fulfilment of the requirements for award of the degree of Master of Technology in Clinical Engineering is a bonafide record of original work done by me under the guidance of **Dr. Manju S.**, Scientist E, Dept. Biomaterial Science and Technology, Biomedical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum.

Trivandrum
14th June 2023

Ankita Modi

ACKNOWLEDGMENTS

I would express my sincere thanks to my project guide Dr. Manju S. for her guidance, timely suggestions and support throughout the project.

I thank Dr. Maya Nandkumar, and Mr. Nagesh for their feedback as external examiners. I extend my thanks to Dr. Deepu, Mr. Sreejith, Ms. Anusree V.S., Mr. Boney Simon, Ms. Reshma, and all staff, Division of Dental Products for their support.

I thank Dr. Renjith S., Ms. Nimmy, Central Analytical Facility for their support in doing mucoadhesive studies. I thank Dr. Rekha M.R and Ms. Rajalakshmi, Biosurface Technology for their support in probe sonication and lyophilisation.

I thank Dr. Manoj Komath, the Head, Department of Biomaterials Science and Technology for all the administrative support.

I thank Dr. Umashankar, Dr. Sachin J. Shenoy, Division of *in vivo* models and testing for their kind help and support in getting tissue samples for ex vivo experiments.

I thank Dr. Sabareeswaran, Histopathology Division for all his kind support. I thank Dr. Lynda V Thomas for her support in doing contact angle measurements. I thank Dr. Anugya Bhatt, Ms. Safena and Ms. Priyanka, Thrombosis Research Unit for all their support.

I thank my project coordinator Dr Arun K Thittai for his guidance and supervision throughout the program. I thank Dr Roy Joseph, Dean and course coordinator, M.Tech Clinical Engineering, SCTIMST for providing all his support throughout the M.Tech. program.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Oral administration is one of the most preferred modes of drug transmission due to it being non-invasive, convenient and ease of patient compliance. Oral mucosa is a potential site for both local and systemic delivery of therapeutics. Mucoadhesive drug delivery systems with customizable release profiles have gained considerable interest among formulation scientists to improve clinical outcomes of drugs. Oral environment is highly dynamic in nature. Multiple gel formulations are available in the market for treatment of various oral conditions such as oral lesions, ulcers, desquamative gingivitis etc. However, retention of drugs in the target gingival region is challenging as those common gel formulations can easily wipe out from the oral moist environment by food, liquids and even saliva. There is a massive need for formulations that can enhance the retention of drugs in the target gingival region. This study aimed to develop a mucoadhesive membrane that could enhance retention of medication in the oral environment for a period of atleast 8h. The mucoadhesive bandage is a three-layer sandwich system made of mucoadhesive thiolated gellan gum, drug particles embedded middle layer made of interpenetrating network of zein-gellan gum film and backing layer made of polycaprolactone that assures unidirectional flow of drug through the mucous membrane. Mucoadhesive bandage prepared using the three-layer sandwich were non cytotoxic to L929 mouse fibroblast cells. A sustained drug release profile was obtained for PLGA nanoparticle and Zein/GG-2S for a period of 8 hours. Favourable biocompatible outcomes, remarkable mucoadhesive strength and high folding endurance with strong mechanical properties confirm that the developed bandage has high potential to be translated into the market following relevant regulatory norms.

CHAPTER 1

INTRODUCTION

1.1. Oral drug delivery

Oral drug delivery is one of the most prevalent modes of dispensing drugs into a physiological system. It encompasses certain benefits like ease of pharmaceutical administration, patient comfort, cost-effectiveness and possibilities of large-scale manufacturing of oral medication forms. Approximately 60% of the usual small-molecule drug formulations commercially accessible are dispensed through the oral route. Recent estimates show that oral formulations constitute around 90% of the international market share of all pharmaceutical formulations deliberated for human consumption. Nearly 84% of the successful pharmaceutical commodities are orally dispensed and are currently valued at $35 billion having an annual growth rate of 10% [1].

The abidance of patients to oral medications is usually more as compared to diverse parenteral routes like that of intravenous, subcutaneous, intramuscular injections, inhalation for asthma medicines [2]. In addition, oral dispensing of drugs can be directed to specific portions within the gastrointestinal tract for localized ailment of diseased cases like that of stomach and colorectal cancers, infections, inflammations, bowel issues, gastro-duodenal ulcers and gastroesophageal reflux complications [3].

On similar lines, oral drug delivery employing the properties of mucoadhesion has shown remarkable growth due to higher residence period of the pharmaceutical dosage and its passivity to degradation by gastrointestinal enzymes [4]. Relatively high blood flow and optimum surface area aids mucoadhesive drug delivery with instant absorption and superior bioavailability. As this method of delivery through the mucous layer surpasses the hepatic

metabolism and escapes from being degraded by gastrointestinal enzymes, it contributes to the transmission of a large number of high molecular weight sensitive molecules like that of peptide and oligonucleotides. Mucoadhesion is defined as the desirability of adherence of pharmaceutical drugs that are to be transmitted via the mucous membrane uniformly over a period of time. This transmission is a consequence of the interaction between medicinal dosage form and secreted mucous or mucosal membrane. The properties of mucoadhesion facilitate a strong bonding of the formulation with the oral mucosa and greater residence period [5]. This mechanism can be better explained via three phases. The first phase facilitates formation of a close adherence between the formulation and the mucous. It is responsible for the proliferation of the formulation over the biological substrate. Second phase leads to an interpenetration between monomeric units and mucous glycoproteins. Mucins arise with the development of physical entanglements amidst the two macromolecular species. Following this, in the third phase, consolidation is achieved that entail the strengthening of polymer-mucin adherence due to establishment of intermolecular interactions like Van der Waals forces, hydrogen bonding, disulphide linkage and electrostatic interactions [6-7].

1.2. Localized drug delivery

Localized drug delivery has become one of the most researched areas. It is a form of targeted delivery that enhances the concentrations of the prescribed drug at the primary location inside the body in correlation to other body tissues. Most of the oral diseases like leucoplakia, candidiasis, ulcers, desquamative gingivitis, and xerostomia require localized drug action for sustained period of time. Localized drug delivery helps to reduce pain, inflammation, dryness, etching by acting locally and instantly at the targeted area. This leads to increased medicinal dose with faster effects and reduced complications as it doesn't affect the other tissues. It is beneficial in the case of those drugs that have limited half-life period. The action of drug is specific and instantaneous. In certain cases, the drug needs to be safeguarded from the

digestives enzymes. Various forms of localised oral drug delivery systems are available which includes patches, nasal sprays, gels, tablets, foams, fibers, microspheres etc. Periochip is one such biodegradable chip encapsulating 2.5 mg of chlorhexidine digluconate to treat periodontitis [8]. The chip is placed using forceps following root surface debridement. The gelatin matrix releases its drug content steadily for a period of 7 to 10 days. Actisite® (tetracycline periodontal) periodontal fiber is available in the market that releases tetracycline hydrochloride for 10 days [9]. However, in this formulation, the fibers have to be removed once root canal complications are overcome. Arestin is another product in the form of dry powder (microspheres) that contains minocycline hydrochloride embedded inside PLGA, used to reduce pocket depth in root canal treatment [10]. Atridox® (doxycycline hyclate) is a liquid bio-absorbable product that solidifies and exhibits controlled drug release for 7 days [11]. It is used to control bleeding, periodontitis and reduction in the probing depth. All these products have remarkable drug release profile.

Drug delivery through the keratinized mucous membrane is highly desirable for localised oral disease treatment. However, none of the oral drug delivery product in the market shows stability for a long period. The mucoadhesive gel formulations available in the market gets washed off easily due to the dynamic oral environment and high mucous turn over [12]. The gel system available in the market does not have a high adherence at the desired location. This requires frequent application of the formulation to achieve desired results. Pardeshi et al., developed valsartan loaded spray dried mucoadhesive intranasal microspheres. The formulation exhibited 74.98% of drug permeation within 90 minutes [13].

1.3. Mucoadhesive polymers and Mucoadhesive theories

Over the years' development of various types of mucoadhesive formulations in the form of semi-solids like gels, tablets and films had gained popularity [14]. They comprise different

forms of hydrophilic polymers of natural origin like that of sodium alginate, gums of xanthan and guar, pectin and chitosan. All these polymers show mucoadhesion but the force of adhesion is extremely poor [15]. Hence, synthetic or semi-synthetic polymers are in focus that provide better mucoadhesive properties after certain chemical modification involving thiolation. Few examples of synthetic polymer include cellulose derivatives, polyacrylic acid, polyhydroxyethyl methylacrylate, polyethylene oxide (PEO), polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA) and thiolated polysaccharides.

Mucoadhesive polymers consist of many functional groups, like that of hydroxyl groups or unionized carboxylate groups etc. In addition, molecular weight and chain flexibility are crucial to enhance interpenetration between polymer chains and mucus. It is reported that optimum degree of hydration, chain entanglement with mucins, sustained system cohesion and ionizable moieties facilitate mucoadhesion. Components like saliva secretion, food intake, local pH, and constituents of the delivery systems impact mucoadhesion. With an aim to enhance permeation via oral mucosa, the scientific community focused on development of adhesive drug conveyance techniques with polymers that have mucoadhesive properties [16].

The phenomenon of mucoadhesion is usually classified into two steps, namely, the contact stage and the consolidation stage. The first stage is achieved by bonding between the mucoadhesive and mucous membrane, followed by proliferation, protuberance and swelling of the formulation, resulting in its strong bonding with the mucus layer. In case of oral drug delivery, the motion of organic fluids in the oral cavity, facilitated by brownian motion plays an important role. When the polymeric system is in close contact with the mucous surface, it encounters repulsive forces like that of osmotic pressure and electrostatic repulsion. Similarly, it will be supported by attractive forces such as van der Waals forces and electrostatic attraction. In order to achieve efficient mucoadhesion, the polymeric particle must conquer this repulsive barrier [17-18].

During the process of unification, mucoadhesive polymeric substance gets triggered in the residence of moisture that plasticizers the setup. This facilitates the mucoadhesive molecules to escape and connect by feeble van der Waals and hydrogen bonds. Basically, the process of unification can be described by two important theories, namely, diffusion theory and dehydration theory. Diffusion theory states that mucoadhesive molecules and glycoproteins of the mucous coherently interrelate because of interpenetration of their chains and establishment of secondary bonds. In order to favour the process, mucoadhesive system has characteristics that favour both chemical and mechanical interlinkages. For instance, certain molecules having building moieties (–OH, –COOH) that support formation of hydrogen bonds with an anionic surface charge, high molecular weight, pliable chains and surface-active features. They initiate proliferation throughout the mucous layer and exhibit mucoadhesive characteristics [19].

In context to dehydration theory, substances that easily gellify in aqueous conditions, when kept in close proximity with the mucus leads to its dehydration because of the difference in osmotic pressure. Variation in concentration gradient extracts water into the formulation before establishment of osmotic equilibrium. This activity blends the formulation and mucus, thereby enhancing contact time with the mucous membrane. Hence, the velocity of water with which it descends gives rise to strengthening of the adhesive bond, avoiding the interpenetration of macromolecular chains. Nevertheless, the dehydration theory doesn't hold good for solid formulations or extensively hydrated forms [19].

Mucoadhesion can be explained by electronic theory as well. This is dependent on the assertion that both mucoadhesive and biological substrates have opposing electrical charges. Hence, when both these entities come in close proximity, they exchange electrons that cause establishment of a double electric layer at the interface. The force of attraction within this electronic double layer affects the mucoadhesive strength. Concept of mucoadhesion can be further explained by the adsorption theory. This explains that a mucoadhesive system attaches

itself to the mucus by physical interactions like that of van der Waals and hydrogen bonds, electrostatic desirability or hydrophobic interactivity. For instance, hydrogen bonds are ubiquitous interfacial forces in polymers having carboxyl groups. These forces turn out to be very crucial in the process of adhesive interaction. Despite being individually weak, numerous interactions due to hydrogen bonding leads to an intense global adhesion [19].

