1. Introduction

The skin is the largest organ in human body, it protects the vital organ from external environment and hence enabling the vital functions to take place in controlled physiological condition [Kurahashi and Fujii 2015, Ng and Lau 2015]. An injury to human body due to violence, accident, or surgery, which typically includes cut or breaking the skin, and often extending into subcutaneous tissue with damage to other structures such as muscles, tendons, nerves, vessels, parenchymal organs and even bone are categorized into wound [Kant et al. 2017, Velnar et al. 2009]. Due to high rate of occurrence in general and growing frequency in the ageing population, the wound has caused the immense social and economic impact worldwide [Velnar et al. 2009]. As per a 2018 retrospective analysis, Medicare had identified around 8.2 million people who had at least one type of wound with or without infections. The estimated cost for the treatment of acute and chronic wound ranged from $28.1 billion to $96.8 billion. Treatment cost was highest for surgical wounds followed by diabetic foot ulcers, with a higher trend toward costs associated with out-patient wound care than in- patient [Sen 2019]. Depending on the healing time, a wound ranges from acute wound to chronic wound. There are also a large number hard-to-heal wounds associated with chronic diseases and abnormalities, such as arterial, venous, diabetic and pressure ulcers, that directly or indirectly end in damage of the cutaneous coverage [Velnar et al. 2009].

Depending on the depth of the skin layer, a wound can be either confined to epidermis or dermis layer. The least damaging wound related to damage done at epidermis layer heals by re-epithelialization without any skin graft. While a full thickness wound (FTW) that

lengthen beyond the two layers of skin (epidermis and dermis) and extend into the subdermal tissues does not heal spontaneously, due to loss of residual cells for regeneration except at periphery of wound [Chong et al. 2007]. Therefore, it takes longer duration for complete re-epithelialization (healing by secondary intention) and often complicated by scarring of the base and significant disability, [Idrus et al. 2014, Marler et al. 1998], or it demands skin regeneration product for prompt wound healing [Norouzi et al. 2015]. Although split-thickness skin graft is usually implanted to promote early wound healing, however its application is restricted by donor sites availability, cumbersome surgical procedures and a tendency of graft to contract with time. Allogeneic or xenogeneic skin grafts can be used as temporary coverage but carry the risk of disease transfer and immune rejection. [Liu et al. 2010a, Ma et al. 2011]. These limitations lead the way towards the development of bioengineered matrices as the skin graft for prompt wound healing.

Wound healing is a complex natural, dynamic, interactive process that consists the participation of various components such as extracellular matrix, soluble mediators, blood and parenchymal cells [El-Ferjani et al. 2016]. Depending on the scale of injury, healing proceeds through three sequential phases of varying and overlapping duration, namely the hemostasis and inflammatory, proliferative, and tissue remodeling [El-Ferjani et al. 2016, Gizaw et al. 2018, Park et al. 2011]. Among those phases, the inflammatory phase is the most critical one, which is marked by release of various pro-inflammatory cytokines, reactive oxygen species, proteases, and growth factors to protect open wound from infection, to improve phagocytic activity, and to assist during wound repair [Pereira and Bartolo 2016, Rasik and Shukla 2000]. Although, A homeostatic state of reactive oxygen species is maintained by endogenous antioxidant system of body [like superoxide

dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)], which scavenges the excess free radicals. However under adverse conditions, such as impaired wound healing associated with full thickness wound and wound heavily infiltrated with microorganism, the free radicals are excessively produced in the wound area. These excessive ROS causes sustained release of pro-inlammatory cytokine and activation of matrix metalloproteases (MMPs), which modify and degrade extracellular matrix (ECM) proteins and can also impair dermal fibroblast and keratinocyte function [Dunnill et al. 2017, Lü et al. 2010]. In these situations, even endogenous anti-oxidants which make the first line defense mechanism against free radicals, fails to neutralize excess free radicals. Consequently, these excess free radicals cause oxidative damage to the fibroblast, primary cell responsible for collagen synthesis and provisional ECM formation [Nafiu and Rahman 2015, Selvaraj and Fathima 2017].

An open wound is highly prone to microbial infection, and it is also considered major reason of tissue regeneration product failure. Infection is either caused by pathogens infiltration at the wound site or by foreign body response of the implant material [Campoccia et al. 2013]. These persistent pathogens infection and released-endotoxin at wound site extend the inflammatory phase and increases level of MMPs, a protease that begin degradation of ECM [George Broughton et al. 2006, Guo and DiPietro 2010]. Therefore, antibacterial biomaterials are one of the greatest interests in the war to prevent the bacterial infiltration and implant-related infections. However, conventional dosage form require very high dose of antibiotics to deliver effective concentration of antibiotic at infected site which also causes other systemic toxicity. Therefore, in order to minimize the

systemic side effect and to achieve effective concentration of antibiotic inside deep tissue, controlled local delivery of drug is anticipated [Feng et al. 2010, Xue et al. 2014b].

