Self-healing Hydrogels as an Efficient Local Drug Delivery System to Treat Periodontitis

MS Thesis

By

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COMSATS University Islamabad

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Self-healing Hydrogels as an Efficient Local Drug Delivery System to Treat Periodontitis

A Thesis submitted to

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In partial fulfillment
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in
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I Shaista Shaukat (CIIT/FA21-R06-013/LHR) hereby declare that I have produced the work presented in this thesis, during the scheduled period of study. I also declare that I have not taken any material from any source except referred to wherever due that amount of plagiarism is within acceptable range. If a violation of HEC rules on research has occurred in this thesis, I shall be liable to punishable action under the plagiarism rules of the HEC.

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It is certified that Shaista Shaukat (CIIT/FA21-R06-013/LHR) has carried out all the work related to this thesis under my supervision at the Department of Chemistry, COMSATS University Islamabad, Lahore Campus and the work fulfills the requirement for award of MS degree.

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Abstract

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Periodontitis is an infection-related, inflammatory condition of the gums. The periodontal tissue is harmed. It can result in tooth loss and a host of other health issues if left untreated. A system that is effective against bacteria and has the ability to regenerate tissue is needed for treatment. Hydrogels that are both affordable and antimicrobial have been created in this study as an efficient local drug delivery system. The synthesis of hydrogels includes polymers polyvinyl alcohol, gelatin, hydroxyapatite, glutaraldehyde and borax as a crosslinkers. The hydrogel is self-healing and has mechanical strength. Metronidazole loaded on it which very effective antibiotic against gram negative strains of bacteria that cause periodontitis. Hydroxyapatite modified with arginine which enhance the wound healing capability of hydrogels. Different characterizations have done for hydrogels FTIR shows dual crosslinking in hydrogels. Drug release study shows the significant release of drug which is helpful in first 24h of healing. Water contact angle measurement shows that the hydrogels are hydrophilic which is helpful in the attachment with cells. Degradation studies confirm the biodegradability of hydrogel. The hydrogels are effective against both E. coli and S. Aureus strains of bacteria. All the characterizations and studies lead to healthy use of hydrogels without any side effect in the treatment of hydrogels.

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LIST OF ABBREVIATIONS

HA	Hydroxyapatite
PVA	Poly Vinyl Alcohol
UV-VIS	Ultraviolet Visible
FTIR	Fourier Transform Infrared
XRD	X-Rays Diffraction
SEM	Scanning Electron Microscopy
MHA	Arginine modified HA
MTDZ	Metronidazole
WD	Without drug Hydrogel

Chapter 1

Introduction

Introduction

1.1 Background Information on Periodontitis

Periodontal disease is the most common oral ailment impacting people of all ages [1]. The occurrence and frequency estimates for periodontal diseases are influenced by the number of teeth, the study sites, bias, and improper case identification [2]. Canadian Health Measures Survey (2007-2009) measured the decline of attachment of periodontal ligament is thought to be a most reliable method for determining the severity of periodontal disease [3]. The NHANES evaluated the probing depth (PD) and adhesion losses (AL) at six sites on all teeth (except third molars) to estimate the prevalence of periodontal illness in the US [4].

The WHO has created a global health data orally archive using the community periodontal index (CPI). Data from large epidemiological studies carried out in numerous countries were used to assess the prevalence of periodontal disease in teenager, adult, and elder populations (Figure11-33). The index score of CPI, which is 0-4, represents the general periodontal health of the population. The CPI scale ranges from zero to four, with zero denoting no periodontal disease, one denoting gingival bleeding upon probing, two denoting calculus and bleeding, three denoting shallow periodontal pockets of 4-5 mm, and four denoting extensive periodontal pockets of 6 mm or more [5].

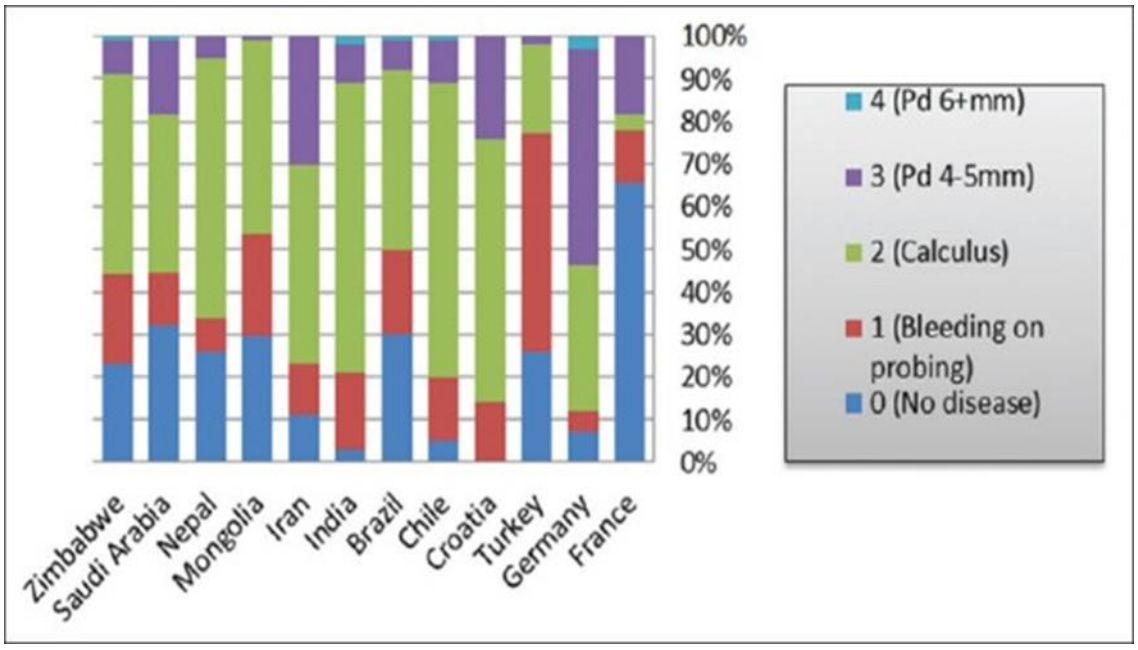


Figure 1. 1 Proportions of 15-19 years adolescents

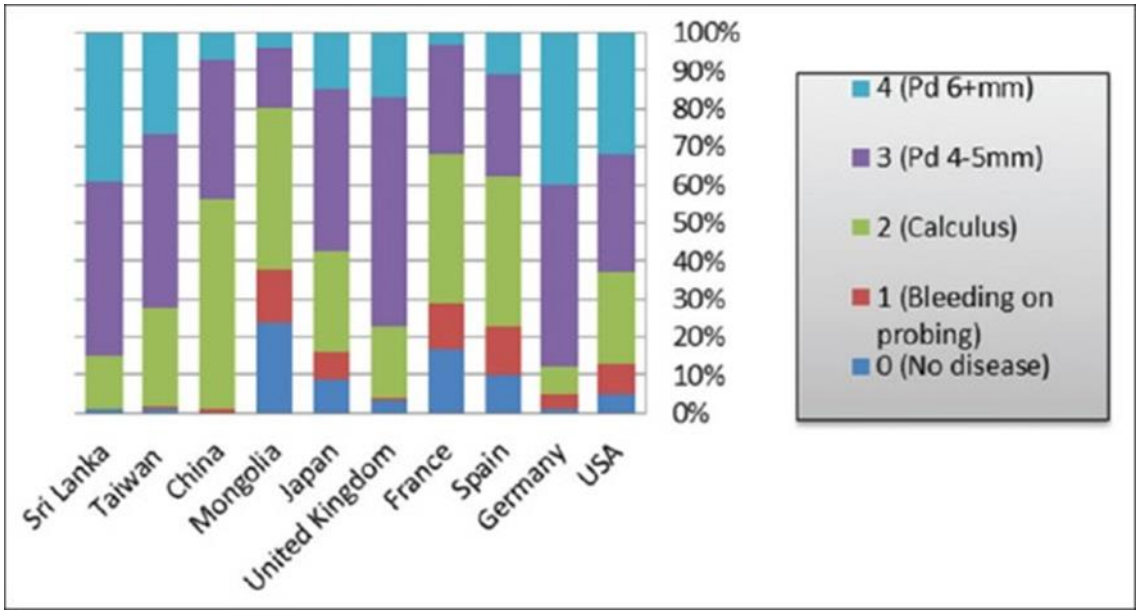


Figure 1. 2 Proportions of 35-44 years

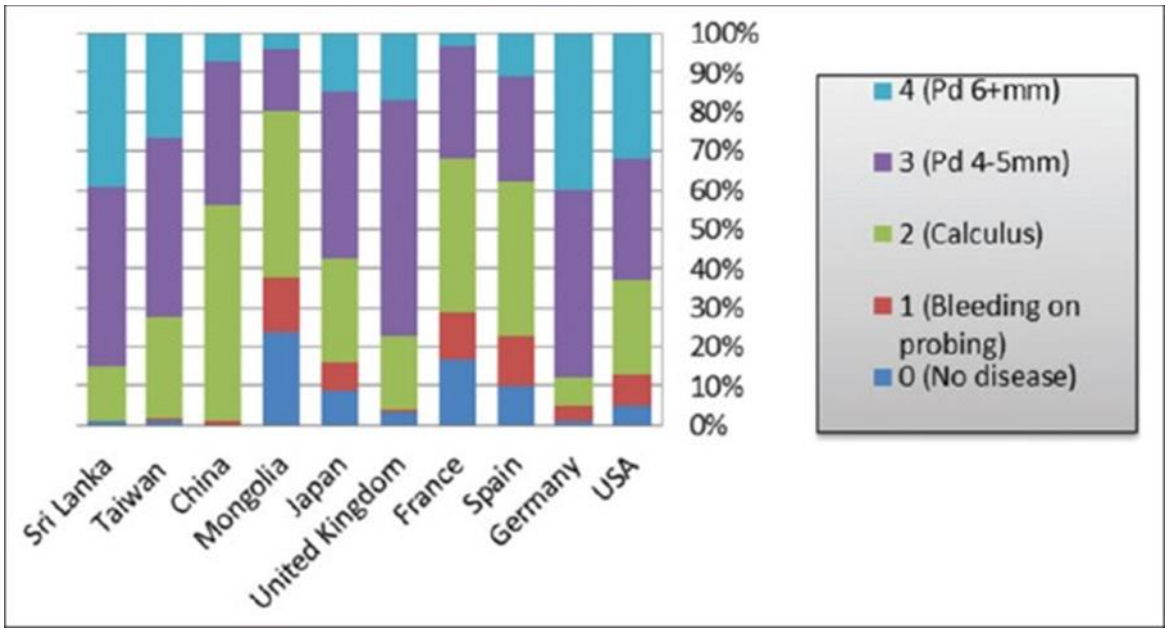


Figure 1. 3 Proportions of 65-74 years older

Calculus and bleeding on probing are more common in teenagers in poor nations than in developed ones (Figure 1-1). In developed countries, the percentage of teenagers with calculus accumulation ranged from 4% to 34%, while it varied from 35% to 70% in underdeveloped nations (Figure 1-2). Similarly, people in wealthy nations had a 14–47% higher likelihood of having calculus deposition than those in developing nations, who has a 36–63% higher likelihood. However, those with 4-5 mm periodontal pockets are more common in wealthy countries.

In both developed and underdeveloped countries, elderly persons (65–74 years) are prone to suffer from periodontal pockets that are 6 mm or greater than adult communities (Figures 1-3) In the entire world, between 20 and 50 % of the population have periodontal illness [6].

1.2 Biofilm and Periodontitis

The hunt for the aetiology of periodontitis began in the fifth millennium BC, but it wasn't until the late 1600s that bacteria were identified as the disease's origin. The general plaque concept, which gained traction in the latter part of the nineteenth century, proposed that periodontitis would manifest if the causing agent could outweigh the body's capacity to detoxify bacterial byproducts [7]. The "Specific Plaque Hypothesis" was put forth much later, in 1976, and claimed that a particular type of bacteria was the cause of this illness. The development of this theory has shed light on the significance of bacterial biofilm in periodontitis[8]. Recent research demonstrates the existence of multispecies biofilms containing bacterial and fungal species interacting across domains. Recent investigations back up the sustainable plaque hypothesis, which holds that typical intake of sugar leads to an acidic oral microenvironment that favours a proliferation of infectious acidophile bacteria like those in the Streptococcus, Lactobacillus, and Actinomycetes genera while suppressing beneficial microbes, leading to dysbiosis. Conversely, fungi like Candida albicans become more virulent when they bind to dangerous bacteria like Porphyromonas gingivalis [9].

The two most frequently recognised hypotheses are the key-stone concept and dysbiosis and polymicrobial hypothesis, which center on the association of various bacteria in a dental biofilm [10]. Streptococci, Actinomyces, and other members of the Firmicutes phylum (Granulicatella, Gemella, and Veillonella) are present in the biofilm that generates on the teeth’s surface. Due to this constant battle for favorable niche demands, the biofilm constantly seems to be in a dynamic, managing condition. Numerous variables that are thought of as "noise" affect the activity of dental biofilm, and depends on the fluctuations of this noise, Dahlen et al. 2020 [11] offered a framework in which the whole of potential determinants is viewed as random noise, acting in the tooth biofilm in ways that either exacerbate inflammation, speed up recovery, or have no effect.

1.3 Symptoms and Variations of Periodontitis

The primary focus of this illness is the gum, which develops an infection and becomes swollen, red, and easily bleeds. because of the pus, foul breath is generated. Teeth become looser due to a deepening gingival pocket, which makes chewing challenging and generally affects dental function.

The stages of infection and the health of the gums are used to categories the different forms of periodontitis. The first type of gingivitis is distinguished by red, swollen gum. With the right medical attention and healthcare, this stage is treatable. If left untreated, it will progress to a more serious form called periodontitis, which causes bad breath by developing pus in the gingival pocket and progressively moving to deeper layers of gum and jawbone. It may be a confined form of aggressive periodontitis that is limited to a particular tooth surface, usually the first molars and incisors, or it may be generic aggressive periodontitis (GAgP), which progresses swiftly and is characterized by the quick deterioration of gingival tissues and bone [12].

Adolescents have a high prevalence of the GAgP and an Actino bacillus actinomycetemcomitans-containing subgingival microbiota [13]. It is a serious complex illness that is still influenced by inherited, immunological, ecological, and microbiological factors. On the other hand, chronic periodontitis (CP) is the very slow loss of teeth and destruction of gingival pockets. Adults are typically more susceptible to it. Systemic periodontitis is frequently brought on by a variety of systemic ailments, such as diabetes and cardiovascular conditions. Necrotizing periodontitis is a type of periodontitis that slowly destroys the oral bones and teeth as a result of immune suppression or situations like starvation [14]. Tooth decay, which commonly emerges very slowly and is frequently asymptomatic and develops at particular locations on the dental enamel, is the main indicator of developed periodontitis. However, the discomfort is brought on by the decay spreading into the tooth pulp and the dentin, which contains nerves. This is brought on by the steadily increasing acidity in gingival and the dental microenvironment, which causes the collagen structure holding down the tooth to disintegrate and the minerals in the teeth to dissolve. The discomfort is usually unbearable and may be ongoing or sporadic [15].

1.4 Periodontitis and Systemic Diseases

Numerous studies have demonstrated a connection between periodontal disease and other systemic illnesses like cancer, diabetes, kidney disease, and even cardiovascular disease. A number of systemic disorders can increase a person's vulnerability to periodontal disease while also increasing the likelihood that various systemic diseases will develop [6].

1.4.1 Cardiovascular Diseases

Periodontitis has been discovered to put people at risk for coronary heart disease since those with periodontal disease are around 19% usually develop cardiovascular disease. Over 65-year-olds are especially susceptible to this vulnerability. Similar studies/case studies conducted by numerous researchers show that patients with periodontal illness are more susceptible to suffer severe heart problems than individuals without periodontal disease [16]. Stroke and peripheral artery constriction are observed to be more significantly related to periodontitis [6]. According to studies, periodontal pathobionts such Fusobacterium nucleatum/Treponema denticola, Tannerella forsythia, Porphyromonas gingivalis, and Aggregatibacter actinomycetemcomitans are responsible for the development of cardiovascular issues [17].