1.4. Different form of oral drug delivery devices

There are various pharmaceutical technologies associated with drug delivery systems like that of polymeric nanoparticles, nanocapsules, nanogels, micelles and liposomes. The primary aim of all such delivery systems is to improvise oral drug absorption. Nanocarriers are colloidal nanoparticles tuned for the delivery of specified dosage of drug to the target site [20]. Size of nanocarriers range between 1 and 100 nm in diameter [21-22]. These nanocarriers render superior biocompatibility because they are passive, thereby, contemplated as a secured approach. They exhibit higher bioavailability with sustained release of drug surpassing the endosome–lysosome system [23]. Certain alteration of the physicochemical features of nanocarriers like that in surface, composition, shape can tune their activity with reduced secondary effects [24]. Hence, it provides a plethora of consequences in the field of drug delivery. Despite a diverse group of nanocarriers being developed, only a few exhibit distinguished features to deliver drugs to the targeted site. Few distinct characteristics of nanocarriers include superior bio-distribution and pharmacokinetics, augmented stability, diminished toxicity with well sustained and targeted drug delivery [25-26]. In general, nanocarriers have a high surface to volume ratio. They are broadly classified as organic, inorganic and hybrid nanocarriers. They can be tuned to conjugate a range of drugs, including ligands for the purpose of drug delivery. Since the beginning of 1990, solid lipid nanocarriers were employed as a befitting carrier for dispensing lipophilic drugs. Solid lipid nanocarriers are synthesised by the mixing of melted solid lipids in water followed by stabilization through

addition of emulsifiers. This is achieved by micro-emulsification or via high pressure homogenization [27-28]. The solid configuration of lipids at room temperature such as free fatty alcohol or acids; steroids or waxes; mono, di, or triglycerides are usually employed for the synthesis of solid lipid nanocarriers [29]. With respect to the production environment and composition, the drug molecules can be integrated into the matrix, shell or core of the solid lipid. Studies identified polymer-lipid hybrid nanocarrier as an efficacious fount for oral drug delivery [30-31]. Nanocapsules are like reservoirs that dissolve and disperse the drug dosage present in the core of the polymer. Nanospheres encompass the pharmaceutical ingredient within the polymer matrix. Both these forms can chemically coalesce or adsorb the pharmaceutical ingredient on its shell [32].

CHAPTER 2

LITERATURE REVIEW

2.1. Gellan gum based drug delivery systems

Mucoadhesive polymers are highly effective for the development of oral, nasal, buccal, or ocular drug delivery systems as it can provide enhanced retention of drug in the mucosal layer [33]. The mucosal layer is made up of mucus, a viscoelastic fluid that lines the visceral organs. mucoadhesive polymers have a strong interaction with mucus glycoprotein membranes via multiple interactions including disulfide linkage [34]. Various mucoadhesive systems in the form of microparticles, tablets, and paste containing drugs such as bethamethasone disodium phosphate, hydrocortisone acetate, clobetasol propionate, curcumin, and triamcinolone acetonide has been reported [35]. Chitosan/Gelatin/Keratin composite containing hydrocortisone sodium succinate as a buccal mucoadhesive patch to treat desquamative gingivitis demonstrated a burst release of more than 75% of the drug within a period of 4 h [36]. Youssef et al., demonstrated topical application of 0.2 % hyaluronic acid gel that appeared to be effective in the control of the symptoms of oral lichen planus [37].

Since decades exopolysaccharides have exhibited remarkable potential in various pharmaceutical applications. Gellan gum is one such extracellular polysaccharide exuded by *Sphingomonas elodea* (ATCC 31461) which was earlier known as *Pseudomonas elodea*. Gellan gum contains tetrasaccharide repeat units, each having two D-glucose units, one D-glucuronic acid and one L-rhamnose. The units are connected in proportion to 2:1:1, connected by β (1 → 4) glycosidic bonds. Commercially, it is accessible in two forms, high acyl and low acyl. Gellan gum has the unique property to form gel at low concentrations as hot solutions are cooled in the residence of gel-promoting cations. Low acyl gellan gum products exhibit characteristics like that of being firm, non-elastic, brittle gels. On the other hand, high acyl gellan gum exhibits

characteristics like that of soft, elastic, non-brittle gels. Altering proportion of the two forms of gellan gum gives rise to a broad spectrum of textures for various pharmaceutical applications [38].

Gellan gum has two types of acyl substituents, l-glycerol and acetyl. Alkaline hydrolysis is employed to get rid of both of the residues, which form deacetylated, low-acetyl or low-acyl gellan gum [39]. Gellan gum solution is prepared by dissolving the same in distilled water and heating to 60° C. Following this, the solution is cooled that aids in conformation changes of the polymer chains, inducing coil-to-helix transition. High acyl gellan gum has acetyl and glyceryl groups situated on the periphery of the helix. This obstructs the polymer chain connectivity and leads to inferior packing [40-41]. Post deacetylation, cations establish bridges between the polymer chains, which gives rise to establishment of a branched network [42-46].

Physical properties of the gellan gum such as gelling temperature, gel strength, texture, clarity and rate of gel formation is dependent on the pH value, presence of mono, di or tri valent ions, type and concentration of cations. Certain gelling characteristics in various media lead to the development of controlled release forms based on gellan gum. On account of the ease in administration, buccal route is the most favoured mode of dispensing medicinal product. Drug liberated from natural hydrophilic polymer matrices is a consequence of complex interaction between swelling, diffusion and erosion [47]. In a liquid environment, polymeric gums hydrate from the periphery towards the centre, forming a swollen, mucilaginous mass that hinders penetration of the fluid into the tablet. This restricts burst release of drug molecules into the neighbouring medium.

Various forms of gellan gum are designed based on its composition, swelling characteristics and concentration gradient. Extra granular gellan gum show fastest dissolution and disintegration rate [48]. On the other hand, sustained and prolonged release formulations are

possible with hydrobhobic modifications of gellan gum [49]. With an aim to prolong the release kinetics of metformin, *Vijan et al*., employed acrylamide-grafted-gellan gum [49]. Here the drug release profile in phosphate buffer (pH 6.8) was in accordance with the Higuchi kinetic model and the release mechanism was controlled by Fickian diffusion. Total drug release was extended up to 8 hours. Another study reported with hard gelatin capsules comprising gellan gum or carboxymethyl cellulose granules with ephedrine hydrochloride [48]. The formulations were evaluated in various media, like 0.1 N hydrochloric acid (pH 1.2), simulated gastric juice (pH 1.5) and simulated intestinal fluid (pH 7.5). Drug release profile showed first order kinetics and Fickian release mechanism. Slow drug liberation was seen in the acidic environment. *Shiyani et al.,* collated the crumbling characteristics of gellan gum in the configuration of an untreated powder and post swelling in water, successive drying and further milling [50]. It was demonstrated that special treatment led to enhanced swelling and hydration of the tablets, resulting in rapid drug release.

Gellan gum formulations exhibit pH sensitivity and showed high stability in acidic media and disintegration in basic media [51-53]. Drug release rate is controlled by its solubility and the amount of the active pharmaceutical ingredient. Alhaique et al., observed that capsules having more hydrophobic drug concentration exhibit instant release at the onset followed by approximate zero order kinetics [54]. Dissolution tests in various media showed that in the acidic conditions, competition between H^+ and Ca^{2+} takes place that alters the gel structure. As a consequence, instantaneous drug release takes place. To prevent burst release of drug, different modifications were introduced including interpenetrating networks structures, chemical modifications to introduce additional inter or intramolecular interactions etc. Zhang et al., synthesised a “highly stretchable and self-healing strain sensor based gellan gum hybrid hydrogel for human motion monitoring. They prepared hybrid hydrogel through one pot method where they have dissolved acrylamide (AAm), gellan gum (GG), MBA(N,N’-

Methylenebis(acrylamide)) and UV Initiator by varying the weight composition. The mixture was stirred at 95^{o}C for 1 hr to get a transparent solution, which was further transferred to mould to get three-dimensional gel networks. They observed that the hybrid hydrogel exhibited excellent mechanical properties and showed a maximum stress of 16.8MPa which was far better than GG and PAAm hydrogel. This is mainly due to uncoupling of gellan gum double- helical clusters during deformation [55].

Mahajan et al., developed a nasal insert composed of chitosan-ondansetron hydrochloride and gellan gum. Ondansetron hydrochloride is a selective 5HT3 receptor antagonist used by patients receiving chemotherapy to prevent vomiting and nausea. Generally, it was absorbed by oral ingestion and showed a bioavailability rate of 60%. However, oral forms of antiemetics are often vomited prior to absorption. The mucoadhesive nasal insert was found to be effective as an alternative to drug delivery. The effect of mucoadhesive composite beads of gellan gum/pectin for the controlled delivery of drugs were reported. The composite beads were prepared by ionotropic gelation technique using Al^{3+} cations as crosslinker and ketoprofen as the drug [56]. Table 1 shows mucoadhesive formulation based localised drug delivery systems and its drug release profile.

Table 1: Different mucoadhesive formulations used for drug delivery applications

No.	**Polymer formulation**	**Drug release kinetics**	**Ref.**
1	Gellan gum: Pectin (4:1, 1:1 or 1:4, mass ratios) at different concentrations (3 or 4%, w/v) Drug: curcumin	Burst release at 10 min. After that, reduction in the release profile and only ~ 35 % of curcumin released out from all the formulations mentioned in the paper. 100% release observed within 720 min.	57
2	Pectin and Gellan gum	Burst release of 35-75 % for all formulations used in the study. 100% release at 12 h	58

	Drug: Triamcinolone Acetonide		
3	Low-acyl gellan gum (GELRITE; MW 2.5 × 105 Da), Carbopol 940P, PEG 400 for one study. Hydroxypropyl methylcellulose for another study Drug: Nebivolol hydrochloride	Drug release from all formulations showed more than 90% in 4 h. Drug release was relatively rapid (~60% in 1h) from gels containing less concentration of gellan gum as compared with the gels that were prepared with a higher amount of gellan gum. ~60% of the drug released within 1 h. Drug release of 90% within 4h.	59
4	Bacterial cellulose/ polycaprolactone Drug: Amoxicillin, ampicillin, kanamycin	Amoxicillin loaded patch showed 99.1 ± 0.6% cumulative release after 336 h, ampicillin loaded showed cumulative release of about 98.1 ± 0.3% after 336 h, kanamycin showed cumulative release of 98.6 ± 0.6% after 336 h.	60
5	HPMC E50 and Eudragit RS100 Drug: Glibenclamide	Membrane permeation of one formulation showed complete permeation of glibenclamide after 10 h, whereas another exhibited complete release of drug after 11 h. Other formulation showed % of permeated drug to vary between 76.15 ± 2.80% and 101.01 ±0.33%.	61
6	Carboxy-methyl cellulose (CMC) Drug: Gatifloxacin	Burst release observed up to 4 h, followed by slow release of the drug for next 24 h. Patch prepared with CMC microwave-treated for 3 min showed 45% release in 24 h, while patch formulated with untreated CMC showed 23% drug release in 24 h.	62
7	Hydroxy-propyl-methyl-cellulose, ethyl cellulose Drug: Methotrexate	Best controlled-release profile of 82.71%. due to presence of hydrophobic polymer ethyl cellulose and hydrophilic polymer hydroxy-propyl-methyl-cellulose in ratio of 1:5.	63
8	Gellan gum, hydroxyethyl cellulose, calcium chloride, propylene glycol, Tween 80 Drug: Moxifloxacin hydrochloride; clove oil to soothe pain	Drug loaded film released 28% of drug during first 3 h followed by cumulative drug release of 82% up to 48 h. Drug and clove oil loaded film showed only 19% of drug release during first 3 h.	64

9	Chitosan (90% deacetylation), polyvinyl alcohol, lactic acid Drug: Diclofenac Sodium salt	Burst release of 17.17% from film in first 5 h. Drug release observed to be sustained reaching cumulative amount of 20.18% ($p < 0.05$) at end of 30 h. There was 19% release of diclofenac from chitosan-based film in 24 h (pH 7.4).	65
10	Polyvinyl alcohol (PVA), sodium alginate (SA), hydroxy-apatite nanoparticle Drug: amoxicillin	Amount of drug released on day 3 was 43%, day 6 was 72% and day 10 was 87%.	66

2.2. Poly-(lactic-co-glycolic acid) (PLGA) based drug delivery systems

Poly-(lactic-co-glycolic acid) (PLGA) is a synthetic biodegradable polymer extensively employed in biomedical research and approved by the US Food and Drugs Administration and European Medicine Agency. Some of the advantages of using this polymer involve its biocompatibility, biodegradability, flexibility, and limited adverse consequences. The two main moieties that make up the polymer include lactic acid and glycolic acid, which can be degraded by three main chemical methods, namely, hydrolysis, oxidation and enzymatic degradation. Some of the dental applications of PLGA are tabulated in table 2. Composites in powdered form of gatifloxacin-loaded PLGA and β-tricalcium phosphate are employed in local delivery for the cure of osteomyelitis. The polymer exhibits characteristics to steadily transport gatifloxacin and possess remarkable antibacterial properties *in vitro* against *Streptococcus milleri* and *Bacteroides fragilis,* microorganisms accountable for osteomyelitis.

