BACKGROUND

Periodontal disease is a pathological condition which initially leads to the formation of small pockets between gums and teeth, gingival inflammation, followed by degeneration of gums, loss of teeth supporting structures like alveolar bone, periodontal ligament, cementum, ultimately culminating in complete tooth loss. This 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. Periodontitis is a major public health concern and has been explicitly indicated as an aggravating factor in cardiovascular diseases, diabetes mellitus and low birth weight or even preterm birth of children. Therefore, timely treatment of periodontal disease is important.

The currently existing periodontal therapy requires the conjunction of exceedingly painful mechanical cleaning along with systemic or localized antibiotic administration. Although systemic administration of antibiotics is beneficial, but it requires high oral doses to achieve effective concentrations in the gingival crevicular fluid (GCF). The high dose of antibiotics, in turn, causes unwanted side effects like hypersensitivity, gastrointestinal intolerance etc. Whereas, local application of mouth rinses, gels, and toothpaste require less dose, but control only supragingival plaques and mucosal infections. Therefore, a more satisfactory approach should be needed to administer antimicrobial drugs directly into the periodontal pocket by using a controlled release device, which would limit the distribution of drugs to its target site, with lesser or no systemic uptake. Current 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 nanofiber membrane loaded with tinidazole (TNZ).

TNZ is one of the most widely used antimicrobial agent against anaerobic periodontal pathogens. It is a 5-nitroimidazole derivative with a half-life of 12–14 h. It has a longer half-life, higher bioavailability and less metallic taste which makes it a promising antimicrobial agent for periodontitis treatment. However, most of the TNZ formulations are available as oral dosage forms, resulting in a low concentration of TNZ in GCF and also causes unwanted side effects. Thus, in the present work, an attempt has been made to develop and optimize TNZ loaded electrospun nanofiber membrane for localized treatment of periodontitis.

OBJECTIVES

The objective of the present research work was:

- To develop, optimize and evaluate TNZ loaded electrospun nanofiber membrane for the effective treatment of periodontal disease.
- To deliver TNZ with slow rate locally in the periodontal pocket and maintain therapeutic drug concentration for treatment duration and thus, to reduce dose size and dosing frequency as well as improve patient compliance.

STUDY DESIGN

The present study was divided into four parts:

Part I: Analytical method development and validation for estimation of TNZ

Part II: Development, optimization and evaluation of TNZ loaded homogeneous electrospun poly (ε-caprolactone) nanofiber membrane.

Part III: Development, optimization and evaluation of TNZ loaded homogeneous electrospun gelatin/ poly (ε-caprolactone) hybrid nanofiber membrane.

Part IV: Development, optimization and evaluation of TNZ loaded homogeneous electrospun chitosan/ poly (ε-caprolactone) hybrid nanofiber membrane.

WORK DONE AND OUTCOME OF THE STUDY

Part I: *Analytical method development and validation for estimation of TNZ*

Simple, rapid, reliable and reproducible analytical methods were developed as well as validated using UV-Visible spectrophotometer for quantitative estimation of TNZ during in-vitro studies. The sensitivity, specificity, accuracy and precision of the developed methods were acceptable as per ICH guideline.

Part II: *Development, optimization and evaluation of TNZ loaded homogeneous electrospun poly (ε-caprolactone) nanofiber membrane (TNZ-PCLNF)*

TNZ-PCLNF membrane were successfully prepared by electrospinning method and optimized by "Quality by Design" approach using Box-Behnken experimental design. The optimized TNZ-PCLNF membrane showed the diameter of 147.6 ± 7.6 nm and EE of 84.36 ± 1.5%. The solid-state characterizations of optimized TNZ-PCLNF membrane using FTIR, DSC, and PXRD pointed towards the encapsulation of TNZ inside the nanofiber membrane without any physical as well as chemical interactions. Surface morphological study using HR-SEM and AFM showed the existence of cylindrical shaped nanofibers with a smooth surface. In-vitro release of TNZ from TNZ-PCLNF membrane in McIlvaine buffer pH 6.6 showed sustained release up to 20 days by the diffusion controlled process. Higher contact angle (123.6±2.8º) revealed that PCLNF membrane is of hydrophobic in nature which would prevent its adhesion to the site of action. MTT assay and CLSM study suggested that nanofiber membrane showed insignificant ($p>0.05$) cytotoxicity on mouse fibroblasts (L-929 cell lines). The optimized TNZ-PCLNF were found stable over the storage time period at room

temperature (30±2 ºC), refrigerated condition (4±1 ºC), and a significant change was observed at accelerated condition (40±2 ºC/75±5 % RH). Further, *in vivo* study by ligature-induced periodontitis in rats confirmed that TNZ loaded nanofiber membrane can significantly ($p < 0.05$) improve continuity of epithelium and transseptal fiber of Interdental papilla in comparison to control group.