1.1 Atherosclerosis

In vitro/in vivo experimental investigation has demonstrated the reliability of a relationship between atherogenesis and periodontal illness, additionally shown the molecular processes through which fatty coatings accumulate in the innermost arteries layers [18]. Because dealing with periodontal diseases does not make people less likely to develop cardiovascular or cerebrovascular problems, the link cannot be said to be mutual.

1.2 Hypertension

The widespread of hypertension among individuals having any form of periodontitis strongly suggests a connection between high blood pressure and periodontal disease. Results of several meta-analyses indicate that periodontal therapy may be able to relieve the patient's hypertension [19].

1.4.2 Diabetes

Diabetes and periodontal disease interact and work in concert one another [20]. The results of a group study involving 628 participants revealed that all people with type 2 diabetes have advanced periodontal disease. In addition, they were discovered having times greater risk of dying from ischemic heart disease [6] than people with minimal or minor periodontal disease. However, it was also discovered that people with type 2 diabetes who successfully treat their periodontitis have improved glycemic control [21].

1.4.3 Chronic Kidney Disease (CKD)

Periodontal disease and chronic kidney disease are symbiotic. According to Ioannidou et al.'s 2011 research, people with such renal issues were 30–60% more likely to experience mild periodontitis [22]. As opposed to patients with normal kidney function, it was discovered that Americans of Mexico with known poorer function of kidney were more prone to periodontitis [23]. Similar to this, periodontitis and impaired renal function are linked in elderly Japanese persons [24]. According to Ricardo et al., CKD patients with periodontitis had a roughly 35% higher chance of dying than CKD patients without the condition [25].

1.4.4 Alzheimer’s Disease

The finding of Porphyromonas gingivalis, a keystone periodontitis bacterium, in the brain of an Alzheimer's patient suggests a possible relationship between these two illnesses. Additionally, periodontitis has two significant pathogenic characteristics, namely inflammation and oxidative damage, similar to those of Alzheimer's disease [25].

1.4.5 Autoimmune Diseases

It has been found that those who have gingivitis and periodontitis are more likely to acquire autoimmune diseases like arthritis. As the underlying pathogenic mechanisms in periodontitis and rheumatoid arthritis (RA) have been discovered to be quite comparable, it is often believed that periodontitis could lead to the development of this autoimmune disease and that those who have rheumatic arthritis suffer higher alveolar bone and tooth loss due to that [26].

A higher frequency of periodontal indicators in people with inflammatory bowel disease (IBD) led investigators to conclude that periodontitis affects the inflammatory

diseases or systemic autoimmune. Particularly Crohn's disease, and IBD a peritoneal inflammation condition [27].

Michaud and colleagues' research [28] has shown that periodontitis increases the chance of developing cancer. Each millimeter of alveolar bone holding tooth cavities lost, elevated the risk of tongue cancer by about five times [29]. Researchers discovered a link between periodontal disease and pancreatic, gastric, esophageal and oral cancers. This connection is much accordant than it is with prostate and lung cancers, according to a literature review. It is more probable that a mix of pathogen species may encourage carcinoma in both the oral cavity and extraoral tissues because periodontitis is a polymicrobial disorder. The periodontal biofilm's bacteria may interact in a cooperative or competitive way. Although a large number of investigations have so far demonstrated the mechanisms of activation of single species participate in cancer, additional research is necessary to establish the integrated actions of many kinds of oral parasites in triggering of carcinogenesis [30].

1.4.6 Immunodeficiency Diseases (ID)

One of the most prevalent symptoms of many primary immunodeficiencies, such as CANDLE Syndrome, Chronic Granulomatous Disease (CGD), Severe Congenital Neutropenia, Common Variable IDs, Hyper IgE Syndromes, Dyskeratosis Congenita, and Chediak-Higashi Syndrome, is periodontitis [30]. Additionally, it is common for people with Papillon-Lefèvre Syndrome (PLS) to have severe destructive periodontitis, which can result in the loss of both primary and permanent teeth. Gingivitis and periodontitis are brought on by ulcerations that develop in Chronic Granulomatous Disease (CGD), creating lesions on the mucosal and keratinized periodontal tissues. Patients with Familial Mediterranean Fever (FMF) typically have moderate to severe periodontal problems. When highly active antiretroviral therapy (HAART) was introduced, it was shown that necrotizing periodontitis in AIDS patients became less severe over time [31].

1.5 Risk Factors of Periodontitis

While the primary cause of periodontitis is the formation of bacterial plaque, a variety of additional variables also have a significant impact on how the condition develops. Two among these risk factors are smoking and diabetes since they both significantly affect the formation of the tooth biofilm that leads to periodontitis [12].

Research studies show that tobacco damages periodontal tissues, hence there is a connection among smoking prevalence and periodontal infection [32].

Experimental findings indicate that diabetes considered as key risk element for periodontitis growth and that periodontal intensity is much higher in patients with type I and type II diabetes. While there is a positive link between the level of periodontitis and the extent of hyperglycemia, the fundamental mechanism is yet unknown. Diabetes and periodontitis have a reciprocal relationship, therefore maintaining good periodontal health is related to controlling blood sugar [33, 34].

Vitamin C supports the health of the gums, and Scurvy, a disorder caused by a severe vitamin C shortage, significantly impairs the production of collagen and mirrors many periodontitis symptoms [35].

Diet and nutrition have a directly contribute to the risk of caries, gum disease, and periodontitis [36], thus, periodontitis and malnutrition are related in a direct manner. Uncontrolled sugar intake and an unwillingness to brush teeth and clean the mouth make the condition worse.

Genetic propensity is one of the main variables controlling periodontitis. An inherited allele that causes excessive IL-1 production is discovered to be linked to a unique genetic marker for the identification of chronic periodontitis [37]. It is clear that periodontitis is somehow genetically controlled since it results from aberrant leucocyte and monocyte activity [38]. Furthermore, it has recently been established that chronic periodontitis contains a heritable component, if not entirely.

Aside from these, other risk factors include AIDS, ageing, diabetes, gender, genetic predisposition, osteoporosis, economic status, and mental stress. Adiposity and periodontitis are linked, according to a number of experimental findings [39]. A person who is fat is more likely to have a variety of comorbidities and issues. Patients with HIV frequently experience necrotizing ulcerative periodontitis (NUP), a sign of prolonged immune system reduction and a susceptibility factor for more severe forms of periodontitis [32].

However, psychological strain seems to be a significant, changeable risk factor for the onset and development of periodontitis [40]. Finally, the issue worsens and nearly

becomes unmanageable as a result of irregular dental exams, an earlier diagnosis of periodontal ailments, and gum bleeding examination.

1.6 Immunological Response of Periodontitis

The oral bacteria alter the host's immunological responses, and the periodontium is impacted by the protective factors brought on by the host reaction. In the absence of an inflammatory stimulus, the pro-inflammatory interleukin-1A and -1B, also known as interleukin-1 (IL-1), which are generated in response to bacterial infection, are blocked by the interleukin-1 receptor antagonist (IL-1RN). When IL-1 and IL-1RN are out of balance, the immune system reacts more strongly, leading to the dissolution of dental bone and the degeneration of gingival tissue.

The neutrophils provide the primary inflammation reaction to dental biofilm, moving from the lamina propria through the junctional epithelium and ultimately out into the gingival crevice. In gingivitis and periodontitis, this neutrophil outflow keeps the T cells and in the gingival and Langerhans cells crevicular epithelium moving through the basement lamina [41]. The (OLF), which is a sign of advanced periodontitis, are formed when (APCs), which include Langerhans cells, B cells, macrophages, dermal dendritic cells and T cells (CD8 + and CD4 +), intrude into the lamina propria [42].

1.7 Genetic Background of Periodontitis

Genetic and environmental variables can affect periodontitis, a multifactorial disease. Aggressive periodontitis is thought to be an autosomal dominant disorder with incomplete penetrance that results from a single gene deficiency. However, despite segregation research showing diverse mechanisms of transmission, several other investigations asserted the X-linked inheritance.

It has been determined that the periodontitis-causing genes are found on the fourth, eleventh, and first chromosomes [4 (4q 11–13), eleventh (11q14), and first (1q25)]. Tumor necrosis factor, IgG2 production, HLA-A9 and B15 antigens, and interleukin-1 gene polymorphism have been demonstrated to have favourable relationships with periodontitis [43].

Genes play a major role in controlling these intricate associations. Three genes— COX-2, GLT6D1, and ANRIL—have been linked to aggressive periodontitis; single nucleotide polymorphisms (SNPs) in COX-2 and ANRIL appear to be linked to chronic

periodontitis as well. On the other side, aggressive and chronic periodontitis are connected, respectively, by SNPs in the DEFB1 and IL-10 genes [44].

Periodontitis comes in a variety of syndromic forms, including aggressive periodontitis, which are caused by mutations in particular genes; periodontitis susceptibility is not brought on by a single mutation. Polymorphism is the result of frequent mutations in a large number of functional variations. interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha) and Interleukin-1 beta (IL-1)gene polymorphisms play a significant influence in controlling inflammation in periodontal disorders. Additionally, it has been discovered that a certain IL-1 gene influences how severe periodontal disease is [45].

Inflammatory conditions, such as periodontal ailments, may be associated with the methylation of cytokine genes and the resulting alteration in cytokine expression, according to studies analysing epigenetic changes. Individuals with periodontal conditions have hypomethylated IL-6 genes. Since chronic periodontitis patients have high levels of IL-6, a key cytokine implicated in bone resorption, IL-6 amplification could have an epigenetic effect. On the other hand, DNA methylation, which stimulates heightened cytokine signalling, can also be brought on by [46].

1.8 Prediction and Diagnosis of Periodontitis

Genetic testing is currently used to identify people with the gene mutations that cause LAD kinds 1 and 2, Chédiak-Higashi syndrome, Ehlers-Danlos syndrome, Haim-Munk syndrome and Papillon-Lefèvre syndrome are among the many syndromic types of periodontitis., in order to predict periodontitis risk [47].

The interleukin (IL)1 level of people who are predisposed to developing periodontitis can be assessed. Researchers discovered increased levels of interleukin (IL)-1 in people with periodontal illness, despite the lack of evidence linking elevated IL-1 levels to periodontal disease. Additionally, it has been discovered that inhibiting IL-1 on its own can lessen periodontal edoema, the degeneration of connective tissue scaffold, and the rapidity of bone resorption caused by periodontal pathogens [48].

For the identification of periodontal illnesses, radiographs, especially panoramic images and periapicals, are frequently used, primarily through the assessment of the level and sequence of bone loss with the identification of calculus accumulation and calculus accumulation in accordance with the generally accepted medical standards [49].

Periodontitis can be diagnosed at a very early phase because to the discovery of biomarkers in all of the saliva. Five biomarkers originating from the host that may be used for the early identification of periodontitis were discovered through recent experimental findings and meta-analyses. The two interleukins are IL-1 (interleukin-1 beta), Hb (haemoglobin), MIP-1 (macrophage inflammatory protein-1 alpha), IL-6 (interleukin-6), and MMP-8 (metalloproteinase-8). [50].

Restoration of periodontal tissues like the periodontal ligament around the teeth, cementum, and alveolar bone is necessary for periodontitis recovery. This calls for expanding the sinus floor, rebuilding the ridge where the future tooth implant will be placed, and fixing any osseous flaws around the implant. Although these methods have yielded encouraging results for tiny and medium-sized faults, they have a substantial influence on the probability of clinical outcomes depending on the desired kind of defect and clinical case selection. With current approaches, predictable regeneration results may or may not be reached under different circumstances. PTEBR, or periodontal tissue engineering and bone regeneration, advocates the usage of scaffolds, cells, signalling molecules, and growth factors to replace missing and damaged tissues. [51].

Three-dimensional (3D) bioprinting could be able to reproduce macroporous internal and bony framework structure of the graft with minimum material loss because of the highly accurate and efficient nature of the advanced manufacturing technology [52]. The periodontium needs to be rebuilt while keeping its natural features because it is a complicated tissue system made up of many parts such bone, gingiva, and cementum [53]. However, the enhanced 3D bioprinting technology is the initial step towards the repeatable creation of space-keeping, cell-loaded frameworks for the therapy of periodontal lesions that may be employed clinically for restorative purposes to meet specific to the patient therapeutic needs [54].

1.9 Possible strategies for periodontitis treatment

Treatment for periodontal disease aims to halt the illness from progressing further, lessen symptoms, maybe restore missing parts, and help patients maintain a healthy periodontal cavity. To ensure its successful implementation, periodontitis treatment employs a wide range of clinical approaches, including psychosocial interventions such as: Oral health instructions that are unique to each person Nutritional therapy to aid

cigarette quitting. Other types of surgeries are used to treat biofilm accumulation. Severe gingival inflammation management necessitates a combination of therapy approaches as well as a lifetime commitment to oral self-care [55].

There are two ways to treat the periodontal membrane,

- Non- surgical Treatments
- Surgical Treatments

1.9.1 Non-Surgical Treatment

Non-surgical therapies for the periodontitis consists of the removal of both layers of the gingival which has blackish sticky material called dental plaque and calculus with the scaling and root planning are did with the hand instruments or some curettes or sonic instruments [56]. The aim of the non-surgical treatment is to treat mechanically the calculus and eliminate the plaque. But it failed to regenerate the periodontal tissues. There are some laser treatments such as carbon dioxide and other doped materials may produce the effect of regeneration but it may show the defect in attachment levels [57]. There are variances in non-surgical treatment of periodontitis

- Scaling
- Root planning
- Antibiotics

1.9.1.1 Root planing and scaling

In scaling and root planing remove the blackish material called tartar and bacteria from the mouth while the root planing smooths root surfaces and unpromising the bacteria to grow into the mouth that causes the swelling or delay in the gums' ability to repair or connect to the tooth enamel. Scalers and ultrasonic equipment are used to scale and design the roots. The scalers are a surgical equipment with a few cutting boards that are used to remove stains, tooth plaque, and calculus [58, 59].

But in both supragingival or in subgingival ultrasonic instruments are used for curing. With the help of water steam, this equipment uses ultrasonic shudder at a rate of roughly 25,000–30,000 cycles per second to get rid of tooth supports deposits [60, 61].

1.9.1.2 Antibiotic

Oral antibiotics are the non-surgical treatment for the removal of various kinds of bacteria in the mouth. Oral antibiotics can assist in the direction of bacterial attacks in the mouth. The medicines for the treatment of bacterial infections are mouth colorants or different kinds of gels that have the antibiotics in the space between the teeth, gums, or into the pockets after deep cleaning. Oral antibiotics are compulsory to remove the diseases-causing microorganism like blackish material called dental plaque [62, 63].

1.9.2 Surgical Treatments

The major goal of dental surgical intervention is to make non-surgical treatments more accessible, as well as to generate a periodontal structure that allows for effective biofilm accumulation control [64]. Surgical intervention has proven a better result in terms of tooth retention than nonsurgical intervention in deep untreated pockets [65] and is especially helpful in areas linked with bone abnormalities [66, 67]. Surgery should only be performed on pockets that are less than 5 mm deep to reduce physical harm to the periodontal tissues. Variations in the results of several surgical and nonsurgical therapy typically diminish with time with proper oral maintenance [68].

Without prior medical therapy, dental surgery appears to produce advantages that are comparable to nonsurgical treatment followed by surgical treatment. Severe swelling during surgery can also make tissue manipulation and visualization during the operation more difficult. The goal of open flap debridement is to attain instilment for the root debridement to get pocket lessening and thorough going flap treatment for devices used up for remedial periodontal disease [69, 70]. Surgically, remaining gaps linked with bone deformities can be minimized. More research is needed to see if using an enamel matrix derivative gives extra advantages. Interproximal abnormalities are a difficult clinical condition for which numerous therapy options have been proposed. To choose the best therapy, a thorough and extensive analysis of the condition is required. To prevent or avoid disease recurrence and tooth loss, supportive dental therapy is required [71].