The study demonstrated that the implantation was successful in constraining the inflammation and assisting osteo-conduction and vascularization of the affected location in rabbit mandible. Sterilized PLGA scaffold has its reliability for constructing tissue-engineered buccal mucosa [67]. PLGA composites having bio-ceramics are employed in direct pulp capping [68-69] either

by encompassing growth factors into PLGA micro particles [69] or by thorough pulp capping with PLGA composites of mechanically exposed teeth. Nevertheless, no hard tissue could be noticed in direct pulp capping with PLGA. Pulp necrosis was significant because of inferior adhesion of PLGA to the pulp regardless of the biocompatibility shown in cellular tests [70]. Hence, PLGA composites encompassing bio-ceramics are a better alternative to PLGA alone in pulp capping, that exhibits superior tissue response in contrast to calcium hydroxide [68, 70]. The favourable outcomes of the PLGA materials encourage more research that focuses on the designing of polymeric substances for sustained drug release for a longer period.

PLGA nanoparticles render its benefits having smaller particle size that supports its perforation into the cells, enhanced entrapment efficiency for more drug release, reduced minimum inhibitory concentration [71]. Minocycline-loaded PLGA nanoparticles exhibits enhanced antibacterial effects as compared to the action of free minocycline [72]. It has shown to render a supportive carrier system for the delivery of antibiotics to periodontal tissues. Inhibition zone of minocycline-loaded nanoparticles (9.2 mm) was observed to be more than that of free minocycline (3.5 mm) against *Aggregatibacter actinomycetemcomitans*, which is one of the most notorious pathogens in periodontal infections [71]. Adding on to that, methylene-blue loaded PLGA nanoparticles exhibited enhanced photodynamic effect as compared to free methylene-blue. It showed efficiency in inhibiting E. *faecalis* biofilm species, which is associated with endodontic failures. The experiments were performed in infected root canals of human extracted teeth [72]. Moreover, methylene-blue loaded PLGA nanoparticles showed enhanced photodynamic effect as compared to free methylene-blue in suspensions of human dental plaque bacteria along with biofilms extracted from 14 patients suffering from chronic periodontitis [73]. Hence, PLGA nanoparticles encompassing methylene-blue photosensitizer has its potential application to treat endodontic infections [72]. It can also be tuned to counter human dental plaque bacteria present in patients having chronic periodontitis [73]. PLGA

nanoparticles enhance bone regeneration procedures, dispatching growth and differentiation factors. Delivery of PLGA nanoparticles encompassing bone morphogenetic protein-2 to bone marrow mesenchymal stem cells influenced greater bone formation in vivo as compared to either embedding of the nanoparticles having bone morphogenetic protein-2 alone or osteogenically differentiated stem cells [74]. PLGA nanoparticles having simvastatin were employed to amplify osteogenesis of bone marrow mesenchymal stem cells, that has its potential application in bone regeneration [75]. PLGA nanoparticles encompassing growth factor has its application in implant therapy, reviving bone formation neighbouring to the surface of a dental implant that penetrates inside the bone [74]. Histomorphometric study resulted in a 44% mean bone-to-implant contact percentage, post 12 weeks of engrafting in rabbit tibiae [76].

Table 2: Different PLGA formulations used for localised oral drug delivery applications

No.	PLGA Formulations	Application	Ref.
1	PLGA (LA:GA = 50:50, MW = 70,000) Drug: Minocycline	Alveolar bone augmentation in ligature-induced experimental periodontitis	77
2	PLGA (MW=12 kDa, 50:50 lactide/glycolide molar ratio), Pluronic1 F-108 Dye: Methylene blue	Photodynamic therapy for treatment of dental plaque	78
3	PLGA (50:50 lactide/glycolide molar ratio) Growth factor: Basic fibroblast growth factor (bFGF)	Cardiac neovascularization, increasing perfusion and improving cardiac function	79
4	PLGA (lactide : glycolide = 85 : 15 and average molecular weight of 240,000 Mw), L-glutamic acid, Hydroxyapatite nanorods Protein: Bone morphogenetic protein (BMP-2)	Bone tissue regeneration	80
5	(PLGA) (85:15), Mw (50 KDa–75 KDa), Gelatin, N-(3-dimethylaminopropyl) N´ethylcarbodiimide hydrochloride and N-hydroxysuccinimide Drug: simvastatin	Enhances osteoblastic activity	81

2.3. Objectives of the work

The study aimed to develop mucoadhesive bandages for the localised treatment of oral disease conditions such as desquamative gingivitis, oral candidiasis etc.

1. Preparation of clotrimazole impregnated multi-layered mucoadhesive bandages using thiolated gellan gum as mucoadhesive layer.

2. *In vitro* evaluations of thiolated gellan gum for its extent of thiolation using different techniques such as FTIR, NMR, Ellman's assay and rheology.

3. *In vitro* evaluations of mucoadhesive bandages for its mechanical properties, contact angle measurements, percentage swelling, *in vitro* drug release kinetics from the drug reservoir layer and PLGA nanoparticles, and *in vitro* cytotoxicity.

3: *Ex vivo* evaluations of mucoadhesive bandages for its mucoadhesive characteristics using pig and rat intestinal mucosa.

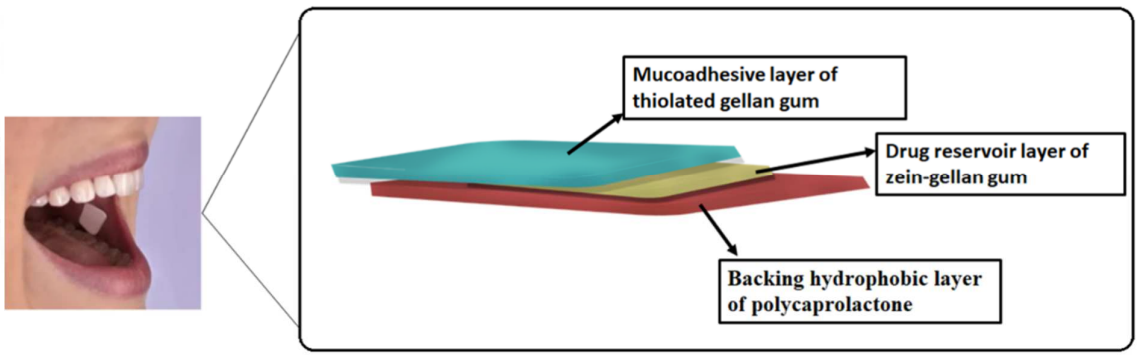

Figure 1: Schematic representation of multi-layered mucoadhesive bandage

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

Gellan Gum (Gelzan™ CM or Gelrite®) Sigma Aldrich, USA, L-cysteine, 1-Ethyl-3-(3-dimethylamino propyl) carbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS), PLGA (50:50) and PLGA (75: 25) and Zein were purchased from Sigma, Aldrich, Bangalore. Aluminium ammonium sulphate dodecahydrate (AlAS) was purchased from Merck Life Science Pvt. Ltd., Mumbai, India. Polycaprolactone (Mn = 80 KDa) and sodium hydroxide were purchased from SISCO research laboratories Pvt Ltd, India. Clotrimazole was purchased from TCI, Hyderabad, India. Deionized water was used throughout the reaction and purification process. All other reagents were of analytical grade and used as such without any further purification.

3.2. Preparation of clotrimazole impregnated mucoadhesive bandage

3.2.1. Synthesis of thiolated gellan gum

Thiolated gellan gum was prepared using different molar ratios of gellan gum and L-cysteine. Briefly, 1% gellan gum (2 g) solution was prepared in DI water by magnetically stirring the solution at 50° C, 400 rpm overnight. To the above solution, required amount of EDC and NHS was added and stirred continuously for two hours. The pH was adjusted to ~8.5 by addition of 0.1 N NaOH solution. Following this, L-cysteine was weighed based on the molar ratio and added to the activated gellan gum solution, followed by continuous stirring for 16 h at 50° C, 400 rpm. The reaction mixture was dialysed against distilled water using a dialysis bag of molecular weight cut off (M.W.C.O) 12 KDa for 48 h with frequent change of DI water. The solution was freeze dried and used for further studies. Thiolated gellan gum was prepared using

different feed ratios of gellan gum: L-cysteine such as 1:0.5, 1:1, 1:2 and were labelled as GG 1:0.5, GG 1:1, GG 1:2.

3.2.2. Preparation of gellan gum films

100 mL thiolated gellan gum solution (GG 1:0.5, 2 %) was prepared in DI water. The solution was stirred mechanically at 800 rpm for 20 min and 0.1 g aluminium ammonium sulfate dodecahydrate (50mg/mL solution in DI water) was added slowly while stirring. This was followed by continuous stirring of the solution for 30 minutes at 800 rpm. The solution was transferred to a glass petri dish (9 cm diameter) and dried in an oven at 40˚C, overnight to get thin transparent film, labelled as GG-0.5S. Similarly, films prepared using GG 1:1 and GG 1:2 were labelled as GG-1S and GG-2S respectively.

3.2.3. Preparation of drug reservoir layer

Drug reservoir layer was prepared using zein-cross-linked thiolated gellan gum. Briefly, 1% solution of thiolated gellan gum (GG 1:1, 66 mL) was prepared in DI water and stirred mechanically at 50 ˚C. To the above solution 34 mg of AlAS in 1 mL water was added slowly at 800 rpm. In the next step, polymer blend was prepared by adding 26.4 ml of 5% zein solution and 37.36 ml ethanol to the homogenous mixture of cross-linked thiolated gellan gum. 1mL clotrimazole (99 mg) solution in acetonitrile was added along with 1.3mL glycerine. The solution was stirred continuously for 30 minutes at 800 rpm and transferred to a 15 cm petri dish. It was dried in an oven at 40^{o}C, overnight.

3.2.4. Preparation of backing layer

Polycaprolactone based backing layer was prepared via electrospinning method. 8 ml of N,N-dimethylformamide (DMF) and 2 ml of dichloromethane (DCM) was added to a 15 ml glass vial. 1 g polycaprolactone (PCL; M_n = 80000) was added slowly to a solution of 8 ml N,N-

dimethylformamide (DMF) and 2 ml of dichloromethane (DCM) in a 15 mL glass vial. The solution was kept for stirring at 400 rpm at room temperature, overnight. Clear transparent solution of PCL obtained was used for electrospinning. Electrospinning parameters were optimised as follows, Voltage = 10.5 kV; Flow rate = 1.5 ml/h; Distance = 12 cm; Syringe diameter = 14.3 mm; Time = 2 h.

3.3. Synthesis of drug loaded PLGA nanoparticles

Polyvinyl alcohol (PVA; Mw= 89-98 KDa) solution was prepared by dissolving 2 g of PVA in 100 ml distilled water at 80° C, 500 rpm. The solution was centrifuged at 2000 rpm for 5 min and collected the supernatant. PLGA solution was prepared by dissolving 30 mg of PLGA in 3 ml chloroform in a 15 ml glass vial. The solution was magnetically stirred overnight at room temperature, 400 rpm. PLGA nanoparticle was prepared by emulsion technique. PLGA solution with and without drug was added to 10 ml of 2% PVA solution in a 50 ml centrifuge tube. The solution was vortexed for 1 min and probe sonicated for 3 min with interval after every 1 minute at an amplitude of 50. Following this, the solution was kept for overnight stirring at 400 rpm. PLGA nanoparticle suspension was divided into 8 eppendorf tubes and centrifuged at 15000 rpm, 4° C, 30 min. The pellet was collected and dispersed in 5 mL DI water. Probe sonicated for 3 min to disperse well. The process was repeated twice. The solution was freezed at -70° C and lyophilized.

3.4. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy analysis was carried out to confirm the thiolation of gellan gum using FTIR Spectrometer (Shimadzu, IR Spirit) in transmission mode. Samples were crushed with KBr powder and compressed using hydraulic press to form a pellet. Transmission spectra were collected in the range of 4000-400 cm^{-1}.

3.5. Particle size

Particle size of PLGA nanoparticle (1 mg/mL in DI water) was measured using Malvern Zetasizer Nano ZS, Malvern Instruments Ltd, with He-Ne laser beam at wavelength of 625 nm.

3.6. NMR Spectroscopy

NMR Spectra were recorded on a 400 MHz Brucker Advance FTNMR Spectrometer. Chemical shifts were recorded relative to TMS as the internal standard. D_2O was used as solvent.

3.7. Contact angle

Surface hydrophilicity of both mucoadhesive film and backing layer were studied using water contact angle measurements (Data physics OCA15 Plus, Germany). First films were mounted on a glass slide using a double-sided tape. Water drops were dispersed on the film surface. Contact angle was measured for both the right and left side of the water drop and the mean was determined. A minimum of five different measurements were taken for each set and averaged.

