CHAPTER 1: INTRODUCTION

1.1 WOUND

A wound can be defined as an injury or disruption to anatomical structure and function resulting from simple or severe break in structure of organ such as the skin and can extend to other tissues and structures such as subcutaneous tissue, muscles, tendons, nerves, vessels and even to the bone (1). Wound can be accidental (e.g. burns, abrasion, skin tears ect.), surgical, occur because of underlying disease for example diabetic and vascular ulcer and some skin conditions may also develop into a wound for example eczema or psoriasis. Wound can be open or closed. Open wounds have exposed body tissue in the base of the wound. Closed wounds have damage that occurs without exposing the underlying body tissue.

Wounds generally fall into two categories: acute wound and chronic wound (2). Chronic wounds are acute wounds have not progressed through the stages of healing normally. They may heal at a much slower rate, heal only partially or reoccur after or complete healing. These chronic wound are almost associated with underlying chronic disease that affect either the blood supply or how the cells function at the wound site (3). There are different types of chronic wounds such as pressure injuries, diabetic ulcers, leg ulcers etc. Disruptions in the integrity of the skin that heals uneventfully with time re considered acute wounds (3). Surgical and traumatic wounds, abrasion, or superficial burns are generally considered acute wound.

A burn is tissue damage caused by heat, steam, fires, gases, chemicals, electricity, sunlight, radiation. Burns are defined by how large an area they cover. Burns can cause swelling, blistering, scarring and in serious cases shock and even death. They also can lead to infections because they damage skin's protective barrier. Globally, burns injuries are the 4th most common type of trauma.

The significance of wound management can be described by following facts:

- According to retrospective analysis of medicare beneficiaries (2018), about 8.2 million people had different types of wounds. Patients suffering from diabetes and obesity are at a high risk of developing chronic wounds. Chronic wound are

mostly seen in the elderly people. In US, 3% of the population > 65 years of age have open wound. There will be about 82 million people (>65 year -old) in developing countries and 48 million people (>65 year -old) in developed countries suffered from diabetics by 2030 which indicates a critical need for advance wound management to better control the future global burden of chronic wounds (4).About 2% of total population of US is estimated to be affected from chronic wound(5). As per 2018 market research report, the global wound closure products market will more than $ 15 billion by 2024 (6).

- The need for treatment strategies for treating chronic wounds is urgent. Diabetic wounds that do not heal are the most severe type of chronic wounds, affecting millions of people annually. Foot ulcers are common in diabetic patient and individuals with compromised blood circulation (7). Many of the roughly 350 million diabetes patients in the world develop foot ulcers, and around 10-15 million cases this ultimately leads to amputation.
- In India, a recent study estimated a prevalence rate of chronic wounds at 4.5 per 1000 population. The incidence of acute wounds was at 10.5 per 1000 population which is more than double form chronic wound.
- Burns is a potential public health problem in the world. As per the Global burden of disease study (2013), there were 33.5 million thermal burn injuries reported in all over the world, resulting 237500 deaths. 90% burn deaths occur in low/middle-income countries (8). According to National program for prevention of Burns injuries, approximately 7 million people sustain burn injuries in each year in India, out of which 0.7 million need hospitalization, 0.25 million get crippled, and 0.14 million succumb (9). According to global Health data (2017), over 61,000 deaths in 2015 due to burns in India. About 1, 80,000 deaths per year due to burn injury were estimated in low-and middle-income countries (10-12).

Treatment of burn injury: Burn treatment varies depending on the cause and severity (13). In preliminary treatment of first degree burns, burns area are gently wash with cool water and some lotion or cream applied on it. Antibiotic ointments or creams are often used to prevent or treat infections in patients with second-degree burns. Using these ointments may require the use of bandages. There are many advanced wound care

products includes impregnated gauzes, foams, honey dressing, hydrogel, silver dressings for the burn treatment. Larger are of third degree burns are treated with different types of skin grafts such as sheet grafts, meshed grafts, full-thickness grafts.