Recently the goal of periodontal surgery is to attain the roots surfaces, also maintain the favorable gingival shape, and also provide oral hygiene, and recover the periodontal tissues by means of the regenerative methods. Combination treatments including operating options with the bone grafts or numerous kinds of bones are replaced such as

bioactive glass, and hydroxyapatite. They all showing different kinds of success in periodontal regeneration [72].

There are some new methods in surgical cures to cure periodontal disease. These are the following surgical treatments.

- Soft bone grafting
- Guided tissue regeneration

1.10 Systemic antibiotics

Systematic antibiotics are used when non-surgical periodontal therapy does not produce the desired results. Systemic antibiotics are used along with non-surgical periodontal therapy with the goal of suppressing harmful bacteria and establishing a wholesome biofilm. If systemic antibiotic usage is being considered, the clinician must choose the stage of the treatment at which systemic antibiotics will be administered. The patient's compliance, side effects, and bacterial resistance must all be considered [56].

1.11 Localized Drug delivery Systems

Systemic antibiotic therapy has demonstrated some promising results in the management of periodontitis. Systemic antibiotics, on the other hand, are now exclusively advised for the treatment of periodontitis that is refractory or fast developing. Some of the disadvantages related to intake of systematic antibiotics are insufficient drug concentration at the place of infection, chances of developing microbial resistance, and many other side effects. The invention of intra-pocket drug delivery systems has become a topic of interest to treat periodontal infections. Periodontal pocket acts as a natural reservoir that can be used for the insertion of drug delivery devices. For drug release from the device Gingival Cervical fluid provides a leaching mechanism. These characteristics make periodontal pocket an ideal site for local drug delivery during the periodontitis treatment.

Intra-pocket drug delivery devices are in demand as they reduce the undesirable side effects, enhance the efficacy of the drug and result in greater patient compliance. These devices also have a potential to maintain efficient level of drug concentration in GCF for a longer time period in treating periodontitis disease.

The delivery vehicles for these systems may be synthetic, semi-synthetic, or of natural origin. Recent advances in polymer sciences have shown synthetic polymers that are

biocompatible and biodegradable and can be tailored to fulfil specific pharmacological and biological needs. There are lot of studies done on development of intra-pocket devices based on polymers that include drugs to treat periodontal disease [73].

1.12 Drug Delivery Systems for Periodontitis Treatment

1.12.1 Fibers

Fibers are thread like structures which are kept in periodontal pocket to provide prolonged drug release, and which are fixed with cyanoacrylate adhesive. Goodson et al. described hollow cellulose acetate fibers loaded with tetracycline hydrochloride. When these system were placed into periodontal pocket, they seemed to have shown good results against periodontal infection because they decrease the number of disease causing bacteria and produced results which could be compared to those of root scaling and root planning, though slightly less dramatic. [74, 75].

Because of the quick drug release of the cellulose acetate fibers caused by the diffusion process, nearly 95% of the drug disappears in the first two hours. Therefore, only using these fibers once will not be enough to sustain the desired level of medication concentration for an extended period of time [76].

1.12.2 Films

Film created by grinding or solvent casting has emerged as a far more widely used intra-pocket delivery technique. The appropriate diameters for larger films can be achieved by cutting or punching them, or they can be inserted into the cavity to the gingival or cheek mucosa surface. Drugs are discharged through drug diffusion, matrix disintegration, or matrix degradation from films that are matrix delivery systems that spread them throughout the polymer. This dose form is beneficial physically for use inside the pocket depending on the size of the pocket to be treated, it is simple to mantain the shape and size of the films. It may be quickly put into the pocket's base while causing the patient the least amount of discomfort possible [77].

1.12.3 Hydrogels

A hydrogel is a porous network which is three dimensional in nature. It forms when polymers crosslink with one another in the presence of a crosslinker. Precursor molecules are often cross-linked covalently, non-covalently, or physically to create the hydrogel. It can attract large amounts of water inside its structure or other aqueous solution without dissolution which helps grow its size. The hydrophilic groups i.e.,

carboxyl, amino and hydroxyl groups help the hydrogel to absorb water and affect its degree of swelling. These traits of hydrogel make it an ideal material for wound dressing to encourage cell division which helps in wound healing process [78].

1.13 Classifications of hydrogels

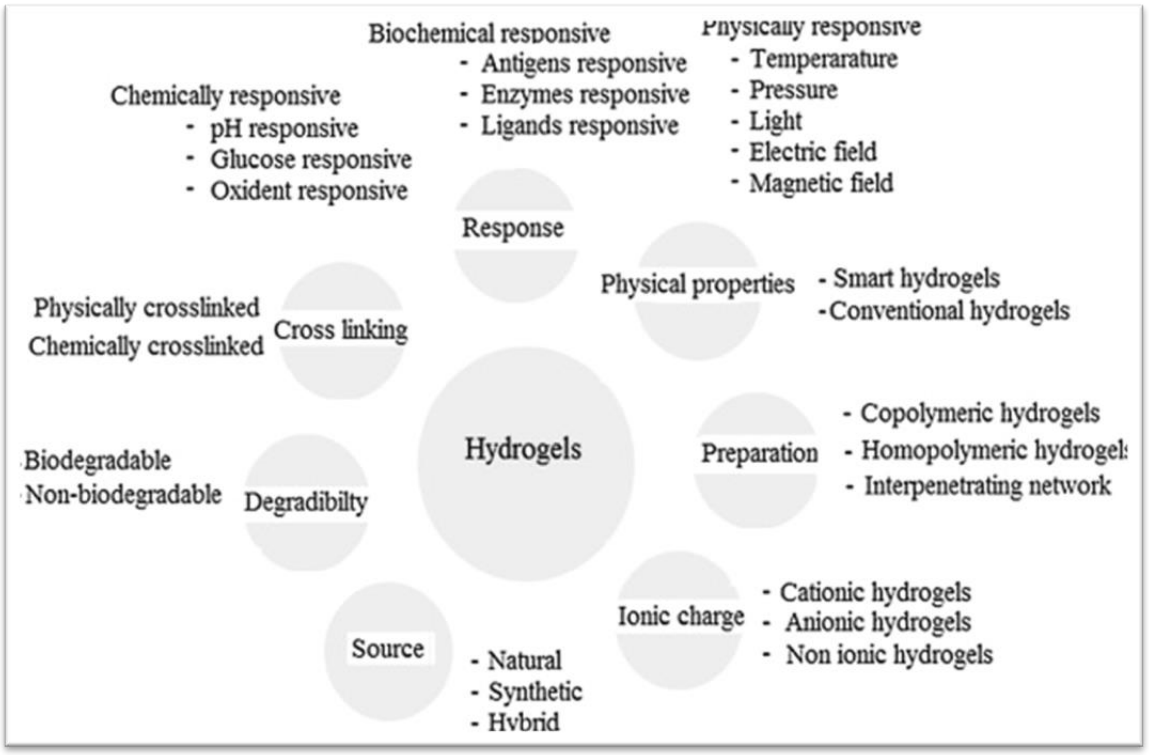


Figure 1. 4 Classification of hydrogels

The basis of the procedure in physical gels is physical crosslinking. This is often accomplished by physical processes such hydrophobic connection, chain aggregation, crystallisation, polymer chain complexion, and hydrogen bonding.

Chemical covalent bridging is a process that is employed (simultaneously or post polymerization) to produce a chemical hydrogel. Physical hydrogels are changeable due to conformational shifts, but chemical hydrogels are persistent and irreversible as a result of configurational alterations.

The dual-network hydrogel falls under a different category and is created by combining hydrogels that have been crosslinked chemically and physically. It has lately been employed to get over the limitations of simply using physical or chemical hydrogels by having an excellent liquid absorption capability over a broad range of pH and a better

responsiveness towards fluctuations in the pH as opposed to chemical hydrogels. Recently, Cong et al. [79] and Yalpani et al. [80] presented the growth of another dual-framework made of graphene polymer composites with exceptional mechanical characteristics.

1.14 Crosslinking in hydrogels

The networks created when crosslinks are formed between the various polymer chains behave in visco-elastic and sometimes pure elastic ways. The section afterwards discusses a variety of physical and chemical science hypotheses in order to assess crosslinking in hydrogels.

Physically crosslinked gels have garnered more interest in recent years. The fundamental justification is the absence of crosslinking chemicals in the production of such hydrogels. These chemicals not only compromise the integrity of the items to be captured (such as proteins or cells), but also are frequently poisonous substances that need to be extracted from the gels before use. Various techniques have been researched to produce physically crosslinked gels.

1.14.1 Crosslinking by radical polymerization

One feature of hydrogels is that the amount of crosslinker can be used to regulate swelling. Additionally, materials that are stimulus sensitive can be created by mixing in a crosslinker with certain characteristics. In addition to the radical polymerization of mixtures of vinyl-monomers, water-soluble polymers derivative with polymerizable groups can also be used to create chemically crosslinked hydrogels. This technique has been used to produce hydrogels using a variety of water-soluble (synthetic, semi-synthetic, and natural) polymers.

1.14.2 Crosslinking by chemical reaction of complementary groups

The solubility property of water-soluble polymers is caused by the presence of functional groups, particularly OH, COOH, and NH2, which can be exploited to create hydrogels. By combining functional groups with complimentary reactivity, such as an amine-carboxylic acid or isocyanate-OH/NH2 reaction, or by creating Schiff bases, covalent connections can be created between polymer chains. Additionally, crosslinking employing enzymes, high energy irradiation, condensation processes, addition reactions, and crosslinking have all been described for chemical hydrogels.

1.14.3 Crosslinking by ionic interactions

Alginate is a renowned example of a polymer which is capable of being cross-linked by ionic forces. The polysaccharide alginate can be connected by calcium ions and carries remnants of mannuronic and glucuronic acids [80]. At physiological pH and room temperature, crosslinking is possible. Alginate gels are therefore commonly utilised as the matrix for the release of proteins [81] as well as the encapsulation of living cells [82]. It's noteworthy to observe that a chelating reagent can destabilize the gels by removing the Ca-ions from them. Alginate microscopic particles are created by spraying a solution of sodium alginate into a water-based solution of calcium chloride. The releasing of proteins from these particles can be regulated by encapsulating the particles with cationic polymers, such as chitosan and polylysine [83]. Poly[di(carboxylatophenoxy)phosphazene] (PCPP) is a synthetic polymer that, like alginate, can be crosslinked with Ca-ions. Gel microbeads are created by misting a water based PCPP solution with an aqueous calcium chloride solution. Hydrogels that are ionotropic degrade in a physiological environment.

1.14.4 Crosslinking by crystallization

PVA is a type of polymer that dissolves in water. When PVA aqueous solutions are maintained at room temperature, a low mechanical strength gel eventually forms. It's interesting that a strong and extremely elastic gel forms after freezing and thawing the aqueous solutions of this polymer [84]. The PVA molecular weight, PVA concentration in the water, freezing temperature, freezing period, and number of cycles all affect the gel's characteristics. The PVA crystallites that form during gel formation serve as the network's physical crosslinking sites. Gels made under ideal conditions can be stored at 37 °C for six months [85].

Hydrogels that have been physically crosslinked are often made from graft copolymers or multiblock copolymers. After can be made of hydrophobic chains with water-soluble grafts or a hydrophobic chain with a water-soluble polymer backbone, such as a polysaccharide. Other crosslinking techniques that have been reported include protein crosslinking [86], hydrogen bonding [79], suspension polymerization [87], irradiation chemical reaction of identical groups [88], and hydrogen bonding [79]. These techniques all use a crosslinking agent, which is frequently toxic and raises objections about the gel's dependability. These factors goes to the generation of physically crosslinked hydrogels, which can be created via a variety of crosslinking techniques,

including ionic interaction crystallisation, hydrogen bonding, protein contact, and hydrophobic interaction [89].

Hydrogels are being investigated by researchers who are intrigued in the basic characteristics of swelling polymeric systems because of their capacity to soak up water, but they have additionally discovered extensive use in a variety of technical domains. These include polymers for protein and contact lens segregation, cell-encapsulating matrices, protein and medication delivery infrastructure, transporters for soil nutrients, cosmetics, and better oil recovery. The reader is pointed in the direction of a wide range of reputable publications and review articles that go over the fundamental characteristics and prospective applications of hydrogels.

According to the composition of the side groups, these hydrogels can be classed as neutral or ionic. The thermodynamic interaction of the water and polymer in neutral hydrogels produces a dynamic force for swelling that affects medical uses by raising the overall free energy [85]. The network has amorphous, semi-crystalline, hydrogen-bonded, super-molecular, and hydrocolloid aggregate physical properties [90].

1.14.5 Interpenetrating network hydrogels

Hydrogels can be categorised as (1) homo-polymers, (2) copolymers, (3) semi-interpenetrating networks, and (4) interpenetrating networks depending on how they were made. In contrast to homo-polymer hydrogels, which are cross-linked frameworks of a single type of hydrophilic monomer unit, copolymer hydrogels are made by the connecting of two co-monomer components, a minimum of one of which has to be hydrophilic in order to make hydrogel swellable. The next step is to build a network, which is then expanded in a monomer to produce interpenetrating polymeric hydrogels. As a result, the later develops a second intertwining network framework [91, 92].

1.14.6 Homo-polymeric hydrogel

Homo-polymers are the frameworks of polymers made from just one type of monomer. These are the fundamental structural element that any polymer network is made up of [93]. Homo-polymers might possess a crosslinked framework based on the kind of monomer and the polymerization procedure. Hydrogels made of polyethyleneglycol (PEG) are responsive to outside stimuli, making them intelligent hydrogels that are frequently utilised in drug delivery systems. PEG hydrogels that have been chemically crosslinked serve as scaffolds for the creation of functional tissues and recombinant

proteins. The release of biomolecules, proteins, medicines, and growth factors can be done effectively and under controlled conditions using this biomaterial [94].

1.14.7 Co-polymeric hydrogel

Co-polymeric hydrogels are composed of a pair of distinct kinds of monomers, preferably among one of them hydrophilic. In order to develop drug delivery infrastructure, Gong et al. [95] created a PECE (poly(ethylene glycol)-caprolactone-PECE) co-polymeric hydrogel that is biodegradable. The ring-opening copolymerization of -caprolactone is the mechanism at play. mPEG served as the initiator, stannous octoate served as the catalyst, and hexamethylene diisocyanate served as the binding agent in the triblock synthesis. This co-polymeric block can generate hydrogel when utilized in-situ.

1.14.8 Semi-inter penetrating network (semi-IPN)

A semi-inter penetrating network is the framework in which one linear polymer can partially penetrate another crosslinked network without the need for additional chemical linkages [96]. Semi-IPNs can better maintain fast kinetic responsiveness to either temperature or pH since they don't have a restricting interpenetrating elastic system, while maintaining benefits like adjustable pore dimensions and postponed release of drugs. The trapping of linear cationic polyallylammonium chloride in acrylamide/acrylic acid copolymer hydrogels produces increased mechanical strength and fully reversible pH shifting of theophylline release as one illustration to support the claim. By using template copolymerization with crosslinking agent N, N′-methylene bisacrylamide, this pH-sensitive semi-IPN was created [87]. Ionic and covalent linkages were both present in the network. The ionic linkages gave the hydrogel greater pH responsive and mechanical strength reversibility, while the covalent bonds preserved the hydrogel's 3-dimensional structure.