3.8. Rheology study

The viscosity of gellan gum solution at different extent of thiolation was studied using a modular compact rheometer, AR 1000N rheometer (TA Instruments, Crawley, UK). Two or three drops of 2% thiolated gellan gum in DI water was taken in the rheometer and placed at 0.2 mm distance. The experiment was performed at different temperatures at a shear rate of 0.1-100 s^{-1}. Temperature was controlled using the Peltier effect with a water bath as heat source or sink. Gellan gum solution without thiolation was used as reference for this study.

3.9. Thiol estimation

Reaction buffer was prepared by dissolving 0.1 M of sodium phosphate and 1 mM of EDTA in 1 L distilled water and adjusted the pH to 8. Ellman's reagent was prepared by dissolving 4 mg of Ellman's reagent in 1 ml of reaction buffer. Cysteine standards were prepared as per table 3.

Table 3: Parameters of the standard solution prepared for Ellman's assay

Standard	Volume of reaction buffer	Amount of L-cysteine	Final concentration
A (Stock)	100 ml	26.34 mg	1.5 mM
B	5 ml	25 ml of standard A	1.25 mM
C	10 ml	20 ml of standard A	1.0 mM
D	15 ml	15 ml of standard A	0.75 mM
E	20 ml	10 ml of standard A	0.5 mM
F	25 ml	5 ml of standard A	0.25 mM
G	30 ml	0 ml	0 mM

Thiolated gellan gum solution was prepared using GG-1: 0.5, GG-1: 1, GG-1: 2 in DI water at a concentration of 0.5 wt. % and used for thiol estimation. 7 test tubes were labelled A-G. 2.5 ml of reaction buffer and 50 µL of Ellman's reagent was added to each test tube. 250 µl of cysteine standard was added to all the test tubes labelled A-G. 7 other test tubes were labelled S1 to S7. 2.5 ml of reaction buffer and 50 µL of Ellman's reagent was added to each test tube. 250 µL each of the polymer samples was added to the corresponding test tube. The solution was incubated for 15 minutes at room temperature. Both standards and samples were measured at 412 nm using UV Spectrophotometer (UV-1800, Shimadzu). Concentration of each polymer sample was calculated using the standard curve.

3.10. Mechanical Properties

Film samples 3 cm×1 cm (l×w) were analysed for its mechanical properties using a universal testing machine (Instron®). The samples were clamped in a sample holder in a vertical position

with a distance of 2 cm in between the two clamps. Load cell of 100 N was applied with a rate of 0.5 mm/sec. The same tests were performed using wet film that was immersed in saline for 30 min.

3.11. Physical appearance

All films and bandages were visually inspected for colour, uniformity, clarity, flexibility, and smoothness.

3.12. Thickness of the films and bandages

Thickness of bare thiolated gellan gum films, drug reservoir layer, backing layer (electro spun polycaprolactone) and mucoadhesive bandages were measured using a screw gage micrometre at three independent locations in the samples. Thickness of each membrane was noted as average ± standard deviation.

3.13. Folding endurance

Folding endurance of the mucoadhesive bandages were determined by repeated folding of the sample (2cm*2cm) at 180° towards one side along the midline. The number of folds before breaking of the sample was observed. This study aids in understanding the efficacy of glycerol as plasticizer and determine the mechanical strength of the bandages. The experiment was performed in triplicate to estimate mean folding endurance for the mucoadhesive bandage formulation.

3.14. *In vitro* drug release kinetics

Drug reservoir layer (Zein-GG2S with Clotrimazole, 1 cm^2, 2 mg) was suspended in PBS having 0.5 wt.% tween 20 and incubated at 37^oC, 100 rpm. The released drug at regular intervals (0.5 h, 1h, 2h, 4h, 6h, 8h) was collected and analysed using UV visible spectroscopy (UV-1800, Shimadzu). Standard curve of clotrimazole was prepared using different concentration of drug in acetonitrile. For drug loaded PLGA nanoparticle, 1mg/ml of the drug loaded PLGA nanoparticle was suspended in PBS having 0.5 wt.% tween 20 and incubated at

37ºC, 100 rpm. After specific time intervals, 100 µl of supernatant was collected and diluted with 400 µl of acetonitrile before reading. The PLGA suspension used in the study was then replenished with 100 µl of PBS and the process was continued. All the experiments were done in triplicate.

3.15. *In vitro* cell culture studies

In vitro cytotoxicity study was performed using L929 mouse fibroblast cells to evaluate the cytocompatibility of the mucoadhesive bandage. Triton X 100 (0.1%) was used as positive control and cell culture media as the negative control.

3.16. *Ex vivo* mucoadhesive study

The intestinal mucosal membrane of Sprague Dawley rat, and pig intestinal mucosa were used to evaluate the mucoadhesive strength of the bandage using a texture analyser (TA.XT plus, Stable Micro Systems). The Institutional Animal Ethics Committee (IAEC, SCTIMST) approved the use of spent animals for harvesting oral/intestinal mucosa. The bandage strips of suitable size were secured to the probe of texture analyser with the help of double-sided adhesive tape. The film was attached to the probe and a force of 50 g was applied for 60 seconds at speed of 0.1 mm/sec. Mucoadhesive strength was determined as the force in dynes needed to separate the film from the mucosal membrane.

3.17. Statistical Analysis

Statistical analysis was performed using Graph Pad Prism. Relevant test methodology (paired t test or one-way ANOVA) was chosen based on number of groups in the particular study.

CHAPTER 4

RESULTS AND DISCUSSION

Thiol modified gellan gum was synthesised at different extent of thiolation via carbodiimide chemistry using different molar ratios of gellan gum and L-cysteine. The schematic representation for the synthesis of thiolated gellan gum is shown in the Figure 2.

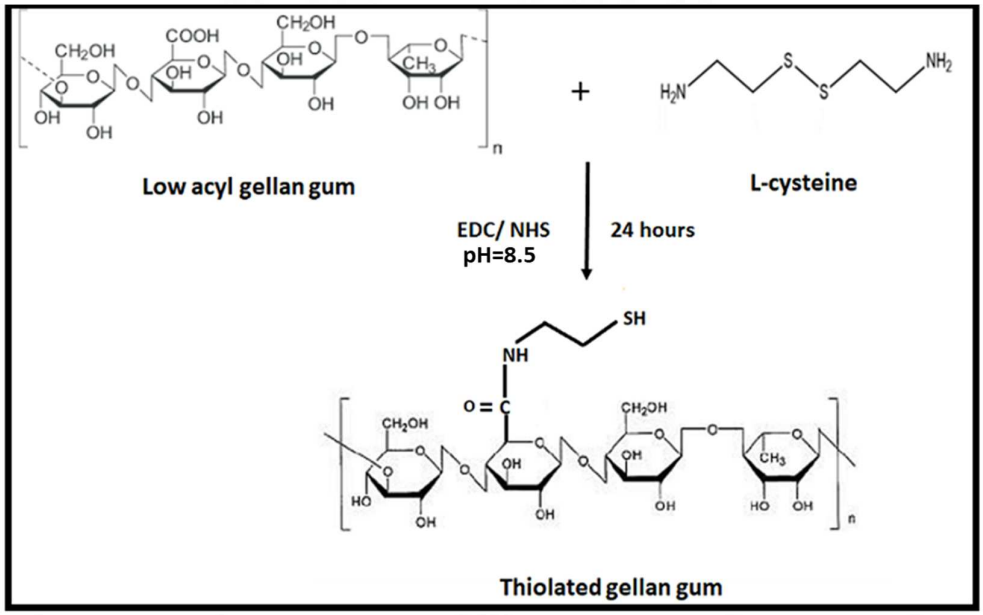

Figure 2: Schematic representation for the synthesis of thiolated gellan gum

Thiol functionalities in the polymer chain was confirmed using FTIR and thiol estimation assay. Figure 3 shows FTIR spectra of gellan gum (GG) and thiolated gellan gum (GG 1:0.5, GG 1:1 and GG 1:2). The peak at 2500 cm^{-1} is assigned to -S-H stretching in GG 1:0.5, GG 1:1 and GG 1:2. However, the thiol peak is not very distinct. A broad peak ~ 3338 cm^{-1} is attributed to O-H stretching in both GG and thiolated GG. –C-H stretching at 2929 cm^{-1} is visible for all the spectra as there is no change in the bond during modifications. The peak at 1614 cm^{-1} is due to the presence of –C=O stretching and no visible change in the peak was noticed before and after

modifications of gellan gum. An additional peak around 2520 cm^{-1} is visible in all thiolated gellan gum spectra and is attributed to the weak –S-H stretching of L-cysteine moieties conjugated into the polymer chain.

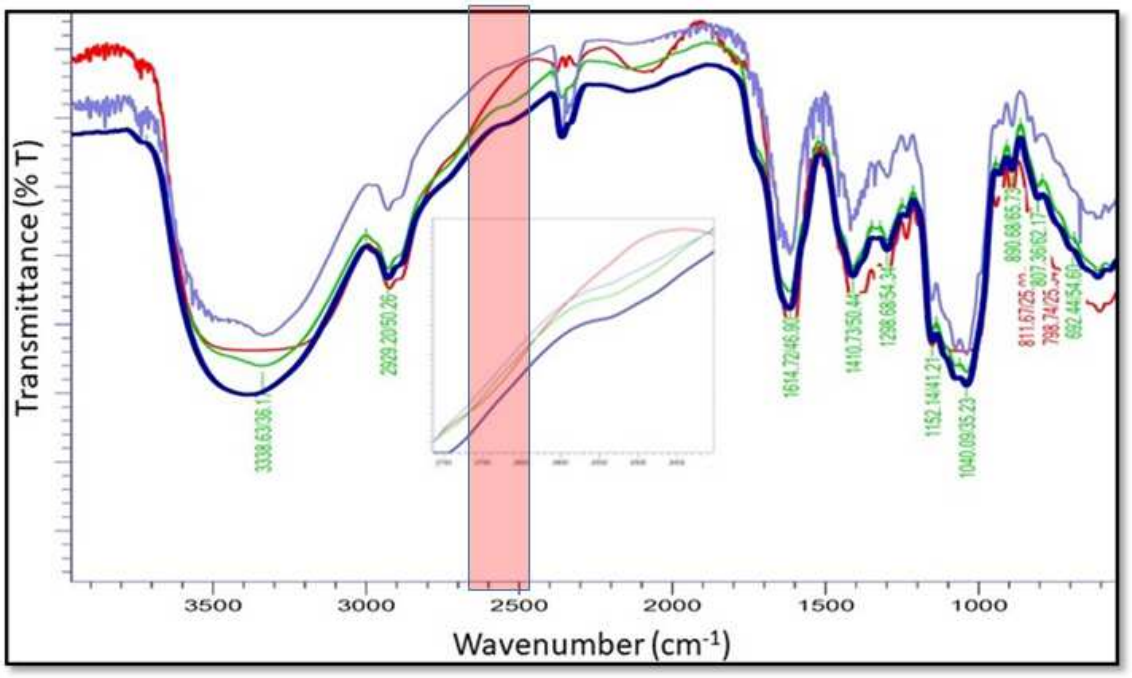

Figure 3: FTIR spectra of gellan gum (GG, red) and thiolated gellan gum (GG 1:0.5 (purple), GG 1:1 (green), GG 1:2 (blue))

Thiol group estimation was performed using Ellman's reagent. The standard curve for thiol estimation showed a linear relationship between absorbance and concentration of standard L-cysteine with an R^2 value of 0.9825 (Figure 4). The standard curve was used to estimate the extent of thiolation in GG 1:0.5, GG 1:1 and GG 1:2. Bare gellan gum was used as the reference. During initial studies, conjugation was done in different media at pH around ~8.5. Thiolation reaction at the feed ratio of 1: 2, gellan gum/L-cysteine in DI water and PBS at pH 8.5 was denoted as GG 1:2 S1 and GG 1:2 S2 respectively. It was noted that GG 1:2 S1 had the

maximum concentration of thiol group (0.1242 mM/mg of GG 1:2) as compared to all other modifications of GG (Figure 4).