1.2 WOUND MANAGEMENT

Wound healing is a complex process involving several inter-related biological and molecular activities for achieving tissue regeneration. The main physiological events include coagulation, inflammation, removal of damaged matrix components, followed by cellular proliferation and migration, angiogenesis, matrix synthesis and deposition, re-epithelization, and remodeling (14). Wound healing is a global medical concern with several challenges including the increasing incidence of obesity and diabetes, an ageing population and the requirement for more effective dressings. Infection is the greatest risk from non-healing wounds. Each of us is exposed every day to common bacteria such as staphylococcus and pseudomonas. These bacteria are present and thus settle easily on our skin. Wound increases the risk for infection, which can lead to serious health problems. Traditional Wound Care, comprised mostly of gauze-based dressings such as woven and non-woven sponges, conforming bandages, non-adherent bandages. Also topical formulations like solution, suspension, cream, gel are used for wound healing (15).

Ideal wound dressing: It is widely accepted that a warm, moist wound environment encourages healing and prevents tissue dehydration and cell death. These conditions also allow the interaction of the cells and growth factors involved in the in the healing process. Therefore, ideal wound dressing should (15, 16):

- Maintain a moist environment at the wound surface
- Provide thermal insulation
- Provide mechanical protection and protect against secondary infection
- Be non-adherent and easily removed without trauma
- Leave no foreign particle in the wound
- Remove excess exudates
- Be cost effective and offer effective pain relief

Over the past few years an ever-expanding list of dressing has come onto the market in an attempt to meet these conditions. Among them are the transparent film dressing, hydrogel, hydrophilic foams, alginates, hydrocolloids, antimicrobial dressing and biological dressing. There is however no magic “one-size-fits-all” dressing. Absorbable haemostats and hydrogel dressings are the part of modern wound management systems which help in haemostasis and moist wound healing respectively.

1.3 SOME BIOMATERIALS USED IN WOUND CARE SYSTEMS

Biomaterials like Gelatin, Sodium hyaluronate, Sodium alginate, Collagen, Chitosan, Growth factors, plasminogens, fibrin, honey, turmeric, calendula, aloe vera are able to interact actively with the wound 'microenvironment', stimulating its healing, and are defined as ‘bioactive medications’.

1.3.1 COLLAGEN

Collagen is a biodegradable and biocompatible protein mostly found in connective tissue. One key component of chronic wounds is an elevated level of matrix metalloproteinases (MMPs). At elevated levels, MMPs not only degrade nonviable collagen but also viable collagen. In addition, fibroblasts in a chronic wound may not secrete tissue inhibitors of MMPs (TIMPs) at an adequate level to control the activity of MMPs. These events prevent the formation of the scaffold needed for cell migration and ultimately prevent the formation of the extracellular matrix (ECM) and granulation tissue. Collagen based wound dressings are uniquely suited to address the issue of elevated levels of MMPs by acting as a ‘sacrificial substrate’ in the wound. It has also been demonstrated that collagen breakdown products are chemotactic for a variety of cell types required for the formation of granulation tissue. In addition, collagen based dressings have the ability to absorb wound exudates and maintain a moist wound environment (17).

1.3.2 HYALURONIC ACID

Hyaluronic acid is a natural biopolymer that alternately consists of D-glucuronic acid and 2- acetamido-2-deoxy-D-glucose and is generally found in mammal's bond tissues and synovial fluids. It has been reported that hyaluronic acid interacts with proteins, proteoglycans, growth factors and tissue components called biomolecules which has vital importance in healing of various types of wounds. This interaction plays an important role in acceleration of tissue repair and wound healing. Hyaluronic acid and its derivatives also play a role in the protection of the injured area against microorganisms due to their bacteriostatic activity (18).

1.3.3 POT MARIGOLD (*CALENDULA OFFICINALIS L.*)

Calendula officinalis L. or pot marigold is a common garden plant belonging to the compositae family. The flowers are the part of the herb used medicinally. The main compounds within calendula are the triterpenoids which are claimed to be the most important anti inflammatory and antiedematous components within the plant (19, 20). Other constituents identified in calendula such as the polyphenols, saponins, micronutrients, flavonoids, and polysaccharides, may also be responsible for the antiedematous, anti-inflammatory, antioxidant, and wound healing effect of the plant (21-27).