1.14.9 Inter penetrating network (IPN)

IPNs are often defined as two closely bonded polymers with at least one of them being produced or connected close to the other one [91]. To do this, a pre-polymerized hydrogel is usually immersed in a solution of monomers and a polymerization catalyst. Thermodynamic mismatch is caused by network sections permanently interacting; this problem can be resolved using the IPN technique to create a constrained phase separation. The interlocking architecture of the cross-linked IPN elements is considered

to provide stability for both the surface and bulk morphologies. IPNs' main advantages include the ability to make hydrogel matrices that are relatively dense and have mechanical qualities that are tougher and harder as compared to those of regular hydrogels, as well as the capability to modify physical characteristics and improve drug loading. Often, medication loading occurs synchronously with the interpenetrating hydrogel phase's polymerization [92]. The IPN pore diameters and surface chemistry may also be altered to customize the drug release dynamics, the hydrogel's interaction with the adjacent tissues, and its mechanical properties [97].

1.14.10 Stimuli responsive hydrogels

The phrase "environmentally sensitive, smart hydrogels" describes hydrogels that alter unexpectedly in their development patterns, network framework, mechanical strength, and porosity in response to external stimulation [98]. Light, temperature, pressure, magnetic and electric fields, mechanical stress, and the potency of different energy supplies are examples of physical stimuli that change molecular connections at critical starting points. Chemical stimuli including pH, chemical substances, and ionic factors change the molecular associations among chains of polymers and solvent and additionally between polymer strands themselves.

A different type of hydrogel known as dual responsive hydrogels is produced by fusing two stimuli-responsive mechanisms into a single hydrogel system. For instance, co-polyvinyl sulfonic acid and polyacrylic acid are examples of a dual reactive polymeric framework [99]. A biochemical stimuli includes reactions to ligands, enzymes, antigens, and other biochemical mediators [98]. The use of stimuli sensitive hydrogels in pharmacy, biological medicine, and biotech makes them interesting biomaterials [100].

1.14.11 pH responsive hydrogels

Patel and Mequanint presented polymeric hydrogels with ionic attached units that have the capacity to either accumulate or release protons in reaction to a change in the pH of the surrounding environment [101]. In a pH sensitive hydrogel, the level of ionization, or pKa or pKb, is considerably changed at a specific pH. This rapid change in net charge of the ionized pendant component generates a sudden volume shift through electrostatic repulsive forces among the ionized groups, which will in response generate a large osmotic inflating force. The two types of pH-responsive hydrogels are anionic and

cationic hydrogels. Whenever the surrounding pH is over the pKa, pendent compounds in anionic hydrogels, such as carboxylic or sulfonic acids, are deprotonated. This results in the pendent groups ionizing and increasing the hydrogel's swelling [102]. As opposed to this, cationic hydrogels that are possess pendent groups similar to amine groups, whereby ionization happens below the pKb, causing swelling as a consequence of stronger electrostatic repulsions [89, 103].

1.14.12 Self-Healing Hydrogels

The self-healing qualities might be imparted in a hydrogel with necessary chemical alterations. Any hydrogel or drug delivery device having the ability to self-heal after receiving recurrent human damage is known as a self-healing hydrogel. When harmed, it will rupture, and after the damage has been repaired, it can be partially or totally returned to its previous state in a matter of seconds to hours. Many studies have been interested in this self-healing behavior. In numerous investigations, materials like microcapsules with their microporous structure filled with self-healing chemicals. Whenever damage occurs self-healing chemicals will be released from the material and help in mending the damaged area. The hydrogel's capacity for self-healing will be constrained once the self-healing chemical has been released from it [104].

By converting physical energy into a chemical or physical response, a particular class of sophisticated substances known as polymers with self-healing properties can restore the damage [105]. In contrast to repairing the interior harm to the polymeric matrix, these polymers can recreate the mechanical properties (such as tensile strength) of the shattered portion through a self-sustaining healing procedure. To achieve this, the polymer must detect the destructive force and autonomously turn it towards the healing process that draws inspiration from nature [106]. An excellent example of this is how the skin injury recovery process in mammals uses a sophisticated blood-clotting pathway and consequent regrowth of tissue to aid in the restoration and repair of physical damage [107]. By using a similar methodology, this principle shows how biomimicry could aid in the creation of self-healing polymers. Polymers are excellent candidates for use as molecules for dynamic self-healing and supporting natural tissue regeneration due to their complicated chain structure. As a result, they can be applied to a variety of tissue engineering projects, including those involving bone, cartilage, skin, brain tissue, and even drug release techniques.

1.14.12.1 Bone

Since bone is the tissue that is majorly transplanted second only to skin, the need for synthetic bone replacements still exists [108]. Despite being the most popular procedure in clinics, autologous bone transplantation has several drawbacks because of the amount of tissue that must be taken and the donor site sickness associated with the elimination and implantation of the bone tissue [109]. Allografts have several advantages than autografts in this regard, including more lenient implanted tissue volume constraints. However, they also have extra problems related to graft rejection.

1.14.12.2 Skin Wound Healing

The body's initial line of defense towards the infection by pathogens is the skin, which also regulates body temperature, possesses mechanoreceptors, and reserves lipids. Hemostasis, inflammation, proliferation, and remodeling are the first four steps of the wound healing process that kick off when the skin sustains damage [110, 111]. To be effective, the wound healing process sometimes requires medical interventions because the injury is too severe or deep.

In this regard, manufactured skin substitutes hold promise for treating chronic or difficult-to-heal wounds. In order to achieve improved qualities, skin tissue engineering has used a variety of methods and materials, including films, membranes, nanofibers, foams, and hydrogels. However, due to their ability to be penetrated into irregular lesions, such as severe burns, and their quick self-healing ability, self-healing hydrogels have become more popular and have seen greater use in recent years [112].

1.15 Polyvinyl Alcohol

Poly vinyl alcohol is a hydrophilic synthesized polymer with bio-degradable and excellent cyto-compatible characteristics. Vinyl alcohol as monomeric units present in unstable form that’s why PVA synthesis is a twostep procedure. Different fractions of PVA polymer can be synthesized by adjusting the hydrolysis stage, which influences the behavior of the polymer composite, stability, crystalline nature, and chemical compositions [113]. Hydrophilicity, potential biodegradation, outstanding cytocompatibility, less cytotoxicity, surface alignment features, and bonding capabilities are some of its distinctive properties. In comparison to PVA with a high enthalpy change, PVA with a low delta H value has a better hydrophilic nature at low temperatures. Non polar nature of acetate groups decrease hydrogen bonding within its

structure which leads to solubility of PVA with high delta H value to solubilize in water at higher temperatures much over 70℃ and make it more difficult for PVA chains to crystalize. PVA has gotten a lot of interest in the biomedical area because of its remarkable bioactivity, notably in tissue regeneration [114].

Hydrogels containing poly vinyl alcohol in their structure have been examined as a replacement for current synthetic implants in tissue engineering. Vrana's research focuses on assessing the sensitivity of vascular cells to variations in the hydrogel matrix produced by doubling the frequency of freeze–thaw cycles. Because PVA hydrogels have large pore size and can disintegrate, Hoffman has been successful in cultivating live cells with them [115].

The physical features of PVA based hydrogels make them suited for biological applications. First, these hydrogels show high cytocompatibility inside body, indicating that when taken into the body, they will not have any adverse effects on living cells. Furthermore, these hydrogels exhibit physical qualities similar to real tissues, such as porosity, elastic modulus, and deformation characteristics, making them excellent for tissue replacements as well as other medical application. These hydrogels have a pH tolerance of around 13 suggesting that they can be employed as sensory biomaterials for regulated drug administration [116].

1.16 Hydroxyapatite

Hydroxyapatite (calcium hydroxyphosphate, Ca10 (PO4)5(OH) 2), is an inorganic constituent of hard tissue (bone). Ca/P mole ratio in pure HA is 1.67 because it includes 39.68 percent calcium and 18 percent phosphorus by weight. There are commercialized HA compounds with Ca/P ratios of more than or less than 1.67. The Ca/P ratio varies, suggesting a transformation between tricalcium phosphate and CaO [117]. HA crystals can be present in the hard tissues of body i.e. bone and teeth. Human bone is covered with HA crystallites as an active composite material in 65 to 70% of cases. In addition, type-I collagen serves as an organic substance of the bone's construction, whereas HA serves as an inorganic material. The network formed as a result of interaction of these two components exhibits a nano-scale composite structure in which HA is dispersed within collagen network. HA crystallites in the bone are 40 to 60 nm long, 20 nm broad, and 1.5 to 5 nm thick, and are shaped like sheets or spikes. The distribution of various HA crystalline lengths and diameters supports the structural integrity, toughness, and

functioning of this tissue [118, 119]. Naturally derived and man-made hydroxyapatite has always been favored as grafts material for hard tissue regeneration.

The biocompatibility of HA, which is characterized by bone development processes, has been shown to assist bone formation in bone regeneration. The bone regeneration characteristic of HA origin for synthesis, directing new bone development on its exterior all the way down to the implant body's pores [120]. The particular shape and porosity of HA determine this bone regeneration characteristic. It stimulates tissue growth, allowing bone formation in non-bony regions. Utilizing HA to cover an implant material improves its early load bearing capacity after insertion, resulting in a reduction in implant failure. By incorporating polypeptide into the surface of the implant, HA enhances the chemical interaction with the connected tissue. Because of its chemical similarity to bone elements, HA may attach directly to bone tissue without the need for an intermediate agent. All of these characteristics of HA indicate that using it as a cellular matrix material is of tremendous interest [121].

Tohamy, Khairy M., et al. explored Sodium alginate /Hydroxyethyl cellulose (HEC)/ Hydroxyapatite (HA) hydrogel for bone regeneration. Prepared hydrogel with higher HA contents showed enhanced cell growth, bioactivity and mechanical properties. Moreover, it played its role to enhance absorbed protein e.g. bovine serum albumin. HEC in the hydrogel increased the porosity, swelling and controlled the degradation rate and cell attachment of prepared hydrogel [122]. Li, Xuefeng, et al. prepared sodium alginate/acryl amide polymer/ferrous ions hydrogel by dual cross-linking strategy. Prepared hydrogel showed extraordinary mechanical properties including high toughness, high strength, healing capability and reversible gel sol transition. Because of the ionic cross linking it could show healing capacity of about 56% [123]. Balakrishnan, B., et al., explored biopolymer alginate/Carboxymethyl cellulose (CMC)/Hydroxyapatite (HA) composite hydrogel with antibacterial properties. Hydroxyl groups in alginate form Hydrogen bonds composite contributing in interfacial adhesion. The fascinating properties of the material used enhance cell growth and decrease the toxicity rate making it favorable as tooth or bone filler for damaged tissue regeneration [124]. Oxidized alginate (OA) was shown by Xu et al. to be a useful crosslinking agent in the fixation of biological tissue. The findings demonstrated improved tissue mechanical strength and demonstrated that OA does not cause microscopic entities to degrade. OA had a lower level of cytotoxicity on fibroblasts

than other compounds. Additionally, the OA surface offered a substrate for fibroblast growth [125]. Balakrishnan, B., et al., prepared oxidized alginate (OA) /gelatin and borax hydrogel for wound healing approach. Hydrogel provided a moist environment which helped in the migration of epithelial cells. Within 15 days hydrogel nearly completely filled wounds with new endothelial cells as compared to the bare wounds [126].

1.17 Gelatin

Another appealing and frequently utilized naturally occurred macromolecule in the biomedical sectors is gelatin. Gelatin has a wide range of beneficial properties, including outstanding biodegradability, non-immunogenicity, and the capacity to encourage cell adherence and proliferation [127, 128] The gelatin hydrogel has been shown in numerous research to be a versatile scaffold for tissue engineering [129]. Gelatin is being utilized to make a wide range of hydrogels joined by Schiff's base for biological uses. Upgraded gelatin, for example, was bonded by oxidized dextran to produce a fast-forming hydrogel coupled by Schiff's base. For regenerating cartilage, this type of hydrogel is frequently employed in tissue engineering [130]. Additionally, the Schiff base reaction was employed to create a hydrogel made of gelatin and oxidized alginate that was utilized for the treatment of osteoarthritis as an injectable matrix. [131]. However, the Schiff base reaction-based self-healing gelatin-based hydrogels are rarely cited. This might be because chitosan has a higher amino content than gelatin, which makes chitosan more suitable for making self-healing hydrogels [132].

In situ gelling hydrogels generated via imine and borate diol ester connections were created by Balakrishnan, B., et al. and used for targeted medication action in tumour therapy [133]. Ionic modification of bacterial cellulose was performed by Khamrai, M., et al., and used to create a gelatin-modified self-healing wound dressing film [134]. A self-healing hydrogel depends on cytidine and boronic acids that is driven by Ag+ ions has been created by Bhattacharyya, T., et al. Such a material has thixotropic and antibacterial qualities and can be employed for drug distribution that responds to stimuli [135].

1.18 Crosslinkers

1.18.1 Borax

Borax, often known by its common name sodium tetraborate, has the chemical formula Na2B4O710H2O. Borax is a member of the monoclinic system and belongs to the C2/c space group. The crystalline spatial arrangement of each sodium ion in the crystal structure of borax has a distorted octahedral geometry and lies at the point of inversion symmetry. Borax is a substance that rapidly dissolves in water and is present as the conjugated acid-base pairs NaB(OH)4 and H3BO3, as indicated in Scheme 1a. Borax is a well-known efficient crosslinking agent that can hasten the formation of hydrogels from compounds having active hydroxyl groups. Additionally, borax can interact with up to four hydroxyl groups in an aqueous solution where it exists as B(OH)4, enabling cross-linking reactions to take place at low concentrations also [136]. In order to create products that are inexpensive, environmentally friendly, and biodegradable, a borax crosslinker is required. However, borax is commonly used as a provider of boron in agriculture. A micronutrient called boron can promote the development of roots in plants and has a big influence on fertilization and photosynthesis [137].

To make Guar gum hydrogel with borax solution as a cross-linker is quite an easy method. However, GG hydrogels that have been cross-linked with borax are exceedingly brittle, lack elasticity, heal slowly, and have poor low-temperature endurance [138].

1.18.2 Glutaraldehyde

Glutaraldehyde is a dual-functional substance having polymerization ability. While it could subsequently interact with other groups, glutaraldehyde can interact with a number of enzyme moieties, especially the thiols, phenols, and imidazoles found in the main amino groups of proteins. But the particular makeup of the crucial structures involved in immobilizing an enzyme or crosslinking a protein is still not completely known. The interaction between glutaraldehyde and proteins suggests that there may be more than one mechanism at work. This is due to the equilibrium ability of glutaraldehyde's principal reactive forms to switch between their monomeric and polymeric forms. The way that each structure reacts to the protein may also differ. For instance, in both acidic and neutral environments, the aldehyde groups from glutaraldehyde may combine with proteins to create Schiff bases. In this instance, lysine's -amino group undergoes a nucleophilic attack on glutaraldehyde. Schiff bases,

however, become unstable in acidic environments and break apart, renewing both the amine and aldehyde groups [139].

1.19 Metronidazole

Metronidazole, sometimes referred to as Flagyl, is an antibiotic and an antiprotozoal u sed in medicine [140]. It can be used alone or in combination with other medications to treat a variety of inflammatory disorders, including bacterial vaginosis, endocarditis, and pelvic inflammatory disease. Many anaerobic infections have been treated with metronidazole. Anaerobic bacteria constitute normal human flora in the oral and intestinal spaces, and become causative pathogens if infection exists at these sites[141]. Because of its specific antibacterial activity against obligate anaerobes and excellent concentration in serum and gingival crevicular fluid (GCF), metronidazole (MZ), a nitroimidazole derivative, has been widely utilised to treat periodontal disease. Due to its ability to suppress bacterial nucleic acid production and hence work against a variety of Gram-negative anaerobic bacteria, metronidazole is frequently recommended for the treatment of periodontal disorders[142].