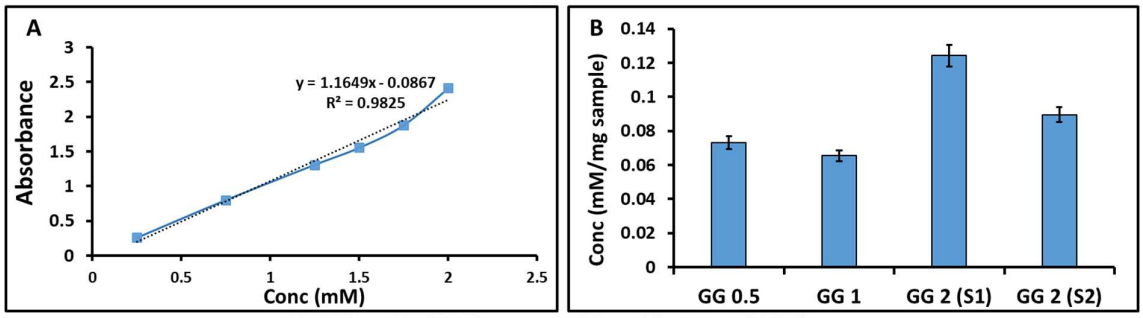

Figure 4: (A) Calibration curve reflecting absorbance *Vs* concentration of standard solution; (B) Normalized absorbance of various thiolated gellan gum samples

The result showed that as the feed ratio of L-cysteine/GG increased more thiol groups were introduced in the polymer chain. Also the extent of thiolation increased while using DI water at pH 8.5 as the reaction media compared to that in PBS. The amount of thiol groups in GG 1:2 while using PBS as the reaction media was only 0.0896 mM/mg of polymer. This is because PBS has high salt concentration, which is not very favourable for thiolation of GG. Based on the extent of thiolation while using different media for the synthesis of GG 1:2 it was concluded that DI water at pH 8.5 was a preferred media for reaction. Following that all the experiments were done in DI water at pH 8.5.

In order to confirm the thiolation GG, 1 H NMR spectra of GG, GG 1:2 was compared (Figure 5). NMR spectra of GG showed four characteristic peaks corresponding to -CH of rhamnose: 5.4058 ppm, -CH of glucuronic acid: 5.12 ppm, -CH of glucose: 4.53 ppm and -CH3 of rhamnose: 1.3 ppm. The NMR spectra of thiolated gellan gum showed singlet peaks near 3.55, 3.77, 3.4 and 2.89 ppm which can be correlated to the presence of thiol moieties [81].

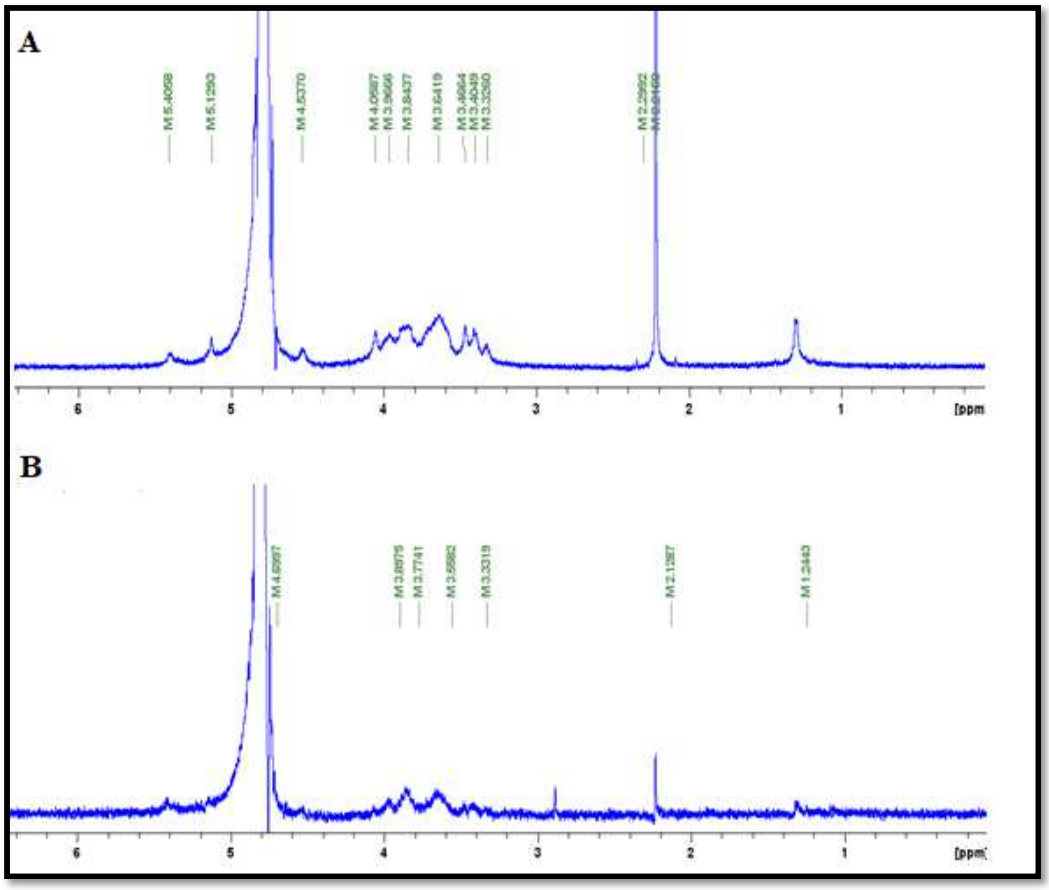

Figure 5: (A) NMR spectra of gellan gum, GG (B) thiolated gellan gum, GG-1:2

The viscosity of 2% gellan gum and thiolated gellan gum was studied using a rheometer at different temperature. It was shown that with increase in the shear rate, viscosity decreased drastically. As evident from the figure 6, all these solutions exhibited pseudo plastic shear thinning effect as in the case of non-Newtonian fluids. GG 1:2 showed higher viscosity compared to that of GG 1:0.5 and GG 1:1 at the shear rate of 0.1 s^{-1}. In addition to that a reduction in viscosity was observed with increase in temperature (Figure 6B). It can be attributed to the increased average kinetic energy of molecules in the solution with increase in temperature. This led to the molecules surpassing the intermolecular attractive forces between them and a declination in the viscosity of the solution was observed.

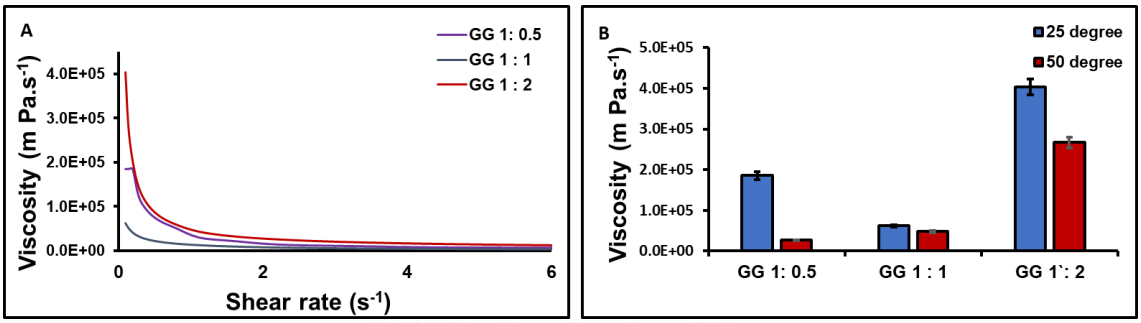

Figure 6: (A) Reduction in viscosity of 2% gellan gum solution with and without thiol modification at different shear rate (B) viscosity of 2% gellan gum solution at the shear rate of $0.1s^{-1}$ at two different temperature

To optimise the crosslinking density to prepare hydrolytically stable GG films, different weight ratio of aluminium ammonium sulfate (AlAS) were used. GG film prepared using different weight ratio of AlAS like, 2 wt%, 5 wt%,10 wt%, 15 wt% and 20 wt% was represented as GG-2, GG-5, GG-10, GG-15 and GG-20 respectively via solvent evaporation method (Figure 7).

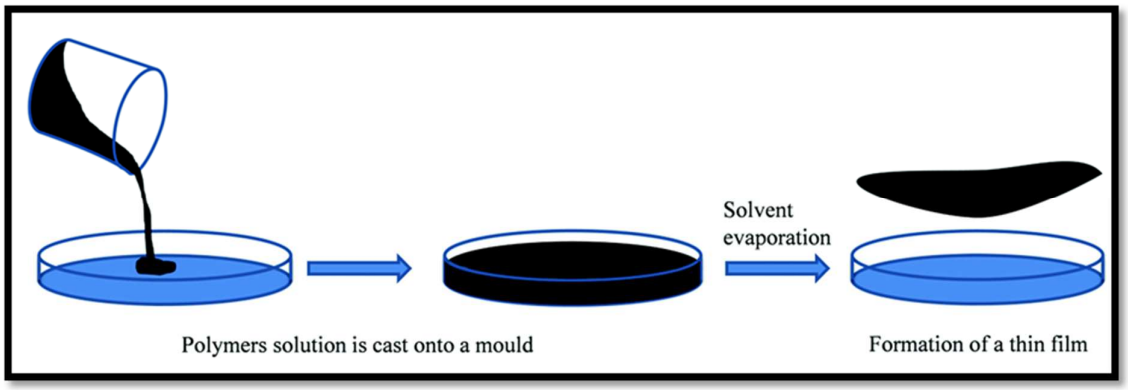

Figure 7: Schematic for the preparation of crosslinked gellan gum film via solvent evaporation

Free swell capacity of GG films both in saline and DI water is shown in Figure 8. According to the result as the percentage crosslinking increased free swelling capacity decreased in DI water. However, there were no significant changes observed between samples in saline.

Importantly, reduced free swell capacity of gellan gum hydrogel in saline was noted compared to that in DI water. It was attributed to the reduced ionic concentration difference between inside and outside of the hydrogel system because of the high ionic concentration of 0.9 wt% saline. It reduced the diffusion of media freely into the bulk of the cross-linked polymer network. Even though there was a slight reduction in the free swell capacity of GG-5 compared to that of GG-2, the hydrolytic stability was more in GG-5. Based on the hydrolytic stability and free swell capacity in DI water, use of 5 wt% AlAS was finalised for further studies.

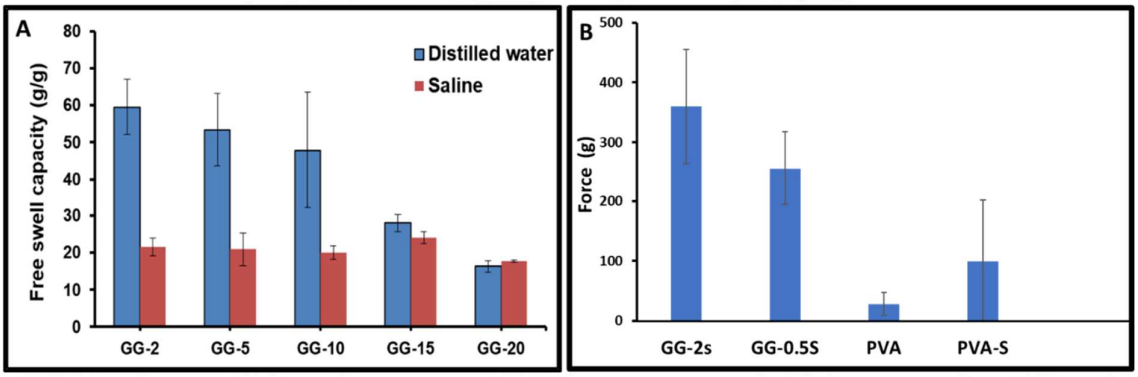

Figure 8: (A) % swelling of cross-linked gellan gum in different media; (B) Mucoadhesion of thiolated gellan gum film compared to polyvinyl alcohol using rat intestinal mucosa

Thiolated GG films were prepared after crosslinking with 5 wt % AlAS. GG films were prepared using GG, GG 1:0.5, GG 1:1, GG 1:2 was denoted as GG, GG0.5, GG1, GG2 respectively. Mucoadhesion studies were done using rat intestinal mucosa and PVA/thiolated PVA (PVA-S) as control. Both PVA and PVA-S films were part of another study of the group. Data showed that GG-2S has high mucoadhesive properties with a force of ~ 300 g compared to other samples prepared using GG-0.5S or PVA/PVA-S. GG-2S at 5 wt% crosslinking was finalised for further studies based on the free swell capacity and extent of mucoadhesion.

