Chapter 1- Introduction and Objective

1.1. Introduction

Skin is the largest organ of human body comprises 15% of adult total body weight. Skin has various functions as protection against external agents, facilitates thermoregulation, fluid balance, provides sensation, vitamin D synthesis and immune activation[1].

A skin wound is disruption of any tissue or cellular integrity due to mechanical, chemical, physical or metabolism related injuries or in simple words wound is a damage or disruption to the normal anatomical structure and function of skin[2]. Wound healing is a cascade of carefully and precisely regulated dynamic and complex steps involving a series of continuous, coordinated events. Although the process of healing is continuous, it may be arbitrarily divided into four phases: (i) coagulation and haemostasis; (ii) inflammation; (iii) proliferation; and (iv) wound remodeling with scar tissue formation[3]. Process of wound healing depends upon various factors such as age, nature of wound, infection, environmental condition as environmental bacterial and fungal load etc. To facilitate accelerated wound healing, the surface of the wound should generally be kept moist[4]. Dressing not only protects against infection, but also helps to maintain a moist environment that promotes faster reepithelization. Sometimes skin is severely damaged and beyond the repair capacity. Since functions of skin are vital for the survival, therefore it must be assisted by tissue engineering to speed up the process of wound healing. Tissue engineering term was first introduced by Langer and Vacant, 1999 as a interdisciplinary field which implies the principles of engineering and life sciences toward the development of biological substitutes to restore, maintain, or improve tissue function [5]. Skin tissue engineering is multidisciplinary

research field which involves biomaterials, scaffold preparation techniques and bioactive agent which leads to controlled stimulation of target cells via systematic combination of molecular signal ultimately culminates in faster wound healing. Skin tissue engineering aims at promoting the regeneration of skin or replacing the falling or malfunctioned skin with scaffold with or without cell and bioactive molecule[6]. Over the past three decades there has been extraordinary advancement in the field of cell biology, biochemistry, physiology, biomaterial research and bioscience etc. These research and finding has increased our understanding regarding the physiology of wound and nature of different biomaterial and its interaction with cell and host tissue etc.[7-8]. These studies contributed positively towards to flourish skin tissue engineering and help towards restoring the normal structure and function of the skin. Many skin substitutes have been developed in the recent decades. These skin substitutes are of different types based on the nature and combination of biomaterial used in the preparation, cellular or acellular, synthetic or natural etc.[9]. These skin substitutes aid in skin tissue generation but majority of them suffer from scar formation, infection, wound constriction and poor integration with host tissue[10].

Skin tissue regeneration is mainly based on selection and fabrication of suitable scaffold. Scaffold provides optimum microenvironment for cell proliferation, migration and differentiation. Scaffold should be biocompatible, biodegradable, non-immunogenic, and nontoxic and exhibit an appropriate physical and mechanical characteristic with appropriate chemistry with good micro and nanostructure is required for good cell attachment and proliferation. Properties of scaffold greatly depends upon the material used for the preparation, methodology used in scaffold preparation, crosslinking agent and crosslinking method[11-12].

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Numerous natural polymers such as chitosan, collagen, gelatin, hyaluronic acid, silk etc. have a great potential to be used in tissue engineering to develop novel wound healing dressing skin substitutes. Moreover, natural biopolymers are, biocompatible, biodegradable and provides biomimetic extracellular matrix for tissue regeneration[13]. Collagen is the most abundant protein in animal tissues and accounts for 70–80% of the dry weight of the dermis. Collagen is the unique, triple helix protein molecule, which forms the major part of the extracellular dermal matrix (ECM). The principal function of collagen is to act as a scaffold in connective tissue. It also offers low immunogenicity, porous structure, good permeability, biocompatibility and biodegradability[14].

The deacetylated derivatives of chitin reported as chitosan, have wide application in wound management because it has capacity to deliver the loaded drug in tissue surface. Moreover, chitosan is hypoallergenic, antibacterial, biocompatible, and biodegradable with high mechanical strength and facilitate quick blood coagulation at the site of injury[15-16].