Chapter 2

Literature Review

2 Literature Review

Researchers et al have worked on the hydrogel delivery system. For the clinical usage and for drug delivery purpose this system is therapeutically helpful. Hydrogels also seems beneficial to give a means of controlling the release of different therapeutic agents, such as cells, macromolecules, and tiny molecules. Because of their programmable physical properties, controllable degradability, and ability to shield labile medicines from degradation, hydrogels offer a platform on which diverse physiochemical interactions with the encapsulated medications are used to govern drug release. The discussion over here is that which setups are used to preserve the drugs by the interactions of different mechanisms and can be integrated to control within time and space. Poor targeting and brief circulation periods (less than 12 hours), which limit oral administration, the extensively used and most popular method of delivering medications, are typical limitations. The size of hydrogels also depends on the requirements by which we required. Hydrogels may be cast or designed into practically any shape and size according to the need of dilevery route the human body. Three classification of hydrogels have been made according to the size namely macroscopic hydrogels, microgels and nanogels with the directions on the order of micrometers and nanometers as well[143].

Researchers et al have made contributions to the periodontal treatment which is based to the mechanical scaling and root planing to eliminate certain periodontal infections, primarily anaerobic Gram-negative bacteria. Antimicrobial drugs are frequently administered orally in high doses if the periodontal abscess is detected. By the action of this approach there have been increase in risks of antibiotic resistance and systemic side effects and decrease efficacy. To control the above-mentioned issue, The goal of this study is to improve thermosensitive hydrogels' ability to deliver the antibiotic metronidazole (MTZ) precisely and locally to the site of an oral infection. To create the thermosensitive hydrogels, 28% w/v Pluronic F127 was mixed with different methylcellulose and silk fibroin concentrations. Investigated gel attributes include gel strength, viscosity, and sol-gel transition time. The drug dissolving profiles were also identified, along with their theoretical models and gel dissolution features. All the hydrogels formulations bear sol-gel transitions at 37°C within a minute. The viscosity of the material has been increased by increasing the methylcellulose(MC) but due to this action the strength of the hydrogel decrease[144].

Researchers et al have worked on the enhancement proliferation and migration of host cells by the process of hydrolyzation of self-assembly peptide (SAP) with functionalized motifs. By the fact that natural materials have risks of infection biomimetic matrices are preferred in tissue regeneration. SAP hydrogels have three-dimensional environment which enhance the proliferation and migration of many kinds of cells. The animals have been allocated to the following 4 subgroups: (a) Unfilled (n = 10), (b) RADA16 (n = 10), (c) PRG (n = 10), and (d) PDS (n = 10). Under general and local anesthesia, standardized bilateral periodontal defects (2 × 2 × 1.7 mm) have been surgically created mesially of the maxillary first molars (M1) according to the method The M1 root was denuded of its PDL, cementum, and superficial dentin. After rinsing with sterile saline and drying, the defects received 50 μL of 2.5% RADA16, PRG, PDS or left unfilled. Resorbable sutures were used to close the flaps. Acetaminophen was given to control pain. Following surgery, sacrifice has performed at 2 or 4 weeks. At four weeks the epithelial downgrowth in the hydrogel groups has been significantly reduced compared to the unfilled group[145].

Researchers et al have made contributions of irreversible defects in the periodontal ligament (PDL) which is caused by the periodontitis. The main hurdle created to the clinical treatment of periodontitis by the regeneration of it. The predominant treatment to enhance PDL regeneration is to use hydrogel to feed for releasing anti-inflammatory drugs. Alg-PBA has been esterified by reacting Alg with PBA to create [42]. 0.1 M MES buffer was used to first dissolve 1 g of alg into a 1% (w/v) solution. Following that, 0.5 g EDC and 0.7 g NHS were added to the Alg solution, along with 500 mg PBA that had been dissolved in 5 ml of methyl sulfoxide. Using 1M NaOH at 25C, the pH of the combined solution was maintained at 4.5–5.0 for 24 hours. After the reaction was complete, the solution was centrifuged for 0.5 hours at 7500 rpm/min to remove any unreacted PBA. The supernatant was then lyophilized after being dialyzed (MWCO 3500) for 5 days against deionized water. By using 1 H NMR spectroscopy (400 MHz JEOL), it was confirmed that the alteration of alginate was successful. The molar ratio of the phenylboronic acid unit to the alginic acid group(that is, the ratio of the integral value of the phenylboronic acid unit to the integral value of the alginic acid group) is used to calculate the degree of modification of phenylboronic acid on alginic acid[146].

Researchers et al have worked on a moist wound environment. It has been seamed that the functionally active wound hopefully provides a moist wound environment.

Additionally, it offers defense against secondary infections, cleans wound exudate, speeds up tissue regeneration, and improves the effectiveness of wound healing. based on biodegradability, biocompatibility, safety against toxins, antimicrobial properties, and biological adhesiveness chitosan-based hydrogels has been taken as an ideal material for the enhancement of wound healing. Chitosan provides a non-protein matrix for 3D tissue growth and activates macrophages for tumoricidal activity. There have been prepared an injectable hydrogels which consists of nanotigecycline and chitosan platelet-rich plasma. Chitosan with a mixture of other polymers or its modification form which contains an active substances enables to promote the effectiveness of wound healing[148].

In vivo evaluation of a combination therapy for the treatment of periodontal disease consisting of systemic administration of parathyroid hormone and locally administered neutral self-assembling peptide hydrogel. The WST-1 assay was used to evaluate the viability and proliferation of rat periodontal ligament cells while they were suspended in a neutral SAP nanofiber hydrogel (SPG-178). In forty Wistar rats, periodontal abnormalities were induced distally to the maxillary first molars. Either 1.5% SPG-178 was used to fill the defects, or the defects were left unfilled. Injections of either PTH (1/34), or saline, were given to the animals every other day. At 2 or 4 weeks after surgery, microcomputed tomography, histological, and immunohistochemical examinations were carried out in order to assess the level of healing that had taken place. When compared to cells that were either treated with 0.8% SPG-178 or were left untreated as controls, cells that were grown in 1.5% SPG-178 showed significantly higher levels of viability and proliferation after 72 hours. In vivo, the administration of systemic PTH led to a significantly increased bone volume in the Unfilled group at both 2 weeks ($p = .01$) and 4 weeks ($p .0001$) as compared to the saline control. At 4 weeks, a significantly greater bone volume was observed in the PTH/SPG178 ($p = .0003$) and PTH/Unfilled ($p = .004$) groups than in the Saline/SPG178 group. This difference was statistically significant ($p = .0003$). At 4 weeks, histological examination revealed that the PTH/ SPG178 group had significantly more bone formation than the other groups. Increased proportions of PCNA+, VEGF+, and Osterix+ cells were found in the treated locations of the PTH/SPG-178 group's tumors. These findings lead us to the conclusion that sporadic administration of systemic PTH and the local delivery of neutral SAP hydrogel both contribute to enhanced periodontal repair[149].

Periodontitis is a persistent inflammatory disease that is brought on by pathogenic biofilms and the immune response of the host. It is responsible for the destruction of tooth-supporting tissues, such as the gingiva, periodontal ligament, and alveolar bone. Chewing and saliva secretion are two of the physiological actions of the oral cavity that drastically restrict the amount of time that therapeutic medications are able to remain in the region of a periodontal lesion. In addition, the numerous and varied mechanisms that cause periodontitis make it difficult to treat the condition in an efficient manner. As a result, the foundation for an effective treatment of periodontitis is the development of sophisticated local drug delivery methods as well as sensible therapeutic tactics. Because of their biocompatibility, biodegradability, and the ease with which they can be administered into the periodontal pocket, hydrogels have generated a significant amount of attention in the field of periodontitis treatment. Recent years have seen a shift in the focus of hydrogel research toward smart stimuli-responsive hydrogels. These hydrogels are capable of undergoing flexible sol-gel transitions in situ and controlling drug release in response to stimulation by temperature, light, pH, ROS, glucose, or enzymes. In this review, we introduce in a methodical manner the research and rational design of new smart hydrogels that respond to stimuli for periodontitis treatment. In addition to this, we go through the most recent and cutting-edge therapeutic approaches using smart hydrogels, which are based on the pathogenesis of periodontitis. In addition, the difficulties and potential future research areas for intelligent hydrogels for treatments for periodontitis are explored with a focus on the development of effective hydrogel delivery systems and prospective clinical uses[150].

Plasmonic nanoparticles have distinct optical and chemical properties, and as a result, novel synthetic methods for their synthesis are continually being developed in order to manage their size, shape, colloidal stability, and surface functionalization. This is because plasmonic nanoparticles have these qualities. In this study, a biphasic approach for the production of gold nanoparticles (also known as Au NPs) is presented. This process is based on the controlled release of a reducing agent from a supramolecular polymer hydrogel. To be more specific, gold nanoparticles are generated by exploiting the reducing capabilities of polyphenols to facilitate the diffusion of gallic acid (GA) from a poly(vinyl alcohol) (PVA)/GA hydrogel into a solution containing Au3+. The process of the creation of gold nanoparticles (Au NPs) is explored under various situations, such as altering the stochiometric ratio between PVA and GA and the

concentration of Au3+. It has been established that even a minor alteration in the circumstances can result in distinct morphologies and sizes in the gold nanoparticles. Using this method, it is possible to produce Au NPs with a size of less than 20 nm, which is significantly smaller than the Au NPs that can be produced by simply mixing GA with Au3+ in any concentration ratio. For example, the usage of 5 weight percent GA in PVA-GA hydrogels in conjunction with 0.25 millimoles of Au3+ solution can result in the production of spherical and highly monodispersed GA-functionalized Au NP colloidal dispersions with a size range of 7 to 2 nanometers. This provides a substrate that is ideal for use in biomedical applications.

The findings are explained by referring to a variety of conventional diffusional models, the morphological characterization of the PVA-GA hydrogels, and UV-vis spectroscopy in conjunction with electrodynamic simulations[151].

It is crucial to have conductive fabrics because they may be used to construct clothing and other textiles that can conduct electricity. This enables the clothing and other textiles to be utilized in a variety of applications including wearable technology, medical devices, and military equipment. The development of conductive fabrics through the use of hydrogels can boost the possibilities for expanding the application of this technology in a variety of fields, most notably the biomedical sector. The sol-gel method is utilized to produce cotton non-woven alginate conductive hydrogel for the purpose of this research. In order to examine the effects of different concentrations of sodium alginate and silver nitrate on the performance of a non-woven conductive hydrogel, we employed three different concentrations of sodium alginate (0.5%, 1%, and 1.5%) and silver nitrate (0.5%, 10%, and 15%). Characterization of these created composite structures of conductive hydrogels included testing for surface resistance, X-ray difraction, scanning electron microscopy, and Fourier transform infrared spectroscopy (FTIR). According to the findings, the composite that contained 1% weight of sodium alginate and 15% weight of silver nitrate had the best surface resistivity values that were lower than 100 Ohm per square. This conductive hydrogel may be applied to non-woven fabrics, and it has the potential to be employed in both smart textiles and other types of technological textiles[152].

When there is an infection in the periodontal pocket, there are two significant problems associated with drug delivery: administration into the periodontal pocket and a high

fluid clearance rate in the periodontal pocket. the compartment or pocket. The purpose of this study was to design and investigate a novel gelatin-based hydrogel system that was crosslinked using a carbodiimide. This method was intended to transport chlorhexidine (CHX) directly into the periodontal pocket through injection, and then in situ gelation was to follow. The effects of the concentration of CHX on the release profile of the hydrogel, as well as its physical, mechanical, and biological properties, were the primary focus of this work. CHX is a common antiseptic agent that is regarded as the "gold standard" in the dental industry. Its release profile showed that there was a burst release of 39% within the first two hours, which was then followed by a release rate that gradually decreased over the next six days. The experimentally determined release profiles were flawlessly represented by a mathematical model that is based on the two-stage desorption theory ($R2 > 0.99$). Results showing fibroblast viability of at least 70% were obtained. achieved after 24 and 48 hours, demonstrating that the technique is suitable for use in living organisms. Injectability and biocompatibility tests showed that both non-loaded and CHX-loaded hydrogels were successful. showed the desired gelation periods of 7.5–10.6 seconds, which means it is suitable for filling a peri-odontal pocket. They demonstrated outstanding mechanical qualities, such as burst resistance, for example.strength (ability to seal) ranging from 233 to 357 mmHg, tensile modulus ranging from 47 to 69 kPa, compressive modulus ranging from 58 to 104 kPa, and tensile strain ranging from 42 to 113%. To sum everything up, The CHX-eluting hydrogels that have been examined have a great potential to be used in minor pockets as well as deep pockets. Furthermore, it is anticipated that these hydrogels will be useful for successfully treating a wide variety of periodontal infections[153].

Chapter 3

Material and Methods

3 Material and method:

3.1 Materials:

Table 3-1 Chemicals used

Materials	**Company**
HA synthesis	
$Ca(OH)_2$	ACROS ORGANIC
$(NH_4)_2HPO_4$	DAEJUNG
Modified HA Synthesis	
Arginine	
Calcium Nitrate Tetrahydrate	DAEJUNG
Hydrogel synthesis	
Polyvinyl alcohol	MERK
Gelatin	DAEJUNG
Glutaraldehyde 25% Aqueous	DAEJUNG
Borax	SIGMA-ALDRICH
Drug loading	
Metronidazole (MTNDZ)	BDH
For Swelling and degradation studies	
PBS	bioWORLD
Anti-bacterial activity	
Sodium Chloride	Fisher Chemical
Tryptone	BIO BASIC INC.
Yeast Extract	BIO BASIC INC.
Agar	Neogen

3.2 Instrumentation

Table 3-2 Instruments used

Instruments and Equipment	Company/Brand

Hotplate	VELP Scientifica/Corning PC-420D
Weighing balance	OHAUS
Drying oven	Classic 1040
Thermometer	Einbaulange 70mm
Contact angle	Biolin One attension theta flex
FESEM	Thermo Fischer Product Apero FESEM
Freeze dryer	SP SCIENTIFIC
Zeta sizer	Malvern
FTIR spectrometer	Nicolet iS50 FT-IR
X-ray diffractometer	Rigaku
Thermogravimetric analyzer	BXDSC-TGA-1250 (BAXIT, China)
Pycnometer	Quantachrom Instruments
UV-Microplate Reader	Multiskan SkyHigh

3.3 Hydroxyapatite synthesis

The synthesis of HA was carried out by in-situ co-precipitation using 1.67 M $Ca(OH)_2$ solution and 1 M $(NH_4)_2HPO_4$ solution prepare separately in deionized water by adding 11.11g into 150ml and 11.88g into 150ml water respectively. Using a dropping funnel, add (NH4)2HPO4 solution dropwise (10 drops per minute) to Ca(OH)2 solution. For one hour, the reaction mixture was stirred. The reaction was left to age for 48 hours. Filtered the reaction mixture to separate precipitates (ppts) and washed thoroughly with distilled water till neutral pH of the filtrate was achieved. The HA precipitates were dried at 80 ^{0}C for 24 hours. After that ppt was grind into fine powder to obtain particles of size less than 100 micron.