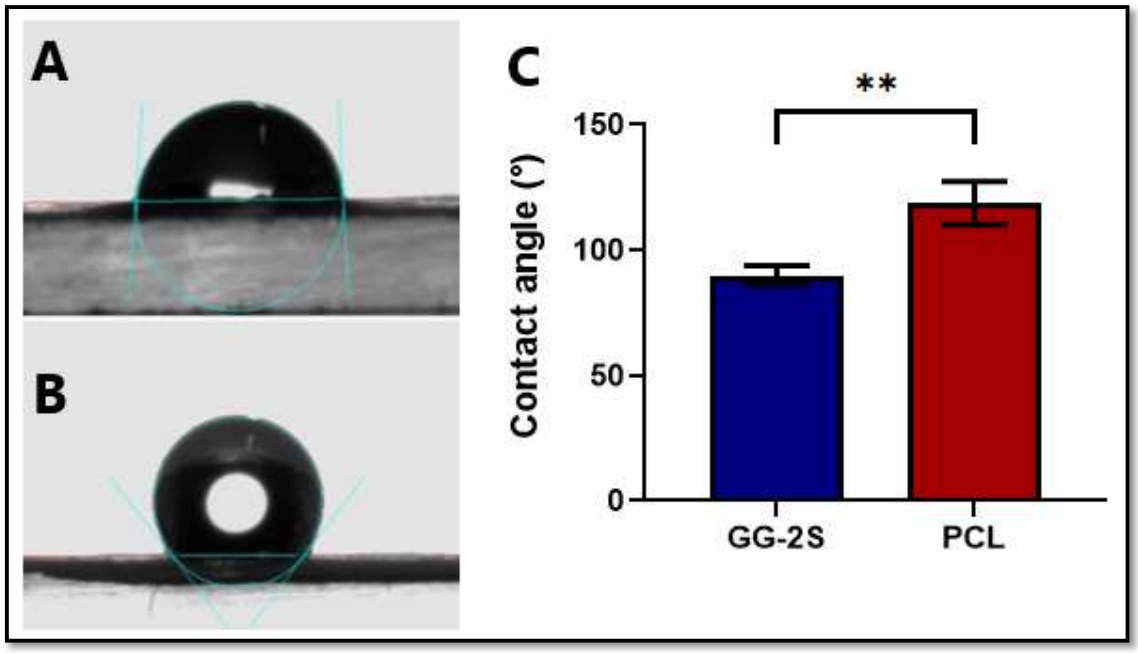

Figure 9: Water contact angle of (A) thiolated gellan gum film (GG-2S; θ=89°) and backing layer of electrospun polycaprolactone (θ=118°) (C) graphical representation of contact angle of thiolated gellan gum layer and PCL backing layer (Statistical analysis was performed using Mann Whitney test, n=6 and p=0.0022).

Thiolated gellan gum layer is highly hydrophilic as evident from the water contact angle (Figure 9). Thiolated gellan gum film (GG-2S) showed a water contact angle θ = 89°. Zein was used as a second component in the film to improve the hydrophilic/hydrophobic balance. The backing layer of electrospun PCL membrane showed a water contact angle of θ = 118°. The hydrophobic nature of the backing layer will ensure the unidirectional flow of the drug molecule through the thiolated GG-2S layer. Figure 10 showed the percentage cell viability of thiolated gellan gum film (GG-2S and GG-0.5). More than 90% cell viability was observed for both GG-2 and GG-0.5. The negative control (NC) and positive control (PC) showed an expected cell viability value of 100 % and < 10 % respectively. The result gave us a preliminary evidence of biocompatibility and confidence in moving forward with the current modifications.

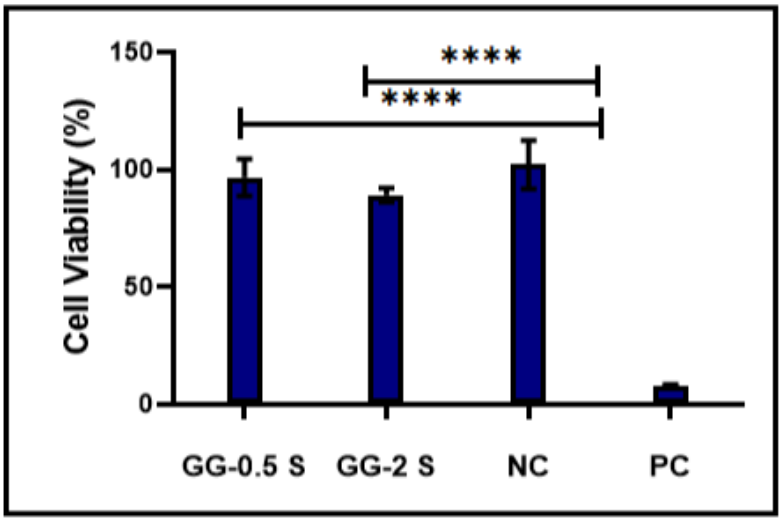

Figure 10: Percentage cell viability of thiolated gellan gum films using L929 mouse fibroblast cells (statistical analysis was performed using one-way ANOVA, n=5; $p < 0.0001$)

The thickness of GG-2S, and Zein/GG-2S film was ~ 0.014 mm, and 0.019 mm respectively. The tensile strength of GG-2S and Zein/GG-2S were analysed in the dry condition. The data showed a reduction in the tensile strength of Zein/GG-2S compared to GG-2S.

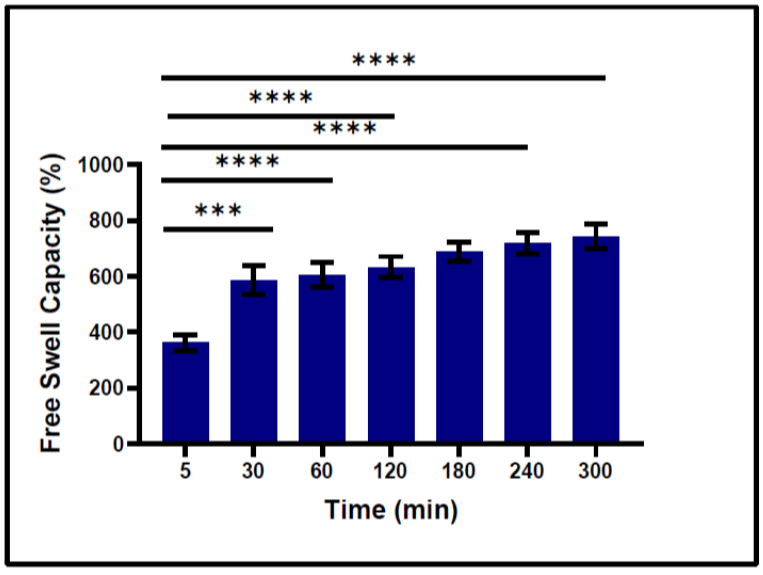

Figure 11: Percentage free swell capacity of the bandage in saline at different time point (statistical analysis was performed using one-way ANOVA, n=3; $p < 0.0001$)

Free swell capacity of mucoadhesive bandage in saline was measured (Figure 11) and analysed using one-way ANOVA. The data showed that free swell capacity increases with time. However, swelling saturation happened within 1 h and no statistical difference in percentage swelling after 1h. The tensile strength of GG-2S was 63.1 ± 40.36 MPa. Whereas Zein/GG-2S had a tensile strength of 29.11 ± 3.44 MPa only. However, Zein/GG-2S showed a significant improvement in the percentage tensile strain at maximum load, 15.67 ± 2.14 % (Figure 12). Whereas, GG-2S showed a reduction in the percentage strain at maximum load compared to that of Zein/GG-2S, 6.92 ± 3.92 %. It can be attributed to the interpenetrating network structure of Zein/GG-2S with enhanced elasticity compared to GG-2S film. Mechanical properties of Zein/GG-2S is good enough to survive in the dynamic environment of oral cavity. To check the properties of Zein/GG-2S in wet condition, tensile strength was measured after incubating the film in 0.9% saline for 30 minutes. The result showed a tensile strength reduction of more than 95% for Zein/GG-2S film in the wet state compared to that in the dry state (Figure 12).

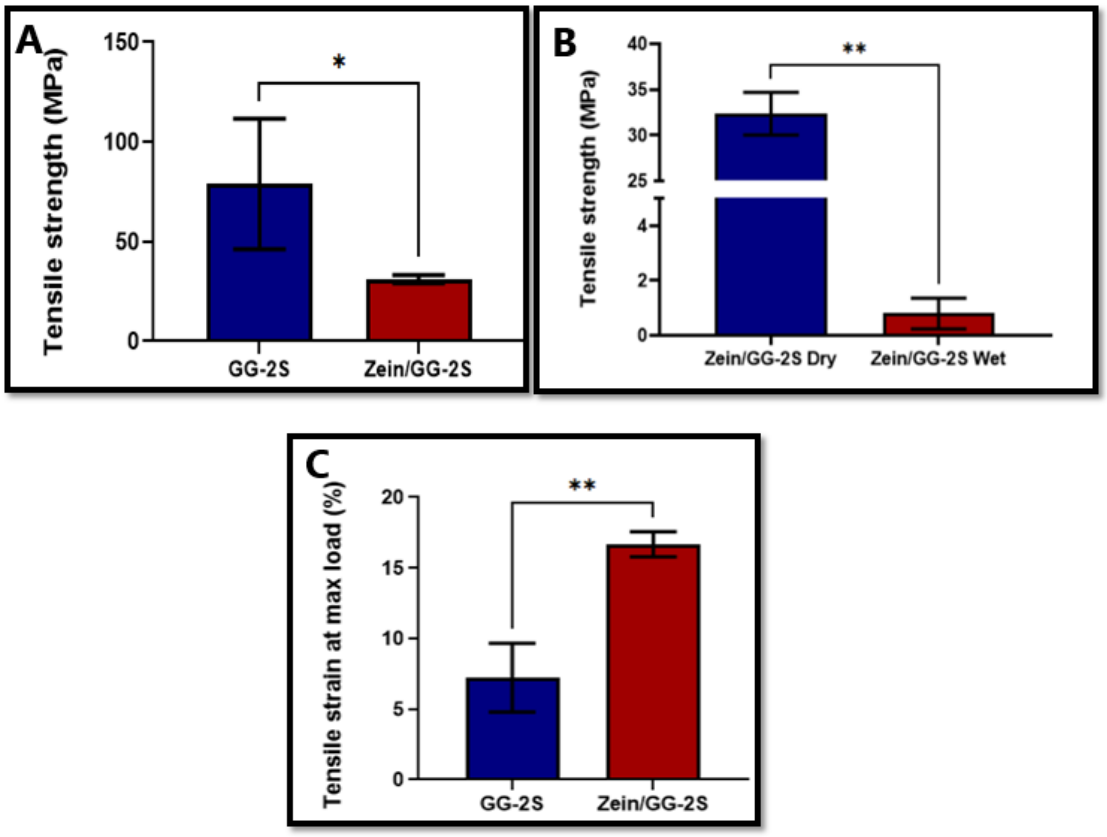

Figure 12: (A) Tensile strength of GG-2S and Zein/GG-2S; (B) Tensile strength of Zein/GG-2S in the dry and wet stage (C) Percentage tensile strain at maximum load for both GG-2S and Zein/GG-2S (statistical analysis was performed using paired t test, * means $p \leq 0.015$; ** means $p \leq 0.002$)

Initially the loading efficacy was determined using high performance liquid chromatography (HPLC). However, due to some technical constraints, the standard curve and drug release study was once again performed using UV –Visible spectroscopy. As observed in figure 13 A, clotrimazole showed linear relationship at lower concentration below 40 mg/L. With increase in drug concentration, the graph saturated and linearity was lost. Concentration of drug in a polymer sample was calculated by measuring the absorbance of released drug at each time point using beer lamberts law (Equation 1).

$$A = €\beta C \text{ ----------1}$$

Where, A = absorbance, € is molar absorption coefficient, β is length of light path, C is concentration of drug. Standard curve for clotrimazole was drawn at wavelength of 210 nm. Figure 13B showed drug release profile of Zein/GG-2S film and PLGA nanoparticle in PBS having 0.5 wt% tween 20. The data showed a burst release of 36 µg/mL and 38 µg/mL within a period of 30 minutes for Zein/GG-2S film and PLGA nanoparticle respectively. After that a sustained drug release of clotrimazole was obtained for a period of 8h. At 8h, ~ 56 µg/mL and ~62 µg/mL clotrimazole was released for Zein/GG-2S film and PLGA nanoparticle respectively. The activity of released clotrimazole need to be anaylsed. However, due to the limited time those experiment will be planned in future studies.

