3. Plan of Work

3.1. Objective

The overall objective of this present was to develop better drug delivery systems for the prompt healing of a full thickness wound which are more patient compliant in terms of comfort and cost. Taking into account multiple factors, such as biocompatibility, biodegradation rate, tensile strength, accessibility, frequent usage in the nanofiber preparation etc, synthetic polymers, such as poly (ε-caprolactone) (PCL) and poly(lactic-co-glycolic acid) (PLGA) in combination with natural polymer viz. gelatin (GE) were chosen as carriers to encapsulate ciprofloxacin hydrochloride and quercetin, while using appropriate solvent which would provide optimum conductivity and help in the electrospinning process as well as produce homogeneous nanofiber membrane.

The basic objectives of the study include;

- To fabricate ciprofloxacin hydrochloride and quercetin loaded nanofiber membrane by electrospinning technique.
- To carry-out *in-vitro* antibacterial and antioxidant evaluations of developed nanofibers.
- To assess *in-vivo* wound healing efficacy of developed nanofibers in a full thickness wound.

3.2. Study Design

To achieve the mentioned objectives, the present study was planned carefully and divided into the following parts:

3.2.1. Pre-formulation Studies

- Development of analytical method for the estimation of ciprofloxacin hydrochloride and quercetin by UV-Vis spectroscopy
- Validation of developed method as per ICH guidelines for the estimation of ciprofloxacin hydrochloride and quercetin by UV-Vis spectroscopy
- Solubility studies

3.2.2. Formulation and evaluation of PCL based nanofibers loaded with ciprofloxacin hydrochloride and quercetin

- Formulation of ciprofloxacin hydrochloride and quercetin encapsulated PCL nanofiber membrane (PCL-CH-Que nanofiber) using electrospinning technique.
- Morphological characterization of the fabricated PCL-CH-Que nanofiber membrane using High-Resolution Scanning Electron Microscopy (HR-SEM).
- Solid state characterization of PCL-CH-Que nanofiber membrane using Fourier Transformed Infra-Red spectroscopy and Powder X-ray Diffraction.
- Evaluation of surface wettability of the nanofiber membranes by measurement of contact angle.
- Determination of entrapment efficiency and *in-vitro* drugs release profile of PCL-CH-Que nanofiber membrane in phosphate buffer (pH 7.4).
- Evaluation of free-radical scavenging efficiency of nanofiber membrane.
- Evaluation of antimicrobial activity of developed scaffold on agar plate.
- Evaluation of biocompatible nature of nanofiber membrane by *in-vitro* hemocompatibility and fibroblast viability.
- Assessment of *in-vivo* wound healing efficacies of fabricated nanofiber membrane:

1. Examination of histological changes in granulation tissues by hematoxylin and eosin staining.

2. Biochemical estimation of SOD, catalase and hydroxyproline content in granulation tissues.

3.2.3. Formulation and evaluation of PCL-gelatin based nanofibers loaded with ciprofloxacin hydrochloride and quercetin

- Formulation of ciprofloxacin hydrochloride and quercetin encapsulated PCL-gelatin based nanofiber membrane (PCL-GE-CH-Que nanofiber) using electrospinning technique.

- Morphological characterization of the fabricated PCL-GE-CH-Que nanofiber membrane using High-Resolution Scanning Electron Microscopy.

- Solid state characterization of the developed PCL-GE-CH-Que nanofiber membrane using Fourier Transformed Infra-Red spectroscopy and Powder X-ray Diffraction.

- Evaluation of surface wettability of the nanofiber membranes by measurement of contact angle

- Determination of entrapment efficiency and *in-vitro* drugs release profile of PCL-GE-CH-Que nanofiber membrane in phosphate buffer (pH 7.4).

- Evaluation of free-radical scavenging efficiency of nanofiber membrane.

- Evaluation of antimicrobial activity of developed scaffold on agar plate.

- Evaluation biocompatible nature of nanofiber membrane by *in-vitro* hemocompatibility and fibroblast viability.

- Assessment of *in-vivo* wound healing efficacies of fabricated nanofiber membrane:

    1. Examination of histological changes in granulation tissues by hematoxylin and eosin staining.

2. Biochemical estimation of SOD, catalase and hydroxyproline content in granulation tissues.

3.2.4. Formulation and evaluation of PLGA-gelatin based nanofibers loaded with ciprofloxacin hydrochloride and quercetin

- Formulation of ciprofloxacin hydrochloride and quercetin encapsulated PLGA-gelatin based nanofiber membrane (PLGA-GE-CH-Que nanofiber) using electrospinning technique
- Morphological characterization of the fabricated PLGA-GE-CH-Que nanofiber membrane using High-Resolution Scanning Electron Microscopy.
- Solid state characterization of the PLGA-GE-CH-Que nanofiber membrane using Fourier Transformed Infra-Red spectroscopy and Powder X-ray Diffraction.
- Determination of entrapment efficiency and *in-vitro* drugs release profile of PLGA-GE-CH-Que nanofiber membrane in phosphate buffer (pH 7.4).
- Evaluation of free-radical scavenging efficiency of nanofiber membrane
- Evaluation of antimicrobial activity of developed scaffold on agar plate
- Evaluation biocompatible nature of nanofiber membrane by *in-vitro* hemocompatibility and fibroblast viability.
- Assessment of *in-vivo* wound healing efficacies of fabricated nanofiber membrane:

1. Examination of histological changes in granulation tissues by hematoxylin and eosin staining.
2. Biochemical estimation of SOD, catalase and hydroxyproline content in granulation tissues.