1 Introduction

Periodontal disease can be described as a pathological condition which initiates formation of small pockets between gums and teeth, gingival inflammation, that is followed by degeneration of gums, loss of teeth supporting structures like alveolar bone, periodontal ligament, cementum, ultimately culminating in complete tooth loss. It is instigated by anaerobic and microaerophilic microorganisms that preferentially propagate by feeding on remnants of left-over food particles stuck in between teeth and gum [Mundargi et al. 2007, Silva-Boghossian et al. 2013]. According to the WHO report (2012), dental caries was the most prevalent condition (affecting 35% of the population), whereas severe periodontitis and severe tooth loss were the 6^{th} and 36^{th} most pervasive conditions affecting 11% and 2% of the population, respectively. This problem is particularly grave and alarming owing to the proximate relationship that exists between oral health and systemic health. It has also been reported that periodontal issues may aggravate cardiovascular diseases, diabetes mellitus and low birth weight or even preterm birth of children [Gomes-Filho et al. 2010, Guimarães et al. 2010, Humphrey et al. 2008, Kopytynska-Kasperczyk et al. 2015]. Therefore, timely treatment of periodontal disease is essential. The current periodontal therapy is aimed at the regeneration of periodontium, which includes the formation of new ligaments, cementum on the tooth root and surrounding bone structure [Ivanovski et al. 2014, Jiang et al. 2015].

Periodontal disease is known to have many stages, ranging from effectively treatable gingivitis to irreversible severe periodontitis inclusive of conditions such as chronic periodontitis, aggressive periodontitis, systemic disease-associated periodontitis and necrotizing ulcerative periodontitis [Armitage 2004]. The primary etiology of gingivitis is poor oral hygiene which prompts the aggregation of a bacterial matrix at the gum line

[Crich 1932]. In some of the patients, gingivitis advances to periodontitis – wherein with the decimation of the gingival fiber, the gum tissues separate from the tooth and the extended sulcus is called as the periodontal pocket. The adherence of oral microbes to the teeth in a substance called plaque initiates the periodontal infection. Plaque can be expounded as a biofilm constituted by oral microbes, salivary glycoproteins and extracellular polysaccharides [Carranza and Camargo 2006, Wiggs and Lobprise 1997]. Minerals extent in the saliva are calcified to form tartar or calculus which is nothing but plaque and contains up to 1×10^{12} bacteria per gram [Schroeder and de Boever 1970]. Microbes present in the biofilm are 1000 to 1500 times more resistant to antibiotics than the free-living bacteria. The plaque present on the tooth surface is known as supragingival plaque whereas the condition where the plaque reaches out under the free gingival margin and into the gingival sulcus is known as subgingival plaque. Supragingival plaque can potentially affect the pathogenicity of the subgingival plaque in the early stages of periodontal disease [Niemiec 2008]. The bacteria dwelling in the subgingival plaque often secretes cytotoxins and bacterial endotoxins, which possess the capacity to invade the tissues and cause inflammation to the gingival and periodontal tissues [Wiggs and Lobprise 1997]. This inflammation ultimately leads to the damage to the gingival tissues emerging in gingivitis. Later, the inflammation causes obliteration of the connection between the periodontal tissues and the teeth, and the stage called periodontitis is reached.

Conventional periodontal therapy demands the concomitance of mechanical cleaning (which is exceedingly painful) with the systemic antibiotic administration. Although the systemic administration of antibiotics is promising, high oral dose is required to achieve effective concentrations in the gingival crevicular fluid (GCF). The high dose of

antibiotics, in turn, causes untoward side effects like hypersensitivity, gastrointestinal intolerance, and development of bacterial resistance.[Laxminarayan et al. 2013, Schwach-Abdellaoui et al. 2000, Singh et al. 2015]. On the other hand, local application of mouth rinses, gels, and toothpaste require lesser dose to be administered, but these systems can regulate only supragingival plaques and mucosal infections. Moreover, such local delivery systems also demand high initial concentration and multiple applications to achieve sustained effectiveness by maintaining drug concentration in the therapeutic window [Jain et al. 2008]. Thus, a more competent approach for the drug to reach the site of infection deep inside the periodontal pockets would be using a controlled release device, which could limit the distribution of drugs to the target site with minor or no systemic uptake.

The present work takes this notion of localized drug delivery forward and is focused on alleviating shortcomings in the treatment of periodontitis by employing a novel electrospun polymeric nanofiber membrane functionalized with Tinidazole (TNZ). Nanofibers have recently been emerged as unique candidates for the treatment of periodontal disease due to their multifold beneficial properties, such as high surface to volume ratio, porous architecture which allows oxygen permeation and morphological similarity to the extracellular matrix, all of which promote regeneration of periodontium [Charernsriwilaiwat et al. 2013, Jayakumar et al. 2011, Qiu et al. 2013]. Moreover, they furnish an initial high dose of drug providing the immediate therapeutic effect, followed by intermittent small doses to maintain minimum therapeutic level throughout the long duration of periodontal treatment. Electrospinning has gained widespread interest in tissue engineering and drug delivery owing to its relative ease of use and adaptability [Hu and Cui 2012]. Electrospinning is a simple and versatile technique for the preparation of a nanofiber network structure. A blend of the polymer solution is loaded into a syringe

and then pushed-out at a slow and constant rate via a pump. A high (10-25 kV) electrical field is applied simultaneously between the needle point and the target collector, a distance of 10-15 cm. Together these parameters cause a Taylor cone to form, deform and then spin. The electrospinning causes micro/nano-scale fibers to be generated as the solvent evaporates. Moreover, by modulating the electrospinning parameters, the drug release profile, as well as the degradation of electrospun nanofiber membrane, can be controlled. Hence, electrospinning has been adopted to fabricate the nanofiber membrane in this study.