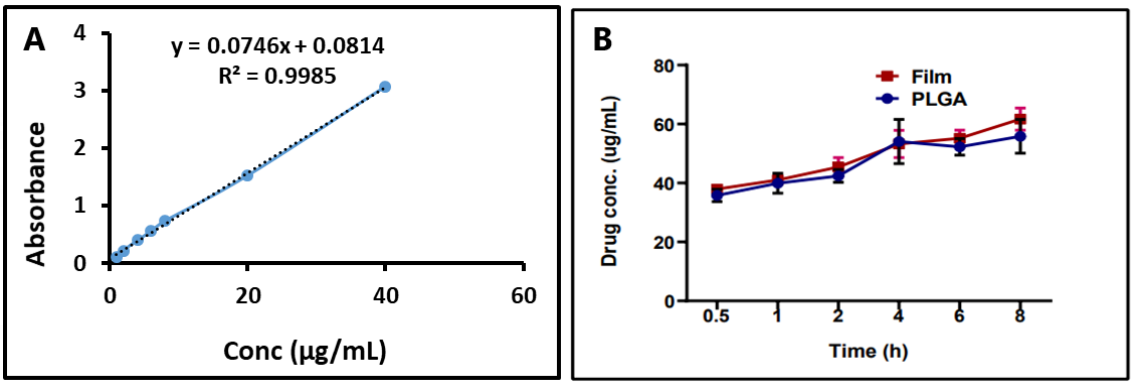

Figure 13: Drug release profile of Clotrimazole from PLGA nanoparticle and Zein/GG-2S film in phosphate buffered saline

The work of adhesion of the three-layer sandwich mucoadhesive bandage and zein/GG-2S were determined using pig intestinal mucosa (Figure 14). The figure 14 B showed different layers of mucoadhesive bandage prepared, top layer of thiolated mucoadhesive GG film (GG-2S), middle drug reservoir layer (Zein/GG-2S) and backing layer prepared using electrospun PCL. Figure 14A showed a work of adhesion of 52 ± 5.6 g.mm for Zein/GG-2S and 199.9 ± 53.4 g.mm for the bandage. Bandage showed a work of adhesion higher compared to Zein/GG-2S.

It may be attributed to the enhanced mucoadhesive nature of thiolated zein layer as compared to that of interpenetrating network structure of Zein and thiolated GG (Zein/GG-2S).

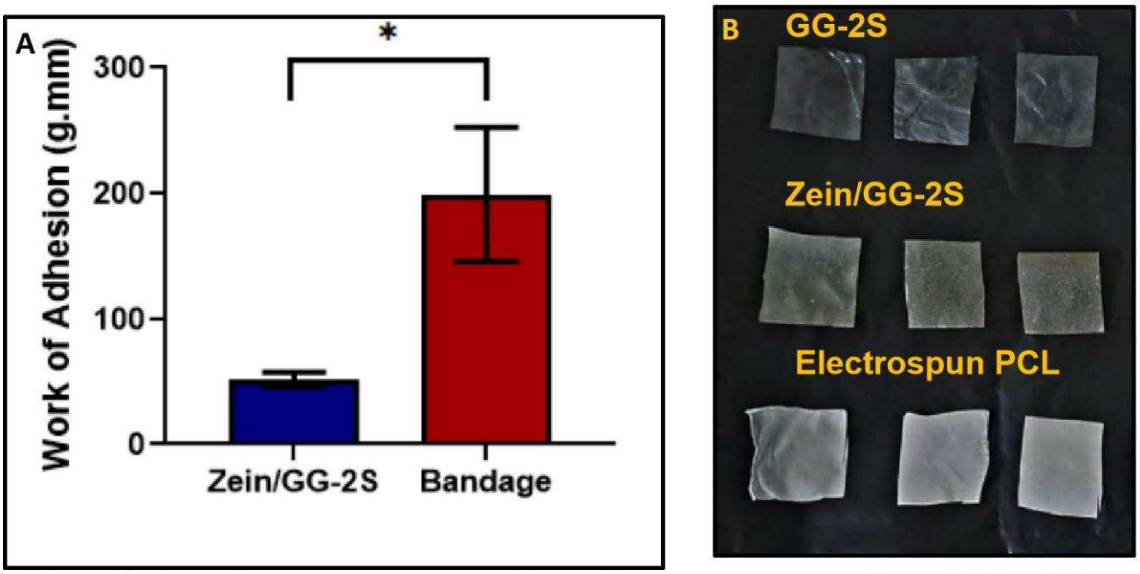

Figure 14: (A) Work of adhesion of Zein/GG-2S and bandage (B) Three different layer of mucoadhesive bandage, ie, mucoadhesive gellan gum layer, middle layer of drug loaded GG-Zein and electrospun backing layer.

CHAPTER 5

CONCLUSION

Oral disease conditions such as candidasis, desquamative gingivitis etc focused on localized oral drug delivery through keratinised mucosal tissue such as gingiva and palate. Multiple mucoadhesive oral formulations are available in the market. However, the mucoadhesive nature of those formulations were poor and need further improvement. The study aimed to develop such localized drug delivery bandages that can have sustained drug delivery for a period of 8 hours. This study was successful in achieving its aim by combining three layers having different characteristics. Contact angle of 89° was obtained for hydrophilic thiolated gellan gum layer and 118° for hydrophobic polycaprolactone layer. The hydrophobic PCL layer could facilitate the unidirectional flow of drug molecule throught he mucoadhesive gellan gum layer. The films showed > 95% cell viability in MTT assay which indicated its cytocompatibility and suitability for oral drug delivery applications. The mucoadhesive bandage developed showed sustained drug release profile for clotrimazole for a period of 8 hours having insignificant swelling. The tensile strength of both dry and wet state of Zein/GG-2S and bandage showed high work of adhesion while using pig intestinal mucosa as the mucosal layer. Favourable biocompatible outcomes, remarkable muco-adhesive strength and high folding endurance with strong mechanical properties confirm that the bandages developed have potential application in translational research.

FUTURE SCOPE

1) Drug release profile of PLGA nanoparticle impregnated drug researvoir layer need to be tuned for controlled release for a period of 8-24 hours.
2) Different composition of drug reservoir layer can be tried, like hydroxy propyl methyl cellulose.
3) Optimization of conditions to prepare electrospun mucoadhesive layer and drug reservoir layer.

REFERENCES

[1] Prasad, V., De Jesús, K., & Mailankody, S. (2017). The high price of anticancer drugs: origins, implications, barriers, solutions. Nature reviews Clinical oncology, 14(6), 381-390.

[2] Ingersoll, K. S., & Cohen, J. (2008). The impact of medication regimen factors on adherence to chronic treatment: a review of literature. Journal of behavioral medicine, 31, 213-224.

[3] Rubbens, J., Mols, R., Brouwers, J., & Augustijns, P. (2018). Exploring gastric drug absorption in fasted and fed state rats. International Journal of Pharmaceutics, 548(1), 636-641.

[4] Lou, J., Duan, H., Qin, Q., Teng, Z., Gan, F., Zhou, X., & Zhou, X. (2023). Advances in oral drug delivery systems: Challenges and opportunities. Pharmaceutics, 15(2), 484.

[5] Brannigan, R. P., & Khutoryanskiy, V. V. (2019). Progress and current trends in the synthesis of novel polymers with enhanced mucoadhesive properties. Macromolecular Bioscience, 19(10), 1900194.

[6] Chatterjee, B., Amalina, N., Sengupta, P., & Mandal, U. K. (2017). Mucoadhesive polymers and their mode of action: A recent update. Journal of Applied Pharmaceutical Science, 7(5), 195-203.

[7] Alexander, A., Tripathi, D. K., Verma, T., Maurya, J., & Patel, S. (2011). Mechanism responsible for mucoadhesion of mucoadhesive drug delivery system: a review.

[8] PerioChip. (2017, July 27). https://www.periochip.com/

[9] RxList. (2021, December 16). Actisite (tetracycline periodontal): Uses, dosage, side effects, interactions, warning. RxList. https://www.rxlist.com/actisite-drug.htm

[10] Arestin + scaling and root planing. ARESTIN + Scaling and Root Planing. (n.d.). https://www.arestin.com/

[11] RxList. (2021a, May 26). Atridox (doxycycline hyclate): Uses, dosage, side effects, interactions, warning. RxList. https://www.rxlist.com/atridox-drug.htm

[12] Shridhar, G. S., Manohar, S. D., Bhanudas, S. R., & Anjaneri, N. (2013). Mucoadhesive buccal drug delivery: An Overview. Journal of Advanced Pharmacy Education & Research Oct-Dec, 3(4), 319-32.

[13] Pardeshi, C. V., Rajput, P. V., Belgamwar, V. S., & Tekade, A. R. (2012). Formulation, optimization and evaluation of spray-dried mucoadhesive microspheres as intranasal carriers for Valsartan. Journal of microencapsulation, 29(2), 103-114.

[14] Kumar, A., Naik, P. K., Pradhan, D., Ghosh, G., & Rath, G. (2020). Mucoadhesive formulations: Innovations, merits, drawbacks, and future outlook. Pharmaceutical Development and Technology, 25(7), 797-814.

[15] Tangri, P., & Madhav, N. S. (2011). Recent advances in oral mucoadhesive drug delivery systems: a review. International Journal of Pharmaceutical Research and Development, 3(2), 151-161.

[16] Shinkar, D. M., Dhake, A. S., & Setty, C. M. (2012). Drug delivery from the oral cavity: A focus on mucoadhesive. PDA J. Pharm. Sci. Technol, 66, 466-500.

[17] Longer, M. A., & Robinson, J. R. (1986). Fundamental-aspects of bioadhesion. Pharmacy International, 7(5), 114-117.

[18] Smart, J. D. (2005). The basics and underlying mechanisms of mucoadhesion. Advanced drug delivery reviews, 57(11), 1556-1568.

[19] Boddupalli, B. M., Mohammed, Z. N., Nath, R. A., & Banji, D. (2010). Mucoadhesive drug delivery system: An overview. Journal of advanced pharmaceutical technology & research, 1(4), 381.

[20] Wu, Z. M., Zhou, L., Guo, X. D., Jiang, W., Ling, L., Qian, Y., ... & Zhang, L. J. (2012). HP55-coated capsule containing PLGA/RS nanoparticles for oral delivery of insulin. International journal of pharmaceutics, 425(1-2), 1-8.

[21] Wu, W., Luo, L., Wang, Y., Wu, Q., Dai, H. B., Li, J. S., ... & Wang, G. X. (2018). Endogenous pH-responsive nanoparticles with programmable size changes for targeted tumor therapy and imaging applications. Theranostics, 8(11), 3038.

[22] Singh, R., & Lillard Jr, J. W. (2009). Nanoparticle-based targeted drug delivery. Experimental and molecular pathology, 86(3), 215-223.

[23] Kingsley, J. D., Dou, H., Morehead, J., Rabinow, B., Gendelman, H. E., & Destache, C. J. (2006). Nanotechnology: a focus on nanoparticles as a drug delivery system. Journal of Neuroimmune Pharmacology, 1, 340-350.

[24] Luo, C., Sun, J., Du, Y., & He, Z. (2014). Emerging integrated nanohybrid drug delivery systems to facilitate the intravenous-to-oral switch in cancer chemotherapy. Journal of controlled release, 176, 94-103.

[25] Nagpal, K., Singh, S. K., & Mishra, D. N. (2010). Chitosan nanoparticles: a promising system in novel drug delivery. Chemical and Pharmaceutical Bulletin, 58(11), 1423-1430.

[26] Gao, L., Liu, G., Ma, J., Wang, X., Zhou, L., Li, X., & Wang, F. (2013). Application of drug nanocrystal technologies on oral drug delivery of poorly soluble drugs. Pharmaceutical research, 30, 307-324.

[27] Malam, Y., Loizidou, M., & Seifalian, A. M. (2009). Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer. Trends in pharmacological sciences, 30(11), 592-599.

[28] Müller, R. H., Mäder, K., & Gohla, S. (2000). Solid lipid nanoparticles (SLN) for controlled drug delivery–a review of the state of the art. European journal of pharmaceutics and biopharmaceutics, 50(1), 161-177.

[29] Üner, M., & Yener, G. (2007). Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. International journal of nanomedicine, 2(3), 289-300.

[30] Hallan, S. S., Kaur, P., Kaur, V., Mishra, N., & Vaidya, B. (2016). Lipid polymer hybrid as emerging tool in nanocarriers for oral drug delivery. Artificial cells, nanomedicine, and biotechnology, 44(1), 334-349.

[31] Bamrungsap, S., Zhao, Z., Chen, T., Wang, L., Li, C., Fu, T., & Tan, W. (2012). Nanotechnology in therapeutics: a focus on nanoparticles as a drug delivery system. Nanomedicine, 7(8), 1253-1271.

[32] Wait, S. D., Prabhu, R. S., Burri, S. H., Atkins, T. G., & Asher, A. L. (2015). Polymeric drug delivery for the treatment of glioblastoma. Neuro-oncology, 17(suppl_2), ii9-ii23.

[33] Edsman, K., & Hägerström, H. (2005). Pharmaceutical applications of mucoadhesion for the non-oral routes. Journal of pharmacy and pharmacology, 57(1), 3-22.

[34] Chatterjee, B., Amalina, N., Sengupta, P., & Mandal, U. K. (2017). Mucoadhesive polymers and their mode of action: A recent update. Journal of Applied Pharmaceutical Science, 7(5), 195-203.