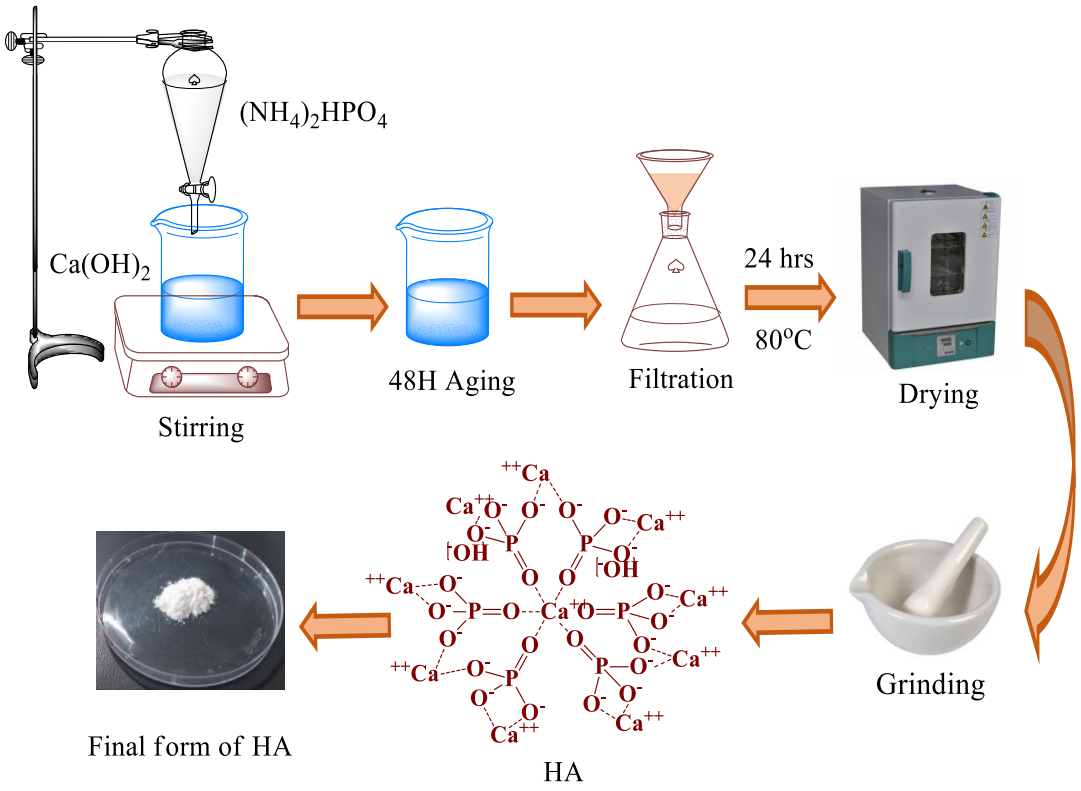


Figure 3-1 Synthesis of HA

3.4 Synthesis of modified HA with arginine

Dissolve 12g of $Ca(NO3)_2.4H_2O$ and 2.65g amount of the amino acid in 30 mL of distilled water. Add aqueous NH_3 (1 M) to adjust the pH to 9. Add water to a volume of 50 mL to give a final Ca^{2+} = 1 M. Prepare a 0.6 M diammonium hydrogen phosphate solution by adding 4.04g diammonium hydrogen phosphate into 30mL water, adjusting the pH to 9 with aqueous NH_3, and making up the volume to 50 mL with distilled water. Add 50 mL of the phosphate solution to 50 mL of the calcium/amino acid mixture to give a white precipitate. Readjust the pH to 9 by adding aqueous 1 M NH_3 (The amount needed will depend on the initial pH of the $Ca(NO_3)_2$. $4H_2O$ solution and the phosphate solution. If these solutions are already at pH 9, no NH_3 solution will be needed). Age the reaction mixture in a hydrothermal reactor for 16 h at 120°C. Obtain a colloidal dispersion of nanoparticles for most amino acids[154]. Filtered the reaction mixture to separate precipitates (ppts) and washed completely with distilled water until the filtrate's pH reached neutral. For 24 hours, the HA precipitates have been dried at 80 ^{0}C. After that ppt was grind into fine powder to obtain particles of size less than 100 micron.

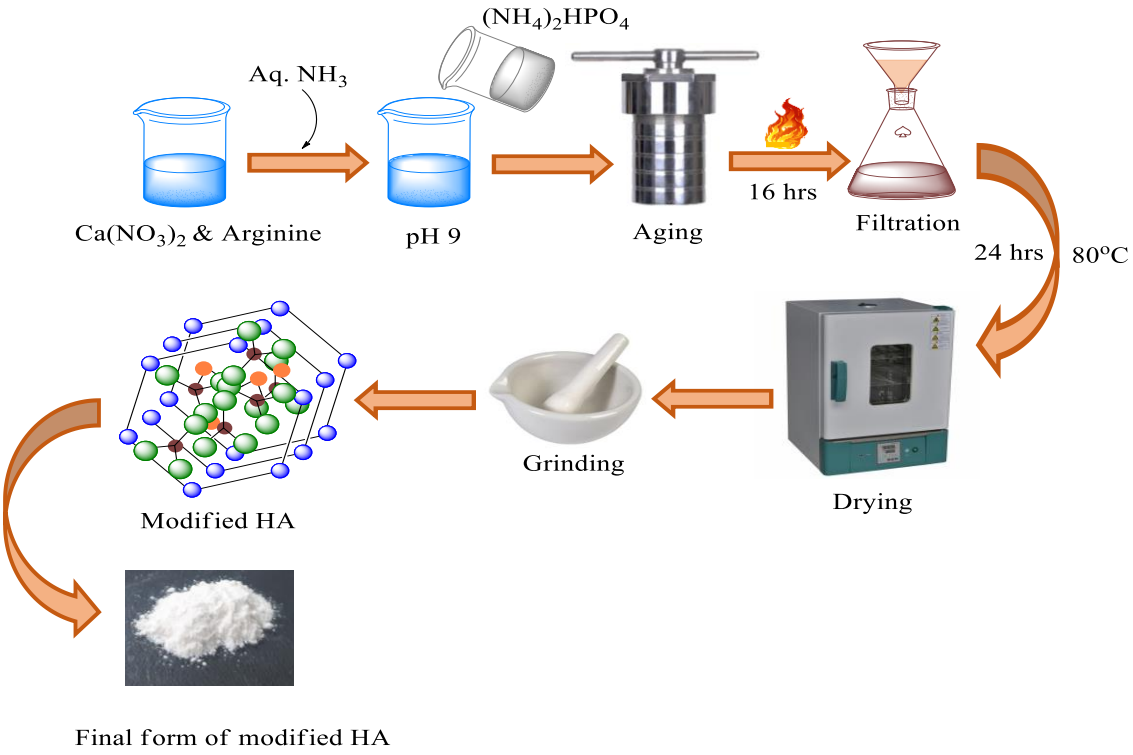


Figure 3-2 Synthesis of modified HA with arginine

3.5 Hydrogel synthesis

A 3:2:32:0.0606:0.06 mass ratio of polyvinyl alcohol (PVA), gelatin (Ge), water (H2O), glutaraldehyde (GA) 0.5% and borax is used for the pure mixture. PVA dissolved in water on water-bath at 90°C with continues magnetic stirring. Then gelatin added at to 50°C temperature and stirred until dissolved. Added borax solution followed by glutaraldehyde dropwise and mixed it well. The different hydrogels prepared on the basis of hydroxyapatite (HA) percentage and HA added after dissolving PVA at 80°C. These hydrogels were named as 0%HA, 10%HA, 20%HA, 30%HA and 40%HA.

3.6 Drug loading on Hydrogel

0%HA, and 30%HA hydrogels prepared with unmodified HA and modified HA(MHA) according the method mentioned above. 3% MTNDZ drug was loaded on Hydrogels by dissolving it into PVA solution during the synthesis process.

3.7 Swelling studies of hydrogels

Swelling is the term used to describe the volume change that occurs when a gel absorbs a suitable solvent, in the case of hydrogels, water. Swelling is feasible because the hydrophilic and elastic properties of the solid hydrogel, which is typically a network of polymer molecules, allow for the absorption of sufficient water to expand the volume

while preventing complete disintegration. Swelling is one of the most crucial properties that affects how well it releases drugs. Weighed and stored in excess of the swelling medium PBS solution were dried hydrogels and then weighed after intervals for 24h. Before weighing wet samples dry with filter paper. The experiments were performed in triplicates, and the results were reported as mean standard deviation.

The following formula was used to determine the swelling's degree:

$$SW\ (\%) = Ws - Wd\ /\ Wd \times 100$$

Where W_s and W_d are the weight of swollen and dry samples, respectively.

3.8 Degradation studies of hydrogels

Completely dried hydrogels were weighed and kept in excess of PBS solution at 37^{o}C in oven. Then take out the samples after 3days, and put them in oven at 37^{o}C to dry and weighed them. Same way used to take readings of 7days, 14days, 21days, and 28days samples. The experiments were performed in triplicates at room temperature and the data obtained were reported as mean ± SD.

The degree of degradation was calculated according to the fallowing formula:

$$DW\ (\%) = Wo - Wd\ /\ Wo \times 100$$

Where *W*o and *W*d are the weight of original and degraded samples, respectively.

3.9 FTIR spectroscopic analysis of Hydrogels

The materials were examined using Fourier Transform Infrared Spectroscopy (FTIR) to identify the presence of particular chemical groups. Completely dried hydrogels analyzed by FTIR Nicolet iS50 using Absorbance mode. The range of wavelength 500 to 4000nm, no. of scans 4cm^{-1} and 256cm^{-1} resolution used to get FTIR spectra of all the hydrogels. Significant vibration bands found in FTIR spectra were correlated with the chemical group after getting normalized[155].

3.10 Thermal Analysis

The thermal gravimetric analysis was performed on TGA BXDSC-TGA-1250 Thermogravimetric analyzer BAXIT, China, with a heating rate of 10°C/min in a nitrogen atmosphere. The samples typically had a mass between 2 and 3 mg. The temperature of the sample pan was increased from room temperature to 800 °C using the balance system equipment.

3.11 X-ray diffraction pattern analysis

A non-destructive method known as X-ray diffraction analysis (XRD) that can give precise details on a material's chemical composition, crystalline structure and physical characteristics. It relies upon the constructive interference of crystallographic sample and monochromatic X-rays. The X-ray diffraction analysis of dried hydrogels samples done with Rigaku MiniFlex 600C X-ray diffractometer using step size 0.02, at scan rate 10°/min of monochromator Cu kα and 2θ range 5°-70°.

3.12 Water Contact angle

To determine the hydrophobicity and hydrophilicity of hydrogels, the wetting behavior of the hydrogels was investigated using a water contact angle device Biolin One attension theta flex. In order to examine how wetting behavior changes over time, we recorded wetting at 0 and 10 seconds[156].

3.13 Drug Release study

For drug reases study standard curve made first. To make a stock solution (1 mg/mL) of metronidazole, 15 milligrammes of the drug will be dissolved in 15 mL of DI water. Different dilutions ranging from 2 g/mL to 60 g/mL will be made from stock solution. These dilution sets will be examined using a Multiskan SkyHigh Microplate Spectrophotometer to determine their absorbance values for metronidazole at a maximum wavelength of 320 nm. The values of their absorbance will then be recorded. The absorbance readings will be plotted against the corresponding concentrations to create a standard calibration curve [157].

Each MTDZ-loaded sample will be weighed out and dissolved into a 50 mL solution of PBS at a physiological temperature of 37 °C. Then, to maintain the constant volume, or sink conditions, a 5 mL sample aliquot will be removed at various time intervals and refilled with 5 mL of fresh PBS solution. At a set max value of 320 nm, the MTDZ concentration will be measured spectrophotometrically. Each sample's in vitro release data will be gathered in triplicate, however data analysis and graphical display take average values into account.[158].

3.14 Anti-bacterial Activity

The antibacterial activity of produced hydrogels against Staphylococcus aureus (ATCC6538) and Escherichia coli (ATCC8739) was investigated using the disc diffusion method. For bacterial strains, 10mL of autoclaved broth solution with a loop

of the appropriate bacterial strain was added and incubated for 24 hours at 37°C. By dilution with autoclaved broth, the OD of the culture was set to 0.1 at 640nm wavelength. The agar solution was prepared and autoclaved to make the agar plates. In order to allow the agar to solidify, hot agar was evenly poured into petri plates and maintained at room temperature in a safety cabinet. On solidified agar, 50µL of bacterial culture were evenly dispersed. The wells were made on the agar plates that had solidified. Following the placement of the samples (200 mg), the labeled Petri plates were covered with paraffin film. The plates were then stored for 24 hours at 37°C in an incubator. The diameter of the inhibitory zones was measured on millimeter scale [159].

3.15 Zetapotential of HA and MHA

The charge and Size of Hydroxyapatite and modified HA were measured with Malvern Zetasizer Nano-ZS instrument at scattering angle of 90^{o}. The suspension of HA and MHA was formed in water with 100PPM concentration [160].

Chapter 4
Results and Discussion

4 Results and Discussion:

4.1 Zetapotential of HA and MHA

Name	Size (d.nm)	Zeta Potential (mV)
Simple HA	817.7	-85.2
Modified HA	1542	-222

Table 4-1 Zetapotential of HA AND MHA

Zeta Potential of both HA and MHA was analyzed and both gives the negative charge on them. The negative Zeta potential value of HA reveals that it negatively charged hydroxyl groups present on its surface. While Arginine can adsorb onto the surface of MHA through electrostatic interactions between its positively charged guanidinium group and the negatively charged phosphate groups on the HA surface. The adsorbed arginine molecules can introduce negative charges to the MHA surface, resulting in a negative zeta potential [161].

4.2 Swelling studies of hydrogels

0% HA and 10% HA samples almost show the same swelling. 20% HA and 30%HA samples show lower swelling than 0% HA and 10% HA. 40% HA show lowest swelling and have highest percentage of hydroxyapatite. The increase in HA decreases the porosity which reduce swelling[162].

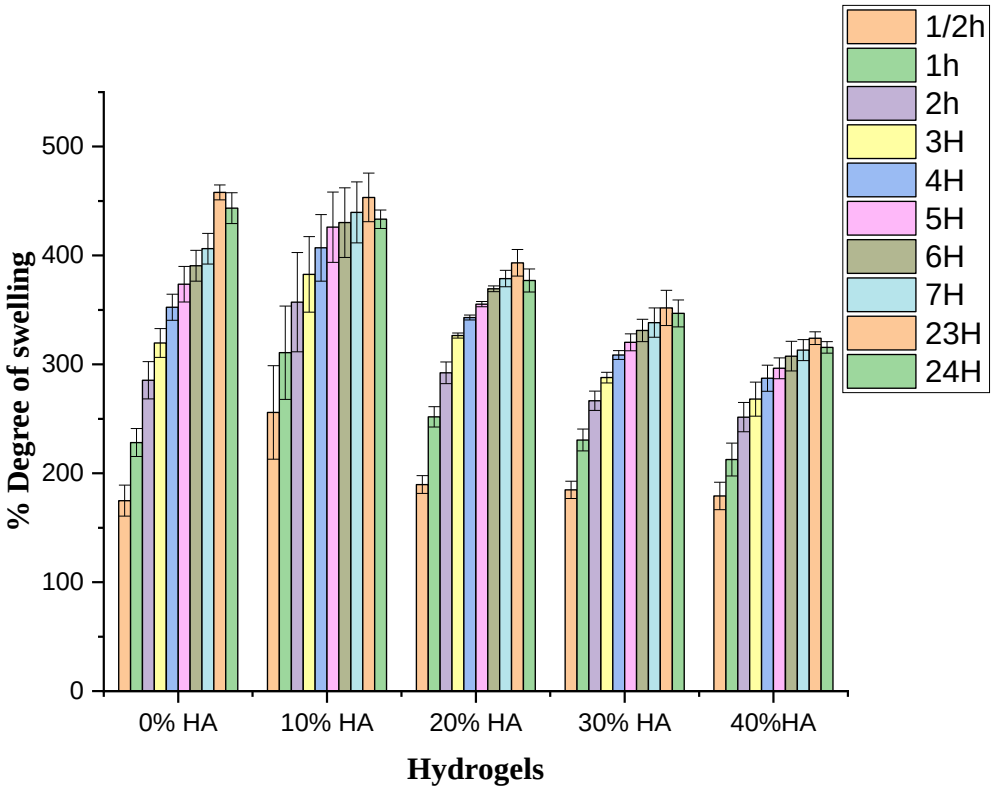


Figure 4-1 Swelling Ratio of Hydrogels

4.3 Degradation studies of hydrogels

In general, the influence of light, water, air, and heat are the major factors in hydrogel degradation. The degradability of hydrogels is primarily determined by the hydrolysis of either the cross-links or the polymer backbone, which results in a decrease in hydrogel weight and degree of crosslinking.

