CHAPTER-1

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Oral diseases such as tooth decay, dental caries, plaque, periodontal diseases, malocclusion and oral cancers are vital public health concerns worldwide (Schwach-Abdellaoui *et al.,* 2000). As per World Health Organization (2012), oral health is elaborated as;

"Oral health is essential to general health and quality of life. It is a state of being free from mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal (gum) disease, tooth decay, tooth loss, and other diseases and disorders that limit an individual's capacity in biting, chewing, smiling, speaking, and psychosocial wellbeing (WHO, 2012)".

Oral diseases were highly prevalent during 1990-2010 affecting about 3.9 billion people worldwide. Out of all oral diseases, dental caries was the most prevalent condition which had affected about 35% of population followed by severe periodontitis, which was 6th most prevalent condition affecting about 11% of the global population (Marcenes *et al.,* 2013). In a recent hospital based survey by Bansal *et al.,* (2015) in India, it was observed that no persons above 44 years had healthy teeth. Highest healthy periodontium was found in 3.9% subjects of 15 to19 years age group. Overall the prevalence of periodontal diseases was observed very high as 96.30% (Bansal *et al.,* 2015).

The prevalence is high not only in developing countries but also in countries like United States (US) where about 25-35% sufferers belong to 35 to 65 age groups. Moreover, the disease advances with age as depicted by higher incidence of 60 to 75% among persons older than 60 years (Schwach-Abdellaoui *et al.,* 2000). Such increasing incidences of diseases would directly affect the growth of periodontal therapeutic market.

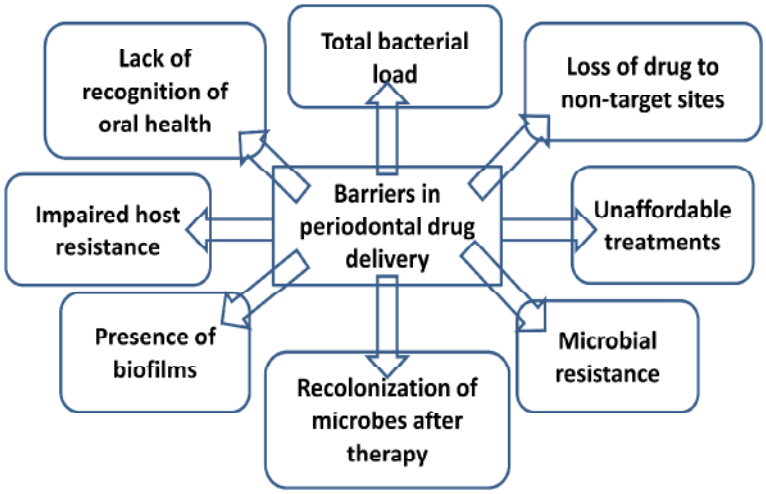

Figure 1.1: Barriers in periodontal therapy

The global data 2010, estimated that the market which valued $1,607 million in 2010 will reach $2,638 million in 2018 at a compound annual growth rate (CAGR) of approximately 6.4% only (Yadav *et al.,* 2015). The major barriers leading to high prevalence of disease and low treatment rates are lack of perception about importance of oral health, limited awareness about treatments, fear of pain during surgery, socioeconomic status, treatment costs, infrequent dental visit, costs of therapy *etc.* (Fig. 1.1) (Singh *et al.,* 2015; Yadav *et al.,* 2015). The biological barriers include development of microbial resistance, recolonization of microbes, biofilms, bacterial loads and impaired host resistance.

Periodontal diseases are a group of inflammatory, microbial induced infection involving damage to supporting tissues of teeth, gingiva, periodontal ligament, and alveolar bone. Concomitantly, loss of parts of the junctional epithelium of periodontal ligaments, destruction of connective tissue attachment and alveolar bone leads to creation of a niche - a pocket between the tooth and the marginal soft tissue (Fig. 1.2) (Oh *et al.,* 2002). These pockets are characteristic feature of periodontitis and are considered as house of periodontal pathogens (Armitage, 1999).