Currently wound management faces two critical challenges-

1. Microbial infection
2. Hypoxia

Microbial infection prolongs the wound healing process thereby increasing the tissue morbidity depending upon the severity of infection and sepsis. Microbial infection or sepsis leads to higher inflammatory period and longer period may delays the wound healing. Therefore, the potential solution to avert such antimicrobial infections and sepsis is to use antimicrobial agents such as antibiotics[17-18]. In skin tissue engineering, common approach is site specific delivery of antibiotic using scaffold as carrier of antibiotics. Site specific

release of antibiotic not only serves the purpose of preventing the microbial growth and sepsis in wound but also prevent the other side effect of orally administered antibiotic. Various antibiotics such as norfloxacin, penicillin, ciprofloxacin etc have been used in tissue engineering application [19-21]. Ciprofloxacin is a synthetic broad-spectrum antibiotic belongs to the fluoroquinolone class; is effectively used against gram-positive and gram-negative bacteria has bactericidal activity against most common pathogens prevalent at wound site such as *P. aeruginosa, S. aureus, E. coli*, etc. Therefore, ciprofloxacin loaded in scaffold, could be used as a wound healing material for sustained and continuous release of the antibiotic to provide protection against infectious agents[22].

Hypoxia is another critical concern which occurs during wound healing process. Since wound healing is an interlinked orchestrated cascade of events that initiates from blood clotting followed by formation of extracellular matrix and terminates with the process of replacing, devitalized and missing cellular structures[23]. Since, this process involves a lot of energy to repair and replacement of damaged part, therefore sufficient oxygen is required to fulfill these processes of wound healing[24].

Low availability of oxygen during the repair process leads to hypoxia, causing the formation of free radicals and singlet oxygen. These Free radicals, singlet oxygen and reactive oxygen species interact with the cellular organelles and cellular components including DNA, proteins, and lipids and causes cellular damage, changing the pattern of bonding between them and ultimately making those inactive[25] . Recent studies have been focused on the development of oxygen releasing biomaterials, which can deliver oxygen slow, sustained and a longer period of time[26-27]. Inorganic peroxides, such as calcium peroxide (CaO_2), sodium per carbonate ($Na_2CO_3 \cdot 1.5H_2O_2$) and magnesium peroxide (MgO_2) are oxygen

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releasing chemicals which can release oxygen in burst due to chemical reaction after coming in contact with water. Therefore, to prevent quick reaction with water and to promote the sustained release of oxygen, inorganic peroxides are encapsulated inside the hydrophobic polymer such as PCL. PCL is a FDA approved, biocompatible synthetic polymer that has been used as coating to ensure the sustained and continuous release of oxygen[28-29].

Second critical issue is the generation of free radicals during the chemical decomposition of inorganic peroxides as reaction intermediates of oxygen production. Therefore, free radicals quenching substance is needed in the oxygen producing biomaterial. Catalase is a natural antioxidant present inside the cell which is stable and has high turnover number; therefore incorporation of catalase enzyme inside the scaffold can effectively quench the ROS[30-31].

During the past years, various biomaterials of natural and synthetic in origin have been studied extensively for skin tissue engineering that has led to production of various commercially available skin substitutes. In the present work, chitosan collagen scaffold was fabricated which has antibacterial and oxygen releasing tendency to overcome the limitation which result in infection and tissue necrosis during wound healing.

1.2 Objective of study

The main objective of study has been the optimization, development and evaluation of different types of acellular scaffolds as skin tissue engineering substitute. The study includes the optimization and evaluation of collagen chitosan scaffold using freeze drying method. Prepared scaffold was physically and biochemically evaluated for its applicability for skin tissue. To study the efficacy of developed scaffold, scaffolds were tested in rat model.

The objectives of my research domain are as mentioned below:

1. To optimize, design, characterize and evaluate biopolymeric scaffolds comprise of collagen and chitosan loaded with ciprofloxacin.

2. To characterize the optimized collagen-chitosan-ciprofloxacin based scaffolds using isolated and grown fibroblast *in vitro.*

3. To study the *in vivo* study of developed scaffold using rat model.

4. To design, characterize and evaluate the ciprofloxacin loaded collagen chitosan oxygen releasing scaffold using physical and biochemical method.

5. *In vitro* study of optimized scaffold seeded with isolated fibroblast using cell culture techniques.

6. Evaluation of efficacy of optimized oxygen releasing scaffold in rat model.