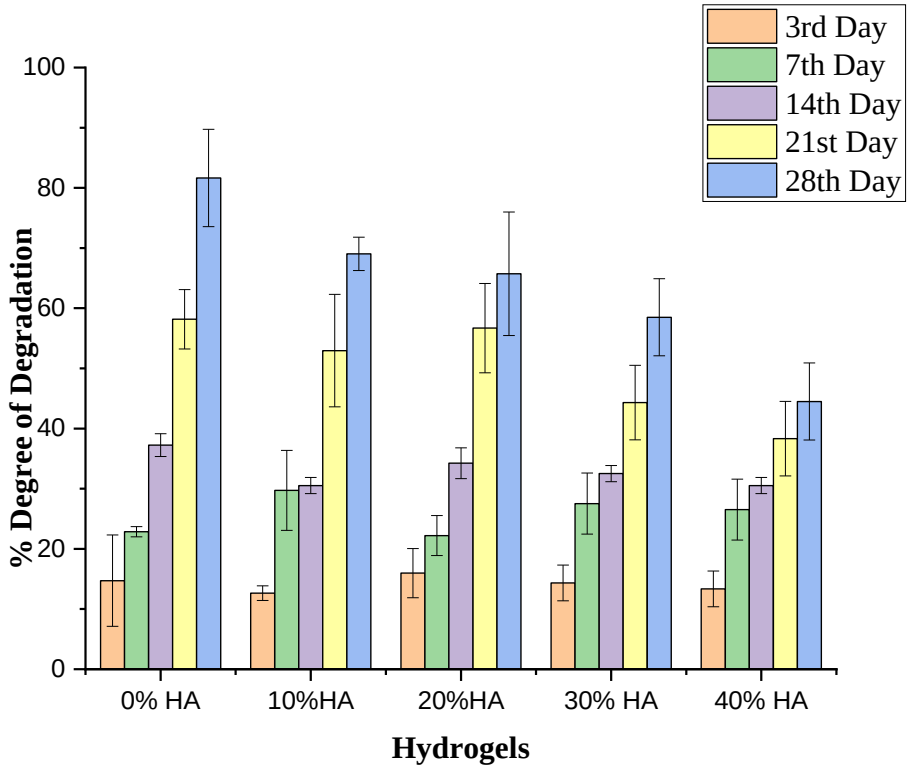


Figure 4-2 Degradation Study of Hydrogels

The degeneration shows the same patterns that the swelling did. In comparison to the sample with more HA, the sample with less HA degraded significantly faster and to a higher extent. The materials with less HA absorbed significantly more water, which accelerated the rate of hydrolysis (and subsequent disintegration). When HA was increased from 0% to 40%, both edoema and degree of degradation were reduced. Once more, this is most likely caused by the material's increased stiffness and the lower amount of water that would otherwise facilitate breakdown.

4.4 FTIR spectroscopic analysis

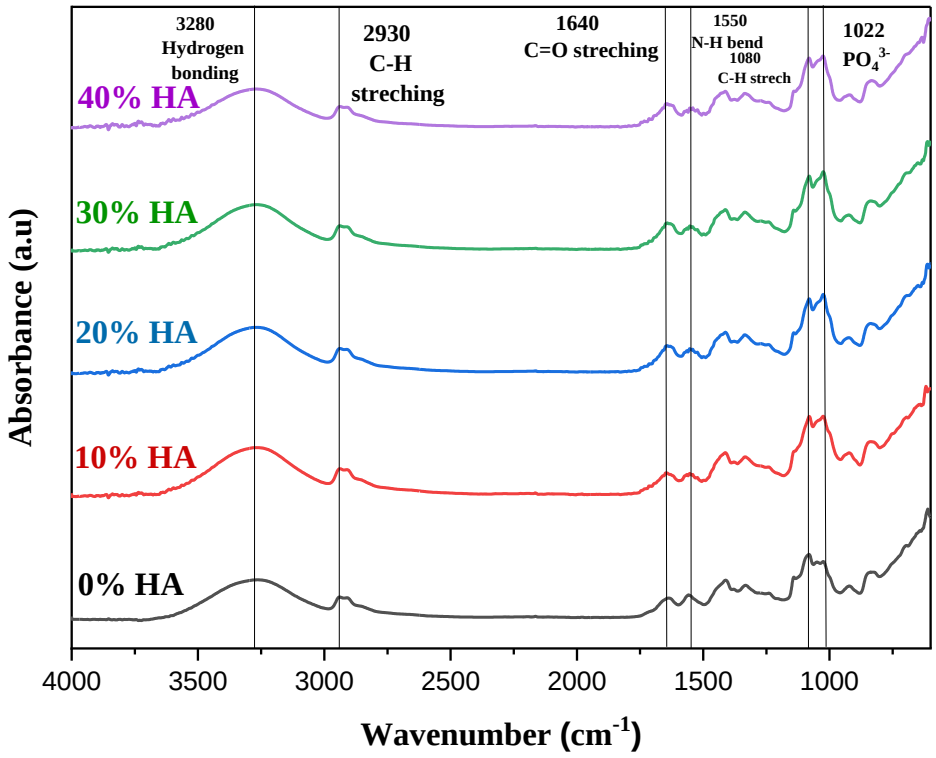


Figure 4-3 FTIR of Hydrogels

Sr NO.	Functional group	Peaks
1.	-OH	3200–3500 cm^{-1}
2.	C-H stretching	2930 cm^{-1}
3.	carbonyl C=O stretching vibration	1640 cm^{-1}
4.	N-H bending	1550 cm^{-1}
5.	PO_4^{3-}	1022 cm^{-1}
6.	C-H	1080 cm^{-1}

Table 4-2 Characteristic peaks of groups

Hydrogel's chemical groups were examined and verified by using FTIR. Glutaraldehyde react with the -NH_2 functional group of neighboring lysine residues in

gelatin and two nearby hydroxyl groups in PVA to generate acetal bridges in the hydrogels[163]. All of the samples of hydrogels showed a peak at 3280 cm^{-1} for pure PVA, which can be attributed to the stretching of inter or intra molecular hydrogen bonds. The acetal interactions between PVA and glutaraldehyde caused this peak to shift in both intensity and position[163-165]. The above figure shows that O-H (3200-3500 cm^{-1}), as the distinctive bonds between PVA and glutaraldehyde. Gelatin presence showed peaks near1640 cm^{-1} and 1550 cm^{-1} due to C=O stretching and -NH bending respectively[166]. The distinct peaks of the asymmetric B−O−C bonding were seen at 1430^{-1} and 1325 cm^{-1}. The intermolecular peaks that depict the typical bonding structure among PVA molecules were seen near 1080 cm^{-1} (C–O–C)[155]. When gelatin crosslinked chemically using glutaraldehyde, the amino group of the protein's lysine residue combines with the crosslinker's aldehyde group form Schiff base (–C=N–)[167]. The hydrogels containing HA show the PO_4^{3-} peak at 1022 cm^{-1} which is absent in the hydrogel with 0%HA.

4.5 Self-healing test

Hydrogel was cut into two halves and kept connected. The self-healing began as soon as two fragments came into touch with one another and complete self-healing occur within 60s as time noted with a stop watch. This demonstrates how well these hydrogels are able to mend themselves.

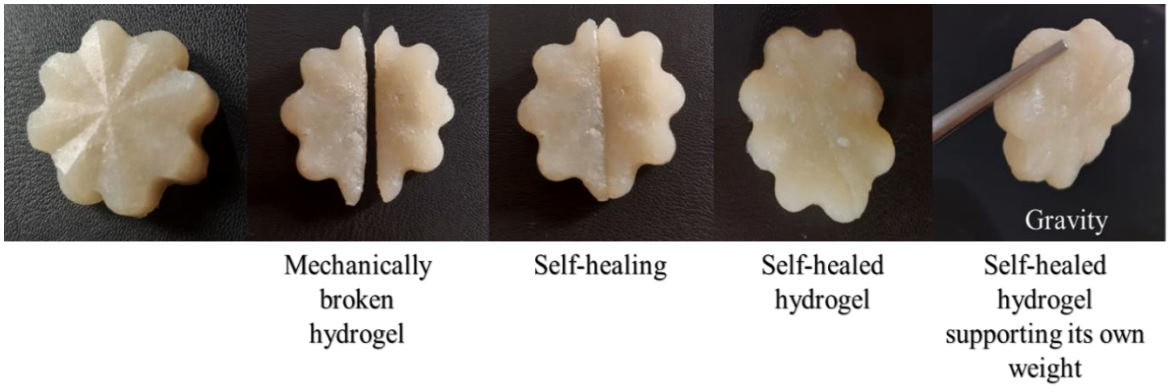


Figure 4-4 Self-healing of hydrogels

4.6 Elastic Deformation

When the hydrogel compressed and then released the pressure, the hydrogel was able to regain its original shape and volumes. The hydrogel exhibits elasticity, as it is able to return to its original shape after being compressed and then released. The hydrogel's elastic qualities can aid in reducing inflammation and fostering tissue regeneration. by

reducing the amount of mechanical stress on the tissue during compression and relaxation[168].

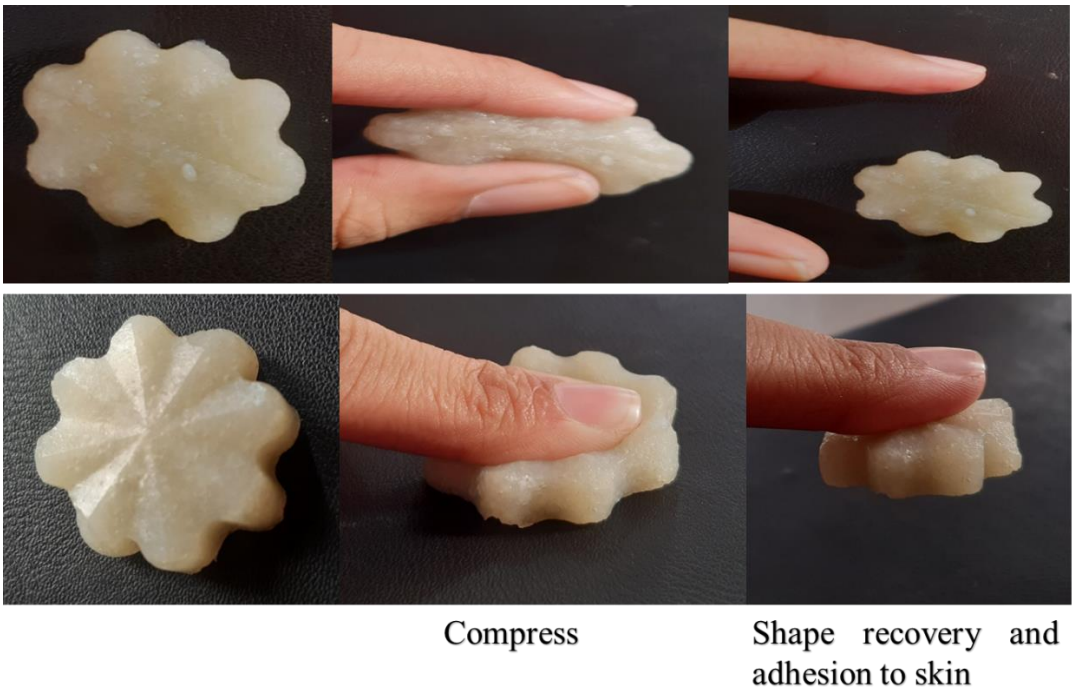


Figure 4-5 Elastic deformation of Hydrogels

4.7 Adhesion test

The numerous excellent mechanical characteristics of the hydrogel also contributed to its ability to adhere to a variety of substrates. The hydrogel property as an adhesive on the surface of human skin was excellent. hydrogel has to stick to surfaces, in this case, human finger. The fact that the hydrogel remains attached to finger even after 3 hours indicates that it has a strong adhesive property. Below figure shows how the hydrogel strip not only clung to the finger surface but also allowed for smooth finger motions and was readily peeled off without leaving any trace. The hydrogel can also be easily shaped or molded by finger movement which suggests that it has a pliable and flexible nature. The hydrogel act as a moldable and adhesive material that can be shaped and molded to fit various surfaces and will remain attached for a prolonged period[169].

Figure 4-6 Adhesion of Hydrogels

4.8 Moldable hydrogels

Hydrogel inserted in a silicon mold and allowed it to set. The hydrogel conformed to the shape of the mold, taking on its form and texture. Once the hydrogel had fully set, it became a molded object with the same shape as the mold. This process of molding the hydrogel allows to create custom shapes and structures for use in a variety of applications[168].

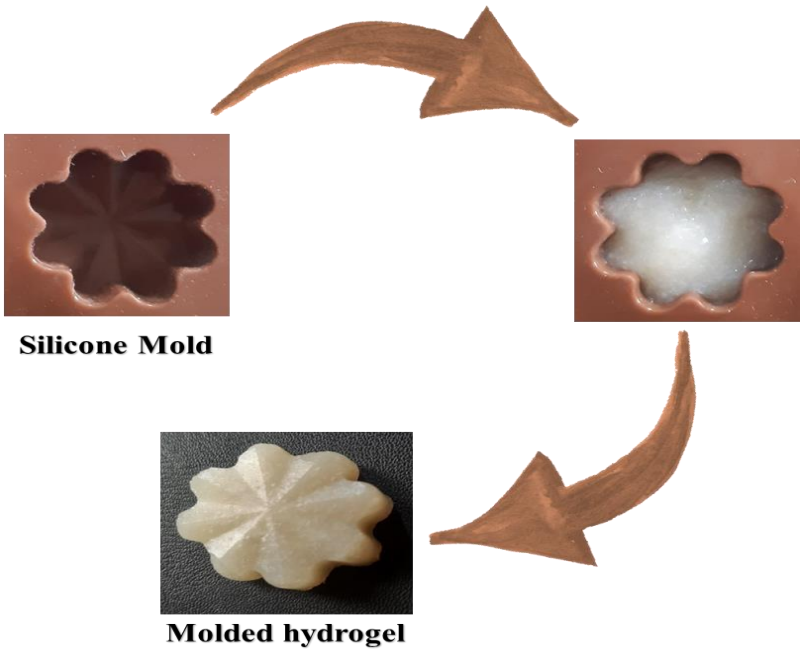


Figure 4-7 Moldable Hydrogels

4.9 Conductivity of hydrogel

The hydrogel may develop self-healing qualities from the migration of borate ions in addition to becoming conductive. A qualitative conductivity test was conducted to confirm this theory. The hydrogel and LED indication were wired together as a closed circuit, as seen in Figure below, the original hydrogel was able to successfully

illuminate the LED indication. When the hydrogel was cut, the indication switched off. The indicator switched on and remained at a similarly steady brightness when the two specimens were reconnected, showing that the hydrogel has outstanding self-healing electrical conductivity[170].

Figure 4-8 Conductivity of hydrogel

4.10 Thermal Analysis

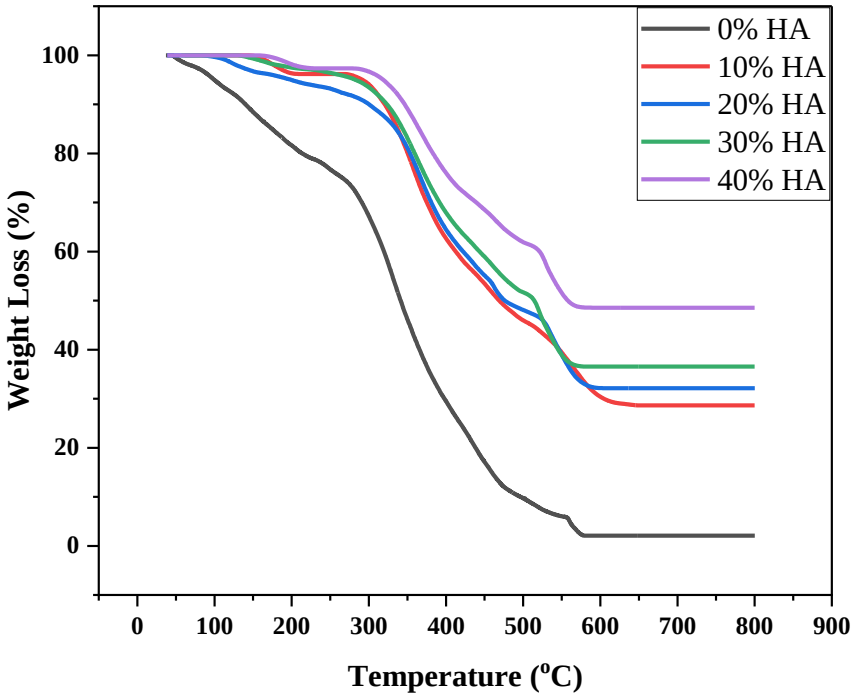


Figure 4-9 TGA of Hydrogels

The first transition occurs from room temperature to 280°C as a result of the removal of free water and result of the continual loss of water prior to decomposition. Consequently, the samples also contain the other type of water in addition to the free water, such as tightly bound water[171]. The greatest weight loss in the temperature range of 280-500°C is seen in the TGA curve of hydrogel, which is thought to be caused by partial breaking of the molecular structure and the disintegration of intermolecular structure [172]. The third rapid transition occur at 520-560°C attributed to the decomposition and degradation of PVA and gelatin. The hydrogel with highest percentage of HA show lowest weight lost and vice versa. The extent of weight loss gives insights into the stability and content of hydroxyapatite within the hydrogel.