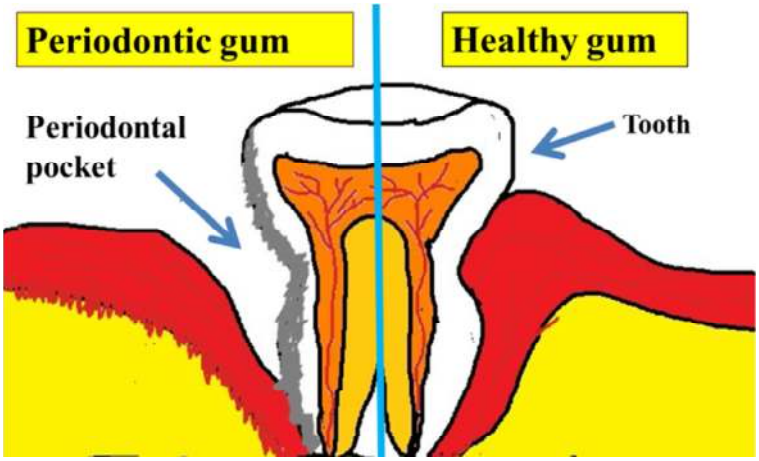

Figure 1.2: Distinct features of periodontic gum and healthy gum (Yadav *et al.,* 2015).

Periodontal diseases are localized opportunistic infection of gums and teeth instigated by predominantly gram-negative anaerobic bacterias such as *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, *Dialister pneumosintes*, *Bacteroides forsythus* and *Treponema denticola*, and pigmented fungi such as *Histoplasma* and *Aspirgillus Niger* (Southard and Godowski, 1998; Yadav *et al.,* 2015).

Although, periodontitis is initiated by microbes, but the most of destruction in the periodontium is caused by activation of host immune response against those microbes. Host derived enzymes like MMPs (Matrix Metallo-Proteinases), inflammatory mediators like cytokines, prostanoids are responsible for destruction of periodontal tissue leading to eventual loss of tooth, confidence and aesthetics of a person (Gulati *et al.,* 2014).

Untreated periodontal infection may spread to other tissues and systemically *via* blood. These oral-systemic connections for many systemic diseases including cardiovascular disease, myocardial infarction, atherosclerosis, overt nephropathy, end-stage renal disease (ESRD), low birth weight of infants and bacterial pneumonia are well established by researchers (Cotti *et al.,* 2011; Craig *et al.,* 2002). These risk factors necessitate the need for development of specialized pharmaceutical application for the treatment of periodontal diseases. "Perioceutics'' is a pharmaceutical field

which deals with the development of drugs and medicine for the management and treatment of periodontal diseases (Honibald *et al.,* 2012; Seshadri and Viswanathan, 2015). As the name suggests it is constituted by two words 'perio (from periodontitis') and 'ceutics' (from pharmaceutics) (Mishra and Yadav, 2015).

First line strategy for treatment of the periodontitis involves conventional mechanical therapy *i.e.* scaling and root planning (SRP), which can improve the overall gingival health and halts the progression of disease. Furthermore, SRP alone could not eliminate microbes infiltrated to epithelium and connective tissue. As a result, for the prevention of recurrent or refractory periodontitis, antimicrobial drugs along with SRP is given by dentists (Southard and Godowski, 1998; Walker, 1996).

Oral administration of antimicrobials provides slow relief, requires frequent intake, increases risk of antibiotic resistance and has poor patient compliance (Southard and Godowski, 1998; Walker, 1996). Further, a lot of side-effects and adverse effects have been associated with systemic delivery of antimicrobial agents. These challenges associated with oral delivery can be addressed by administration of drugs directly to the intended site of action *i.e.* periodontal pockets with significantly lesser dose (Schwach-Abdellaoui *et al.,* 2000; Southard and Godowski, 1998).

Periodontal pockets act as a natural reservoir for drug delivery insertion and Gingival Crevicular Fluid (GCF) supply a leaching medium for the release and distribution of drugs throughout the pocket. These features, together with the fact that periodontal diseases are localized to the immediate environment of the pocket, make the periodontal pocket an ideal site for the targeted treatment of pocket infections with local delivery systems (Schwach-Abdellaoui *et al.,* 2000; Yadav *et al.,* 2015).

Commonly existing mouthwashes, professional irrigation and toothpastes provide immediate relief to the patients but do not maintain drug concentration at the site of action for prolonged time and get washed by high turnover of GCF. An ideal intrapocket formulation should be biocompatible, biodegradable, mucoadhesive, easy to administer, possess broad spectrum of activity and able to provide controlled and prolonged release of drugs.