[35] Bernkop-Schnürch, A. (2005). Mucoadhesive systems in oral drug delivery. Drug discovery today: Technologies, 2(1), 83-87.

[36] Davoudi, Z., Rabiee, M., Houshmand, B., Eslahi, N., Khoshroo, K., Rasoulianboroujeni, M., ... & Tayebi, L. (2018). Development of chitosan/gelatin/keratin composite containing

hydrocortisone sodium succinate as a buccal mucoadhesive patch to treat desquamative gingivitis. Drug development and industrial pharmacy, 44(1), 40-55.

[37] Youssef, M. I., Darwish, Z. E., Fahmy, R. A., & El Sayed, N. M. (2019). The effect of topically applied hyaluronic acid gel versus topical corticosteroid in the treatment of erosive oral lichen planus. Alexandria Dental Journal, 44(1), 57-63.

[38] Sworn, G., & Stouby, L. (2021). Gellan gum. In Handbook of hydrocolloids (pp. 855-885). Woodhead Publishing.

[39] Kang, K. S., Veeder, G. T., Mirrasoul, P. J., Kaneko, T., & Cottrell, I. W. (1982). Agar-like polysaccharide produced by a Pseudomonas species: production and basic properties. Applied and Environmental Microbiology, 43(5), 1086-1091.

[40] Paulsson, M., Hägerström, H., & Edsman, K. (1999). Rheological studies of the gelation of deacetylated gellan gum (Gelrite®) in physiological conditions. European journal of pharmaceutical sciences, 9(1), 99-105.

[41] Mazen, F., Milas, M., & Rinaudo, M. (1999). Conformational transition of native and modified gellan. International Journal of Biological Macromolecules, 26(2-3), 109-118.

[42] Grasdalen, H., & Smidsrød, O. (1987). Gelation of gellan gum. Carbohydrate Polymers, 7(5), 371-393.

[43] Moritaka, H., Nishinari, K., Taki, M., & Fukuba, H. (1995). Effects of pH, potassium chloride, and sodium chloride on the thermal and rheological properties of gellan gum gels. Journal of Agricultural and Food Chemistry, 43(6), 1685-1689.

[44] Quinn, F. X., Hatakeyama, T., Yoshida, H., Takahashi, M., & Hatakeyama, H. (1993). The conformational properties of gellan gum hydrogels. Polymer gels and networks, 1(2), 93-114.

[45] Ikeda, S., Nitta, Y., Temsiripong, T., Pongsawatmanit, R., & Nishinari, K. (2004). Atomic force microscopy studies on cation-induced network formation of gellan. Food Hydrocolloids, 18(5), 727-735.

[46] Morris, E. R., Nishinari, K., & Rinaudo, M. (2012). Gelation of gellan–a review. Food Hydrocolloids, 28(2), 373-411.

[47] Ferrero, C., Massuelle, D., & Doelker, E. (2010). Towards elucidation of the drug release mechanism from compressed hydrophilic matrices made of cellulose ethers. II. Evaluation of a possible swelling-controlled drug release mechanism using dimensionless analysis. Journal of Controlled Release, 141(2), 223-233.

[48] Emeje, M. O., Franklin-Ude, P. I., & Ofoefule, S. I. (2010). Evaluation of the fluid uptake kinetics and drug release from gellan gum tablets containing metronidazole. International journal of biological macromolecules, 47(2), 158-163.

[49] Patil, J. S., Kamalapur, M. V., Marapur, S. C., & Kadam, D. V. (2010). Ionotropic gelation and polyelectrolyte complexation: the novel techniques to design hydrogel particulate sustained, modulated drug delivery system: a review. Digest Journal of Nanomaterials and Biostructures, 5(1), 241-248.

[50] Vijan, V., Kaity, S., Biswas, S., Isaac, J., & Ghosh, A. (2012). Microwave assisted synthesis and characterization of acrylamide grafted gellan, application in drug delivery. Carbohydrate polymers, 90(1), 496-506.

[51] Shiyani, B. G., Dholakiya, R. B., Akbari, B. V., Lodhiya, D. J., & Ramani, G. K. (2009). Development and evaluation of novel immediate release tablets of metoclopramide HCl by direct compression using treated gellan gum as a disintegration-accelerating agent. Journal of Pharmacy Research, 2(9), 1460-1464.

[52] Quigley, K. J., & Deasy, P. B. (1992). Use of deacetylated gellan gum (Gelrite) for the production of sulphamethizole containing beads. Journal of microencapsulation, 9(1), 1-7.

[53] Miyazaki, S., Aoyama, H., Kawasaki, N., Kubo, W., & Attwood, D. (1999). In situ-gelling gellan formulations as vehicles for oral drug delivery. Journal of Controlled release, 60(2-3), 287-295.

[54] Alhaique, F., Santucci, E., Carafa, M., Coviello, T., Murtas, E., & Riccieri, F. M. (1996). Gellan in sustained release formulations: preparation of gel capsules and release studies. Biomaterials, 17(20), 1981-1986.

[55] Liu, S., Qiu, Y., Yu, W., & Zhang, H. (2020). Highly stretchable and self-healing strain sensor based on gellan gum hybrid hydrogel for human motion monitoring. ACS Applied Polymer Materials, 2(3), 1325-1334.

[56] Mahajan, H. S., & Gattani, S. G. (2009). Gellan gum based microparticles of metoclopromide hydrochloride for intranasal delivery: development and evaluation. Chemical and Pharmaceutical Bulletin, 57(4), 388-392.

[57] Prezotti, F. G., Siedle, I., Boni, F. I., Chorilli, M., Müller, I., & Cury, B. S. F. (2020). Mucoadhesive films based on gellan gum/pectin blends as potential platform for buccal drug delivery. Pharmaceutical Development and Technology, 25(2), 159-167.

[58] Fernandes, F. P., Fortes, A. C., da Cruz Fonseca, S. G., Breitkreutz, J., & Ferraz, H. G. (2018). Manufacture and characterization of mucoadhesive buccal films based on pectin and gellan gum containing triamcinolone acetonide. International Journal of Polymer Science, 2018.

[59] Nair, A. B., Shah, J., Aljaeid, B. M., Al-Dhubiab, B. E., & Jacob, S. (2019). Gellan gum-based hydrogel for the transdermal delivery of nebivolol: Optimization and evaluation. Polymers, 11(10), 1699.

[60] Altun, E., Yuca, E., Ekren, N., Kalaskar, D. M., Ficai, D., Dolete, G., ... & Gunduz, O. (2021). Kinetic release studies of antibiotic patches for local transdermal delivery. Pharmaceutics, 13(5), 613.

[61] Manna, S., Dhanalakshmi, D., Bhowmik, M., Jana, S., & Jana, S. (2022). Cellulose Derivative-Based Bioadhesive Blend Patch for Transdermal Drug Delivery. Frontiers in Materials, 9, 366.

[62] Ullah, W., Nawaz, A., Akhlaq, M., Shah, K. U., Latif, M. S., & Alfatama, M. (2021). Transdermal delivery of gatifloxacin carboxymethyl cellulose-based patches: Preparation and characterization. Journal of Drug Delivery Science and Technology, 66, 102783.

[63] Latif, M. S., Azad, A. K., Nawaz, A., Rashid, S. A., Rahman, M. H., Al Omar, S. Y., ... & Abdel-Daim, M. M. (2021). Ethyl Cellulose and Hydroxypropyl Methyl Cellulose Blended Methotrexate-Loaded Transdermal Patches: In Vitro and Ex Vivo. Polymers, 13(20), 3455.

[64] Li, A., Khan, I. N., Khan, I. U., Yousaf, A. M., & Shahzad, Y. (2021). Gellan gum-based bilayer mucoadhesive films loaded with moxifloxacin hydrochloride and clove oil for possible treatment of periodontitis. Drug Design, Development and Therapy, 3937-3952.

[65] Dathathri, E., Lal, S., Mittal, M., Thakur, G., & De, S. (2020). Fabrication of low-cost composite polymer-based micro needle patch for transdermal drug delivery. Applied Nanoscience, 10, 371-377.

[66] Prakash, J., Kumar, T. S., Venkataprasanna, K. S., Niranjan, R., Kaushik, M., Samal, D. B., & Venkatasubbu, G. D. (2019). PVA/alginate/hydroxyapatite films for controlled release of amoxicillin for the treatment of periodontal defects. Applied Surface Science, 495, 143543.

[67] Selim, M., Bullock, A. J., Blackwood, K. A., Chapple, C. R., & MacNeil, S. (2011). Developing biodegradable scaffolds for tissue engineering of the urethra. BJU international, 107(2), 296-302.

[68] Gala-Garcia, A., Teixeira, K. I. R., Wykrota, F. H. L., Sinisterra, R. D., & Cortés, M. E. (2010). Bioceramic/Poly (glycolic)-poly (lactic acid) composite induces mineralized barrier after direct capping of rat tooth pulp tissue. Brazilian oral research, 24, 08-14.

[69] Zhang, W., Walboomers, X. F., & Jansen, J. A. (2008). The formation of tertiary dentin after pulp capping with a calcium phosphate cement, loaded with PLGA microparticles containing TGF-β1. Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials, 85(2), 439-444.

[70] Gala-García, A., Carneiro, M. B. H., Silva, G. A. B., Ferreira, L. S., Vieira, L. Q., Marques, M. M., ... & Cortes, M. E. (2012). In vitro and in vivo evaluation of the biocompatibility of a calcium phosphate/poly (lactic-co-glycolic acid) composite. Journal of Materials Science: Materials in Medicine, 23, 1785-1796.

[71] Kwon, I. K., Lee, S. C., Han, B., & Park, K. (2012). Analysis on the current status of targeted drug delivery to tumors. Journal of Controlled Release, 164(2), 108-114.

[72] Pagonis, T. C., Chen, J., Fontana, C. R., Devalapally, H., Ruggiero, K., Song, X., ... & Soukos, N. S. (2010). Nanoparticle-based endodontic antimicrobial photodynamic therapy. Journal of endodontics, 36(2), 322-328.

[73] Klepac-Ceraj, V., Patel, N., Song, X., Holewa, C., Patel, C., Kent, R., ... & Soukos, N. S. (2011). Photodynamic effects of methylene blue-loaded polymeric nanoparticles on dental plaque bacteria. Lasers in surgery and medicine, 43(7), 600-606.

[74] Jeon, O., Song, S. J., Kang, S. W., Putnam, A. J., & Kim, B. S. (2007). Enhancement of ectopic bone formation by bone morphogenetic protein-2 released from a heparin-conjugated poly (L-lactic-co-glycolic acid) scaffold. Biomaterials, 28(17), 2763-2771.

[75] Wang, C. Z., Fu, Y. C., Jian, S. C., Wang, Y. H., Liu, P. L., Ho, M. L., & Wang, C. K. (2014). Synthesis and characterization of cationic polymeric nanoparticles as simvastatin carriers for enhancing the osteogenesis of bone marrow mesenchymal stem cells. Journal of colloid and interface science, 432, 190-199.

[76] Lee, S. Y., Koak, J. Y., Heo, S. J., Kim, S. K., Lee, S. J., & Nam, S. Y. (2010). Osseointegration of anodized titanium implants coated with poly (lactide-co-glycolide)/basic fibroblast growth factor by electrospray. International Journal of Oral & Maxillofacial Implants, 25(2).

[77] Ma, Y., Song, J., Almassri, H. N., Zhang, D., Zhang, T., Cheng, Y., & Wu, X. (2020). Minocycline-loaded PLGA electrospun membrane prevents alveolar bone loss in experimental peridontitis. *Drug Delivery*, *27*(1), 151-160.

[78] Wang, Y., Liu, X. C., Zhao, J., Kong, X. R., Shi, R. F., Zhao, X. B., ... & Lu, F. (2009). Degradable PLGA scaffolds with basic fibroblast growth factor: experimental studies in myocardial revascularization. Texas Heart Institute Journal, 36(2), 89.

[79] Haider, A., Kim, S., Huh, M. W., & Kang, I. K. (2015). BMP-2 grafted nHA/PLGA hybrid nanofiber scaffold stimulates osteoblastic cells growth. BioMed research international, 2015.

[80] Nath, S. D., Linh, N. T., Sadiasa, A., & Lee, B. T. (2014). Encapsulation of simvastatin in PLGA microspheres loaded into hydrogel loaded BCP porous spongy scaffold as a controlled drug delivery system for bone tissue regeneration. Journal of Biomaterials Applications, 28(8), 1151-1163

[81] More, M. P., Bhamare, M. S., Bhavsar, C. J., Patil, P. O., & Deshmukh, P. K. (2017). Development of novel thiolated carboxymethyl-gellan gum as potential mucoadhesive polymer: Application of DoE. Adv. Mater. Sci, 2, 1-9.