4.11 X-ray diffraction pattern analysis

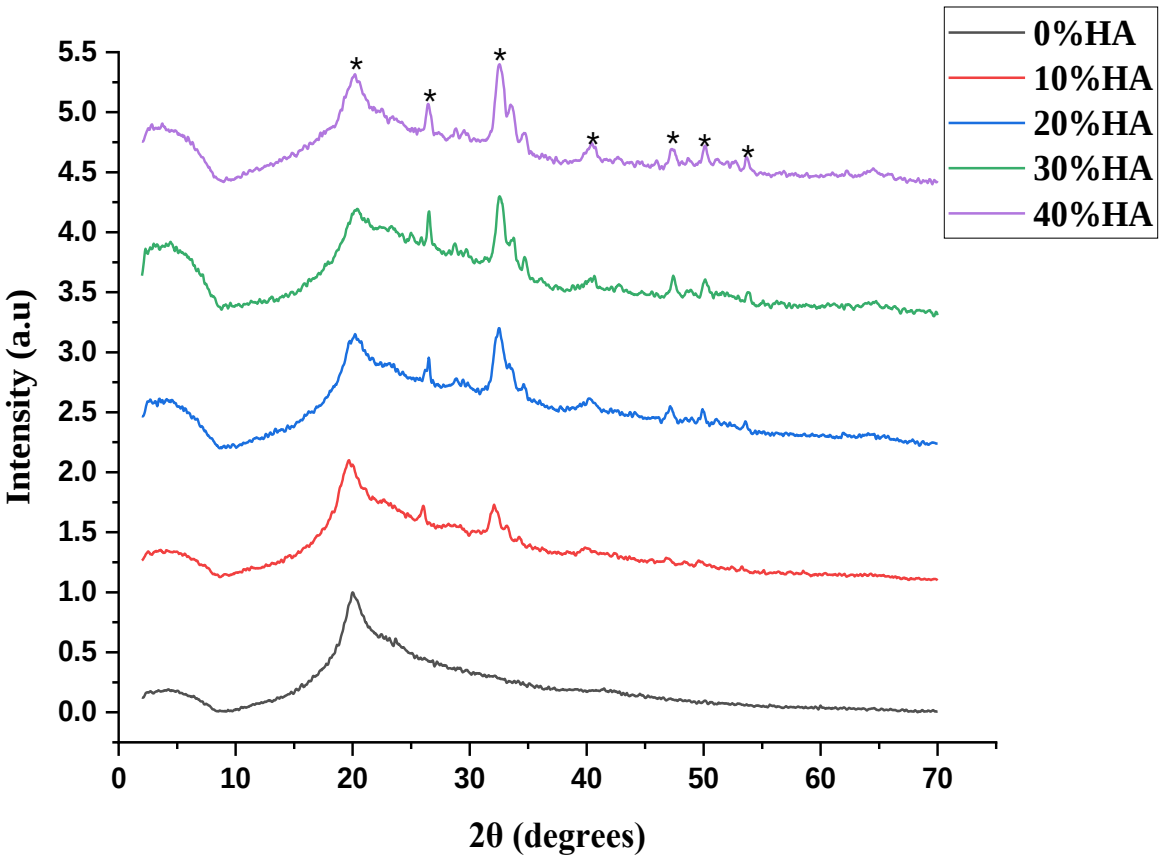


Figure 4-10 XRD of hydrogels

This figure makes it obvious that the PVA/Gelatin matrix is the main reason for the hydrogel's' crystallinity, as XRD pattern exhibits a noticeable peak at around 2θ = 20°[166]. The random dispersion of HA in the hydrogels is attributed to the loss in crystallinity in all of the XRD patterns of hydrogels[173]. Diffraction peaks at 2θ values of 26, 32, 47, 50 and 53.7 relate to the HA[174]. The increase in HA content, increases both the peak intensity and the % crystallinity of PVA significantly for these peaks.

4.12 Water Contact angle

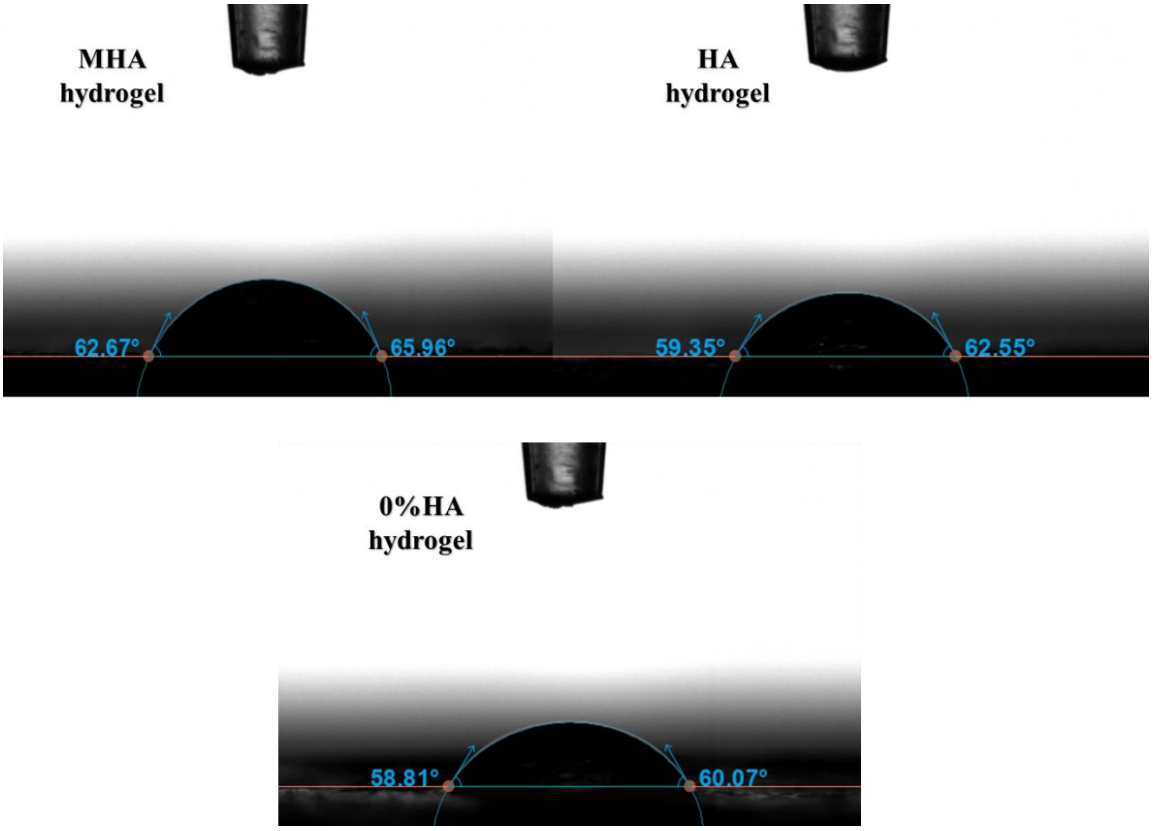


Figure 4-11 Water contact angle of hydrogels

Wetting describes a liquid's capacity to adhere to a solid surface. Intermolecular interactions are what bring the solid and liquid surfaces together. The amount of wetness is determined by the balance of forces between adhesive and cohesive forces. The bonding or adhesion of two dissimilar materials depends on wettability. Material hydrophobicity and hydrophilicity carried by surface forces, referred as wettability that regulate wetness. The adhesive forces between the solid and the liquid cause a liquid drop to spread across the surface. The drop balls are caused by cohesive forces in the liquid and avoid contacting the surface by standing up[175]. If the contact angle is less than 90 degrees, the surface is hydrophilic, and if it is greater than 90 degrees, the surface is hydrophobic. The surface's ability to support cell adhesion, differentiation, and proliferation improves with increasing hydrophilicity [176]. All the hydrogels show hydrophilic behavior. However, the hydrogel with 0%HA showed the highest hydrophilic behavior among all. While the hydrogel having MHA showed the least hydrophilic behavior because the ester linkages present in it are hydrophobic in nature which resulting in the compromised hydrophilic behavior[177].

4.13 In vitro drug release

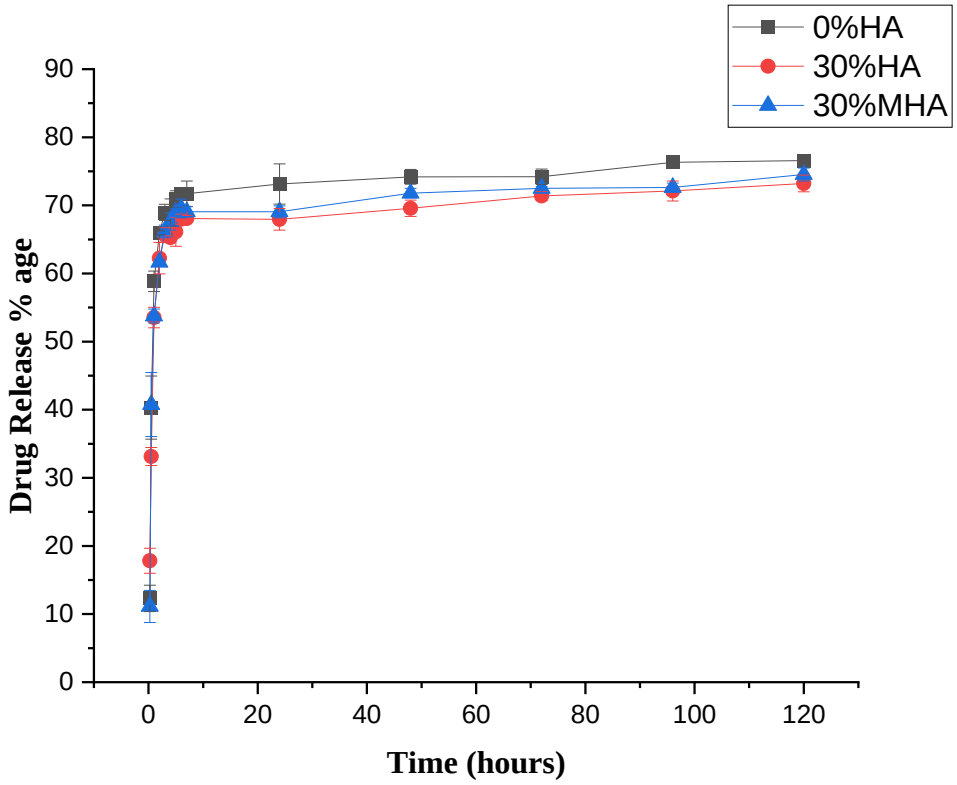
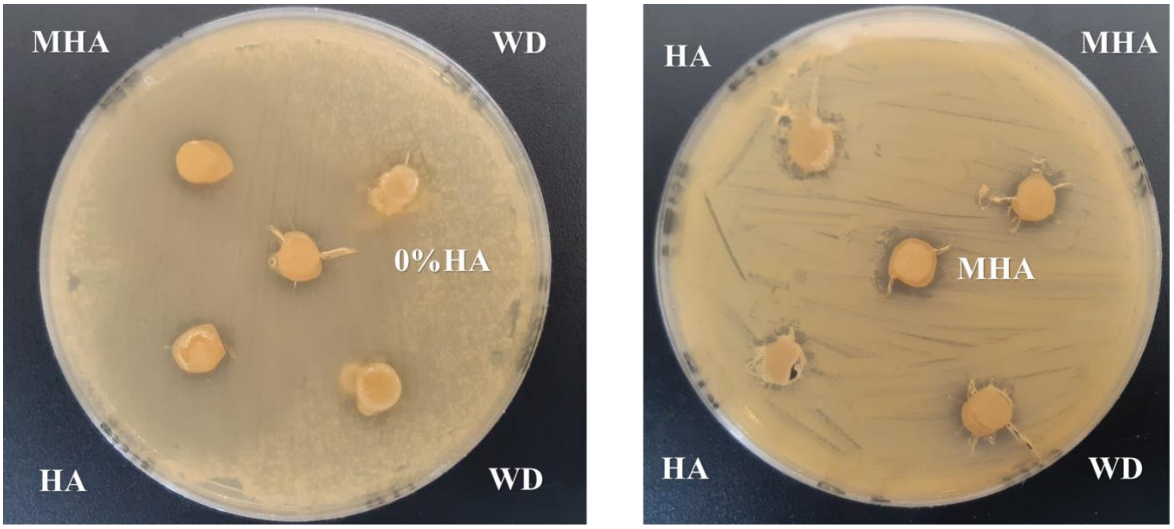


Figure 4-12 Drug release study of hydrogels

There are two drug delivery techniques based on hydrogel: 1) Systems that controlled by stimuli, and 2) Systems that are time-controlled. Their very long response periods are stimuli-sensitive hydrogels' main flaw. Making thinner and smaller hydrogels is the quickest approach to obtain fast-acting responsiveness, but doing so results in loss of mechanical strength and fragility in the hydrogel[100]. In first 24h 73%, 69% and 67% drug released from 0%HA, MHA, and HA hydrogels respectively. Rapid release of a significant portion of the drug within the first 24 hours may be desirable for certain conditions where an initial high drug concentration is required for immediate action. By achieving a substantial drug release within the first 24 hours, the need for frequent dosing can be reduced. This can improve patient compliance, convenience, and overall treatment adherence.

4.14 Anti-bacterial Activity



E.Coli S.Aureus

Figure 4-13 Anti-Bacterial Activity

The drug-loaded hydrogels were subjected to antibacterial tests against the E. coli and S. aureus strains of bacteria. In order to evaluate the anti-bacterial action, disc diffusion was performed. All of the formulations with drug effectively combatted two bacterial strains. The reason for this is that the antibiotic was placed into all hydrogel compositions except one without drug (WD) hydrogel, and the crosslinkers utilized, such as glutaraldehyde and borax were all naturally antibacterial[178], [179].

Chapter 5
Conclusion

5 Conclusion and Future tasks

In the current work, an attempt was made to create a hydrogel for the treatment of periodontitis. Good compressibility, stretchability, moldability, and self-healing properties have seen in the produced hydrogel. The hydrogel has both covalent and non-covalent connections, according to FTIR measurements. Hydrogels have good swelling properties and are hydrophilic by nature, which leads to greater cell adhesion, according to studies on swelling and contact angle. The fact that hydrogels take longer to degrade shows that they can speed up the process of tissue regeneration. According to the drug release study, firstly drug release abruptly then these hydrogels allow for longer and sustained drug release, which reduces the risk of infection recurrence while tissue regeneration is taking place. Additionally, antibacterial activity shows that hydrogels are effective against S. aureus and E. coli, and these hydrogels have good antibacterial properties. All these characteristics make these hydrogels a potential candidate to be used in Periodontitis treatment. Future task consists of Rheology Studies to investigate the viscosity and gelling time and Biocompatibility analysis to check the cytotoxicity of the hydrogel.

Chapter 6
References

6 References

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