Taking into consideration of mentioned issues faced by an open full-thickness wound, i.e., requirement of a tissue regeneration product, an exogenous anti-oxidant to scavenge excess free radicals, and an antimicrobial to resist microbial infection at wound site, we had proposed to develop a polymer-based nanofiber membrane loaded with an antimicrobial and an anti-oxidant for application at wound site to achieve accelerated wound healing.

Among the wide variety of biomaterials, nanofibers play a significant role in wound care and management. Recently, electrospining is a widely employed technique for the production of nanofibres as tissue engineered scaffold. Various properties of an electrospun nanofibers such as it (i) offer high surface area and highly porous structure which quickly start cell signaling and attract fibroblast for extracellular matrix components secretion, (ii) imitate the functional and structural similarity of native extracellular matrix (ECM) that support cell attachment and its proliferation, (iii) protect the wound from microbial infiltration due to extremely interconnected pores, and (iv) act as drug delivery device with controlled and sustained release profile, and with high drug loading, make it a potential candidate for wound dressing [Chong et al. 2007, Kim et al. 2009, Liu et al. 2010a, Liu et al. 2010b, Xue et al. 2014a]. Furthermore, the dressing materials should also preserve the normal state of differentiation within the cellular compartment. To achieve this objective, an engineered matrix must be biodegradable, biocompatible, and should not produce adverse effects in the surrounding tissue [Liu et al. 2010a].

Ciprofloxacin hydrochloride is a most commonly used broad spectrum fluoroquinolone antibiotic for a variety of systemic as well as local bacterial infections. It is also active against the most common causative organisms associated with wound infection such as *Staphylococcus aureus*/*MRSA, Enterococci, Streptococcus pyrogenes* & *Pseudomonas aeruginosa*. Further, low minimal inhibitory concentration value and lower frequency of microbial resistance makes it a promising antimicrobial for wound infection [Jannesari et al. 2011, Kevadiya et al. 2014, Suhaeri et al. 2018, Unnithan et al. 2012]. However, frequent and high oral dosing to achieve MIC at the topical site is notable shortcomings of the oral formulation. Quercetin is a naturally occurring flavonoid, commonly found in fruits and vegetables. High propensity for electron transfers proves it as strong free radical scavenger and potential anti-oxidant. Additionally, it also shows other health-beneficial effects like anti-carcinogenic, anti-allergic and anti-inflammatory [Aceituno-Medina et al. 2015, Arvand et al. 2015, Li et al. 2016]. However, poor water solubility, low bioavailability, short half-life, physiochemical instability, high temperature and oxygen limits the potential benefit of quercetin. Therefore, we had proposed to encapsulate these medicaments in nanofibers and deliver by alternate route i.e. topical route, so that the respective shortcoming of medicaments could be avoided. Meanwhile, the temperature during electrospun process increases marginally and therefore has no anticipated effect on the stability of formulation and drugs encapsulated.

In the present study, we had developed ciprofloxacin hydrochloride and quercetin loaded PCL based, PCL-Gelatin based and PLGA-Gelatin based nanofibers by electrospinning technique, and hypothesized that fabricated nanofiber based scaffold would (i) release the drugs in sustained and controlled manner, (ii) decrease bacterial growth and hence check

degradation of ECM, (iii) scavenge free radical and thus diminish the inflammatory response, (iv) degrade and get assimilated in the granulation tissue, and (v) facilitate the complete wound closure in an accelerated manner. Further, we evaluated the proposed hypothesis in a rat model after excising full thickness wounds.

Taking into consideration multiple factors, such as accessibility, frequent usage in the nanofiber preparation and other positive benefits, PCL, gelatin (GE) and PLGA were chosen as carriers to encapsulate ciprofloxacin hydrochloride and quercetin, while using appropriate solvent that would provide appropriate conductivity and help in the electrospinning process as well as produce homogeneous nanofiber membrane. Electrospinning solutions of different compositions were prepared in respective solvent(s) system. DMSO was used as co-solvent for solubilization of quercetin. The final solution was filled in a 5mL syringe fitted with a 24G needle and was maintained at 8-10cm distance from the collector. The electrospinning solution was pumped at a flow-rate 0.5-0.8 mL/h from the syringe and electrospun at a voltage of 14-16kV. The randomly oriented nanofibers were collected on fixed collector and evaluated for various parameters like fiber morphology and diameter distribution by analyzing the SEM images, membrane porosity by utilizing ImageJ software, solid state characterization by FT-IR & P-XRD, surface hydrophilicity by contact angle measurement, entrapment efficiency and *in-vitro* drugs release profile in buffer (pH 7.4), *in-vitro* antioxidant by DPPH scavenging method, antibacterial activity (*S. aureus*) by measuring growth inhibition zone on agar plate, biocompatibility study in terms of hemocompatibility and MTT assay, wound healing efficiency in a full thickness wound, histological changes in granulation tissues, and biochemical estimation of SOD, catalase and hydroxyproline content in granulation tissues.