Many intrapocket delivery systems had been developed including, inserts, films and strips (Kassem *et al.,* 2015; Khan *et al.,* 2015), gels and other semi-solid formulation (Bansal *et al.,* 2009; Esposito *et al.,* 1996), nanoparticles, microparticles (Govender *et al.,* 2005), *in-situ* forming implants (Do *et al.,* 2014), and fibers or nanofibers (Khan *et al.,* 2016). All the drug delivery systems have their own pros and cons discussed in next chapter. Inserts, films, strips, fibers or nanofibers have poor penetration into deeper pockets due to less flexibility, do not cover whole pocket area and get folded during insertion into pocket. However, multiparticulate and gels or *in-situ* gels are flexible systems which could be inserted easily into pockets using syringes, reaches deeper and are capable of filling the pocket area. Only few marketed products are available for localized pocket administration including Arestin®, Actisite®, Atridox®, Periochip®, Dentomycin®, Atrigel® and Elyzol 25. The periodontal treatment using these systems is less affordable by common people and they are mostly marketed in developed countries not available in India. Also, all these products contains single antimicrobial agent which could either be effective for obligate/facultative anaerobic or aerobic microbes.

Microparticles and nanoparticles are the most important multiparticulate systems used in periodontal drug delivery. They are the divided dosage forms with mini drug depots which can be used as per the need (Shukla *et al.,* 2011). Although, nanoparticles (< 1 µm) appear more alluring in drug delivery but, manufacturing and characterization cost of nanoparticles increases the total cost of treatment. Unlike nanoparticles, microspheres (1µm to 1mm) are more stable and easy to formulate and handle without the need of sophisticated instruments. Besides, they provide benefits of small size of nanoparticles as well as high encapsulation efficiencies for the delivery of two antimicrobial drugs due to their comparatively large size which could be advantageous for periodontal drug delivery where prolonged treatment is desirable (Jha *et al.,* 2011; Pandey *et al.,* 2015). Though they cannot easily be used for intravenous or systemic delivery (because they could agglomerate and cause clotting), but they are effective for local or targeted delivery, like pocket insertion, subcutaneous injection and can be used in sustained release systems (Yadav et al., 2017a; Yadav et al., 2017b).

Multiparticulate based *in-situ* gel systems have gained much considerable attention in recent years as one of the most promising drug delivery systems owing to their unique potentials of combining the characteristics of a hydrogel system (e.g., hydrophilicity and extremely high water content) and small size (Ballauff and Lu, 2007; Hamidi *et al.,* 2008). The thermosensitive *in-situ* gel systems provide important advantages, namely: (i) easier to administer, (ii) efficient spreading within the periodontal pockets, (iii) form solid implants as per the shape and size of pockets of patients, (iv) use of biocompatible and biodegradable polymers could provide controlled release of drugs for prolonged time without the need of removal of empty remnants (Do *et al.,* 2014).

Owing to above mentioned informations, this thesis describes polymeric multiparticulate based intrapocket drug delivery systems of ornidazole (OZ) and doxycycline hyclate (DX). OZ is a nitroimidazole derived antiprotozoal drug and has been chosen as drug of interest for this work as it has potent antibacterial activity against 94% of oral anaerobic bacteria residing in the deeper pockets and alveolar canal such as *Bacteroids* species, *Fusobacterium* species, *Peptostreptococcus* species, *Clostridium* species, *Provotella* species, *Porphyromonas* species, *Actinomycetes*, *Propionibacterium* species and *Eubacterium* species (Goldstein *et al.,* 1978; Kamma *et al.,* 2000; Ogrendik, 2006). It is a derivative of metronidazole, but exhibits longer elimination half-life ($t_{1/2}$ = 11-14 h), more water-soluble, administered with lower frequency of dosing, has lower Minimum Inhibitory Concentration (MIC) against periodontal pathogens, and is relatively safe with minimal side-effects than metronidazole ($t_{1/2}$ = 7.3 h) (Goldstein *et al.,* 1978; Hizarciolu *et al.,* 2004; Rossignol *et al.,* 1984; Singh *et al.,* 2003). Previous studies suggested that single dose of OZ has produced comparable effects to seven day dose of metronidazole due to its longer half-life (Jaswal *et al.,* 2008; Oren *et al.,* 1991). It has also been preferred for surgical prophylaxis because of its excellent penetration into lipidic tissues as compared to other nitroimidazole derivatives (Hizarciolu *et al.,* 2004).

Use of OZ alone in periodontal formulation would target only obligate anaerobic bacteria. Thus, to increase the spectrum of activity (both facultative

anaerobic and aerobic bacteria), DX has also been included as another drug of choice in the formulations. Reportedly, metronidazole and doxycycline combination therapy was found more effective in prevention of recurrent periodontitis than systemic administration of metronidazole alone (Aitken *et al.,* 1992). DX is US Food and Drug Administration (FDA) approved, potent semisynthetic tetracycline derivative which acts by interrupting bacterial protein synthesis with activity against *A. actinomycetemcomitans* (Müller *et al.,* 2002). DX is highly active against both gram-positive and gram-negative bacterias and is in use for more than forty years for the treatment of anthrax, respiratory, genitourinary, skin and soft tissue infections, and prophylaxis of malaria (Castro *et al.,* 2009; Vargas-Estrada *et al.,* 2008).