Retention on the mucosal surface and controlled drug release at the site of action can thus be contemplated as prerequisites for the drug delivery systems aimed at localized periodontal therapy. A prolonged retention time of the delivery system in the periodontal pocket can be acquired by using bioadhesive or mucoadhesive polymers which would provide intimate contact between the dosage form and the absorbing tissues, ultimately leading to enhanced retention time. Maximizing the bioadhesive forces of the system, therefore, remains a significant goal in the developmental phase of long-retentive drug delivery systems. In addition to the bioadhesivity, it is also desirable that the drug is released in a controlled manner from the dosage form. Controlled drug delivery systems should provide a continuous delivery of drugs so that a predictable and reproducible kinetics exists for a predetermined period. The potential advantages of this concept include the minimization of dose-related side effects and improved patient compliance. Furthermore, nanofibers have several unusual properties that can be exploited to enhance periodontal drug delivery. Initially, Goodson et al. prepared tetracycline hydrochloride loaded ethylene vinyl acetate fibers, which exhibited *in vitro* drug release till 9 days [Goodson et al. 1983]. Afterwards, many researchers attempted to develop nanofiber membrane using various polymers and antibiotics for the

treatment of periodontitis [Jiang et al. 2015, Reise et al. 2012, Samprasit et al. 2015, Xue et al. 2014a, Xue et al. 2014c, Zamani et al. 2010].

TNZ is one of the most pervasively used antimicrobial agents against anaerobic periodontal pathogens [Maestre et al. 2007, Tian et al. 2016]. It is a 5-nitroimidazole derivative with a half-life of 12–14 hr [Wood et al. 1982]. It has a longer half-life and higher bioavailability which makes it a promising antimicrobial agent for periodontitis treatment. Although the majority of the TNZ formulations are available as oral dosage forms, these formulations not only result in a low concentration of TNZ in gingival crevicular fluid (GCF) but also causes undesired side effects. In this perspective, the proposed research work was endeavoured to develop and optimize various nanofiber-based drug delivery systems loaded with TNZ which would directly deliver TNZ locally at a slow rate to the periodontal pocket and thus maintain the essential therapeutic drug concentration for entire treatment duration with the ultimate aim being reduction of dose size and dosing frequency as well as improved patient compliance. Accordingly, present thesis embodies studies on the development, optimization, and characterization of three different types of nanofiber systems namely poly (ε-caprolactone) nanofiber (TNZ-PCLNF), gelatin-poly (ε-caprolactone) hybrid nanofiber (TNZ-PGHNF) and chitosan-poly (ε-caprolactone) hybrid nanofiber (TNZ-PCHNF).

The traditional approach used for the optimization of a pharmaceutical product or process by changing one variable at a time (OVAT) has been proved to be not only uneconomical regarding time, money, and effort, but also unfavorable to fix errors [Singh et al. 2005]. Conventional OVAT method of formulation and process optimization ascertains the effect of individual factors on the responses while it excludes the estimation of interaction effects among the factors. To circumscribe the

shortcomings of OVAT, a holistic Design of Experiment (DOE) approach has been preferred which expresses the responses as a function of various factors involved including interaction terms, not only using mathematical expression but also with graphical illustration. By applying DOE, one can ensure that the quality is well built into the product and is not merely established by testing the end product [Lawrence 2008]. DOE identifies various critical variables and collects maximum information of essential variables while using lesser number of runs to rapidly avail the high-quality pharmaceutical products [Verma et al. 2009]. Formulation development and its optimization involves the thorough understanding of the effect of the formulation independent variables viz. polymer content, drug concentration, the ratio of organic solvent, flow rate, distance between needle tip to the collector, applied voltage, etc. on the formulation response variables such as diameter and entrapment efficiency of the nanofiber. Response surface methodology (RSM) can be defined as a collection of mathematical and statistical techniques based on the fitness of a polynomial expression to the experimental data, which must describe the behavior of a data set with the aim of making statistical anticipation. The critical formulation and process variables obtained after preliminary screening were applied to response surface methodology (RSM) based Box-Behnken experimental design, for statistical optimization of the nanofiber.

Taking various factors into account such as availability, frequent usage in the nanofiber preparation and other beneficial advantages; poly (ε-caprolactone) (PCL), gelatin (GE) and chitosan (CH) were selected as carriers to encapsulate TNZ while using appropriate solvent composition (formic acid/acetic acid) which would provide sufficient conductivity and help in the electrospinning process as well as produce homogeneous nanofiber membrane. In this study, TNZ encapsulated various polymeric electrospun

nanofiber membrane were prepared by the electrospinning method. These nanofiber membranes were characterized by various physicochemical characteristics, morphological properties and also evaluated for *in vitro* drug release profile in McIlvaine buffer. Antibacterial efficacy of the nanofiber membrane was evaluated on selected strains of bacteria. Eventually, MTT assay and confocal study were performed to evaluate the viability of nanofiber membrane. Moreover, ligature-induced periodontitis in rat model was used to study the progression of periodontitis and to assess the efficacy of developed nanofiber membrane. After developing and comparing all the three types of nanofiber-based drug delivery systems, the optimized formulation was subjected to clinical study in patients suffering from periodontitis to assess the efficacy and therapeutic potential of developed nanofiber-based drug delivery system.