Part III: *Development, optimization and evaluation of TNZ loaded gelatin/poly (ε-caprolactone) hybrid nanofiber membrane (TNZ-PGHNF)*

TNZ-PGHNF were prepared by electrospinning technique and optimized with BBD experimental design. Box–Behnken design was employed for evaluating the influence of formulation and processing variables on entrapment efficiency (EE) and diameter of the nanofiber. The optimized batch selected by desirability approach was subjected to physicochemical characterization such as FTIR, DSC and PXRD which revealed entrapment of drug in a molecular dispersion form and devoid of any chemical interaction. Electron microscopy showed the smooth structure in nanometre range. Optimized TNZ-PGHNF nanofiber membrane exhibited a diameter of 160.54±11.8 nm and EE 82.75±1.6%. In-vitro release of TNZ from TNZ-PGHNF membrane in McIlvaine buffer pH 6.6 showed sustained release up to 15 days by the diffusion controlled process. Further, reduction of contact angle (from 123.6±2.8º to 55.2±1.6º) and increase in mucoadhesive force 120 gm/cm^2 (optimized batch) revealed that incorporation of gelatin enhanced the hydrophilicity as well as mucoadhesivity of the nanofiber membrane which would facilitate its adhesion to the site of action and instigate proliferation of cells. In vitro antibacterial study showed significant antimicrobial activity against *S. aureus* and cytocompatibility with L929 cell lines were observed. TNZ-PGHNF were found physically and chemically stable over the storage

time period without any significant change ($p>0.05$) in their physicochemical attributes, stored at room temperature (30±2 °C), refrigerated condition (4±1 °C) whereas significant change ($p<0.05$) was found at accelerated condition (40±2 °C/75 + 5 % RH). Moreover, *in vivo* study by ligature-induced periodontitis in rats confirmed that TNZ loaded nanofiber membrane can significantly ($p < 0.05$) improve continuity of epithelium and transseptal fiber of Interdental papilla in comparison to control group.

Part IV: *Development, optimization and evaluation of TNZ loaded chitosan/poly (ε-caprolactone) nanofiber membrane (TNZ-PCHNF)*

TNZ-PCHNF membrane was prepared by electrospinning method. A 3-level, 3-factor Box-Behnken design was employed for evaluating the influence of formulation and processing variables on quality of final formulation. Optimized nanofiber membrane was subjected to solid-state and surface characterization studies using FTIR, DSC, XRD, SEM and AFM, which revealed that TNZ was entrapped in an amorphous form inside smooth and uniform cylindrical nanofibers without any physicochemical interaction with excipients. The optimized TNZ-PCHNF membrane had a diameter of 143.55±8.5 nm and entrapment efficiency of 83.25±1.8%. In vitro drug release and antibacterial study demonstrated capability of the developed nanofiber membranes for efficiently delivering TNZ in a sustained manner up to 18 days, and its ability to inhibit bacterial growth, respectively. Further, reduction of contact angle (from 123.4±2.5º to 27.4±2.3º) revealed that blending of CH with PCL increases hydrophilicity as well as mucoadhesivity of the nanofiber membrane. MTT assay and CLSM study suggested that optimized nanofiber membrane was devoid of cytotoxicity on mouse fibroblasts. TNZ-PCHNF were found physically and chemically stable over the storage time period without any significant change ($p>0.05$) in their physicochemical attributes, stored at

room temperature (30±2 °C), refrigerated condition (4±1°C) whereas significant change ($p<0.05$) was found at accelerated condition (40±2 °C/75 + 5 % RH). *In vivo* study by ligature-induced periodontitis in rat confirmed that TNZ loaded nanofiber membrane can significantly ($p < 0.05$) improve periodontal marker as compared to control group.

After developing and comparing all three types of nanofiber based drug delivery systems, our results suggest that TNZ-PCHNF has a better potential to deliver TNZ locally into the periodontal pockets, based on homogenous distribution of diameter, hydrophilic and mucoadhesive nature of nanofiber, entrapment efficiency drug release performance further, clinical study was also conducted in patients suffering from periodontitis to assess the efficacy and therapeutic potential after approval from Ethical Committee, (Dean/2015-16/EC/1569). Results of clinical study on patients proved therapeutic efficacy of the nanofiber membrane by eliciting a significant ($p<0.05$) decrease in clinical markers of periodontitis.

Thus, on the basis of our research findings, it could be concluded that the performance of the developed TNZ loaded CH-PCL hybrid nanofiber membrane with the hydrophilic and mucoadhesive property was found to be promising for the treatment of periodontal infections by prolonging the periodontal residence time and thereby better therapeutic effects. In addition, they provide intimate contact between dosage form and periodontal pocket which may result in high drug concentration in the local area. Hence, the developed TNZ loaded nanofiber drug delivery system is proved to be a novel approach for the better treatment of periodontal disease as it reduces the dose size, dosage frequency, dose-related side effects, bypasses the usual surgical procedures and improves patient compliance.