Thus, DX is also selected as drug of interest for this study as it has both antibiotic properties and host-modulatory action which has produced significant risk reduction of recurrent periodontitis (McCulloch *et al.,* 1990). Host-modulatory action involves inhibition of MMPs, B-cell function, interleukin-1, nitric oxide and collagen synthesis produced by plaque microorganisms, which are responsible for the damages to connective tissue of gums (Smith and Cook, 2004). It has dose dependent action with MIC of 25-100 µg/ml for antibacterial activity, whereas 5-10 µg/ml is essential for host-modulatory action (McCulloch *et al.,* 1990).

There are no registered trademark preparations containing DX and OZ for intrapocket delivery in the market. The local drug delivery systems present in other countries are expensive that a common man cannot afford it. Those who do not receive effective treatment suffer from permanent tooth loss and periodontic pain. The challenge is to develop an efficient and reproducible intrapocket delivery system that would deliver antimicrobials slowly for prolonged time and be easy to use (Aitken *et al.,* 1992).

Further, this thesis presents utility of the natural, biodegradable polymer such as chitosan based crosslinked microspheres formed with various crosslinking agents *viz.* sodium alginate, tripolyphosphate, glutaraldehyde and vanillin for simultaneous delivery of OZ and DX for the treatment of periodontal pocket infections. Based on the beneficial aspects of natural polymers the crosslinking property, chitosan has been

exploited. Due to ionization at acidic pH chitosan become positively charged molecule (ammonium ion) and is able to interact with negatively charged carboxylate containing molecules. Microspheres were chosen as drug delivery vehicles due to their simplicity, cost-effectiveness, non-toxicity as discussed earlier. They can be directly filled into the deep periodontal pocket or can be incorporated into gels for injection into pockets.

The development and optimization of microspheres involves a vital understanding of the effect the various formulation variables *viz.* polymer concentration, crosslinker concentration, agitation speed, agitation time, surfactant concentration *etc.* on the desired properties (response) of the formulation such as particle size, entrapment efficiency, drug release parameters *etc.* The traditional one variable at a time (OVAT) formulation and process optimization technique determines the effect of individual factors on the responses (Singh *et al.,* 2005). In the absence of the effect of interactions of factors on the response, OVAT technique provides only a suboptimal solution at the level being tested (Leardi, 2009). Therefore, systematic design of experiments (DoE) is a holistic approach for understanding the effect of various factors on the responses simultaneously through generated mathematical equations, contour and surface plots, and analysis of variance (ANOVA) results.

DoE based Plackett-Burman Factorial Design was employed to screen the significant process variables affecting the desired responses of the microspheres. Microspheres were designed and optimized based on predicted optimum levels of the independent variables of the factorial design. The optimum crosslinked chitosan microspheres were further designed and developed based on the screened significant process variables and finally optimized using Box-Behnken Experimental Design based on desired responses including minimized particle size, maximum entrapment efficiency, maximum time for 80% drug release ($T_{80\%}$) and mucoadhesion.

All the optimized microspheres batches were characterized for their physicochemical and solid state attributes by Fourier-Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD), Nuclear Magnetic Resonance (NMR) (in case of covalently crosslinked

microspheres *viz.* vanillin), Electronic Dispersive X-ray Analysis (EDXA) and Scanning Electron Microscopic (SEM) techniques.

Furthermore, many supportive studies such as pH, mucoadhesion, swelling, erosion, antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* and cytocompatibility studies by Sulphorhodamine (SRB) assay were done to prove the applicability of optimized microspheres. Vanillin crosslinked microspheres provided more controlled and prolonged delivery of drugs as compared to other crosslinkers suggesting it as final optimized batch. The final optimized microspheres were incorporated into thermosensitive *in-situ* Pluronic® gels (MLIG) to further improve its injectability and patient compliance. The formulations can fully fill the pocket with maximum access to the deeper sites. The microspheres loaded *in-situ* gels were evaluated for their physicochemical properties and stability study parameters. The formulations were tested for their biocompatibility and gingival tissue regeneration abilities on rats also. Furthermore, clinical studies of optimized MLIG were performed on the periodontal patients to evaluate long-term management of periodontitis significant benefits in the reduction of deep pockets, plaque index, and gingival index in patients with severe chronic periodontitis.