1.4 SELECTED FORMULATIONS FOR EFFECTIVE WOUND HEALING

1.4.1 HYDROGEL

Hydrogel is a three dimensional network of hydrophilic polymers. Hydrogels consist of swellable hydrophilic materials such as starch, cellulose; synthetic polymers like polyvinyl alcohol, polyacrylic acid, Polyvinylpyrrolidone, poly(methacrylates) or other plant or animal derived polysaccharides and contain up to 96% water(28). Hydrogels are capable of absorbing large volumes of water because of the presence of

hydrophilic chains, which allow them to swell extensively without changing their gelatinous nature. They can be used on dry, sloughy, or nectrotic wound.

The advantages of hydrogels in wound and burn treatment can be listed as follows

- Bioadhesion of gels to the surface of the wound is high and this also eases the treatment due to increased contact with the wound. Hydrogel dressing provide cooling, soothing effect; reduce pain associated with dressing changes due to its high water content (29).
- Their structures facilitate the moisture and water vapour permeability necessary to heal the wound area (30). Moist treatment of wound is considered to be the gold standard of therapy for acute open wounds as well as chronic wounds (30).
- Difficulties that are particularly related to the application to open wounds are not seen in these preparations (31).
- They can easily be removed from the application site when adverse events are seen (31).

1.4.2 SCAFFOLDS / FILMS

Hybrid scaffolds comprised of polymeric substrates coated with bioactive materials, collagen, silk fibroin, as well as advanced tissue engineered substrates impregnated with endothelial progenitor cells, and nanomaterial-based scaffolds are used as advanced wound dressings to initiate and expedite wound healing (32, 33). These pharmaceutical dosage forms, which are available in thickness ranging from μm to mm, are prepared by different methods using one or more polymers. Films and scaffolds are the ideal dressing materials and available commercially (34). Films/membranes with a homogeneous polymeric network structure are used to treat the damaged area and generally protect the wound and burn area against external factors. Dermal scaffold would mimic the tissue's natural extracellular matrix. Scaffolds are manufactured using natural or synthetic polymer like chitosan, collagen, gelatin, PLGA, hyaluronate. In the case of major burns where injury damages the deep dermis and no sources of cells for regeneration remain; there is a requirement to provide a dermal scaffold to fill in the deep wound (34).

1.5 AIMS AND OBJECTIVES

1.5.1 AIMS: The aims of the research work were

1.5.1.1: To develop safe and effective hydrogel sheet containing calendula flower extract for wound management

1.5.1.2: To develop safe and effective calendula flower extract loaded biodegradable collagen film for wound management.

1.5.2 OBJECTIVES

- ❖ Overall:
- Development and evaluation of biomaterial based formulations containing *Calendula officinalis* flower extract for wound management
- Perform safety and efficacy study of developed formulations

.

- ❖ Specific:

i. Development of calendula flower extract loaded biodegradable film and hydrogel sheet
ii. Optimization of formulations using Design of Experiment Techniques
iii. *In-vitro* and *In-vivo* performance assessment of the formulations for the evaluation of safety and efficacy.

1.6 HYPOTHESIS

- Biodegradable film containing biomaterials such as collagen and sodium hyaluronate would mimic the tissue's natural extracellular matrix which plays an active role in normal wound healing process and tissue regeneration. Calendula flower's constituents like polyphenols, triterpenoids and flavanoids which are responsible for its significant therapeutic activity such as anti-inflammatory, antioxidant, anti-odemetaous etc., will give synergistic effect in wound healing with collagen and sodium hyaluronate.
- Hydrogel sheets are easy to remove, easy to apply, have good moisture donating and absorbing capacity and water vapor permeability which helps in wound healing process. By incorporating *Calendula officinalis* flower extract into hydrogel sheet, there will be improved and increased would healing efficacy of hydrogel sheet due to anti-inflammatory, antioxidant, anti-odemataous activity of *Calendula officinalis* flower extract.

1.7 PLAN OF WORK

i. Literature review
ii. Selection and procurement of materials
iii. Preliminary studies
iv. Development and optimization of formulations
v. Physicochemical Characterization of developed formulations
vi. *In-vitro* cell cytotoxicity study
vii. Scratch assay
viii. *In-vivo* efficacy study
ix. Stability study
x. Paper publication & thesis write up

1.8 REFERENCES

1. Gonzalez A, Costa T, Andrade Z, Medrado A. Wound healing–a literature review. Anais Brasileiros de Dermat 91: 614–620. 2016.

2. Cooper P. A review of different wound types and their principles of management in Wound Healing: A systematic approach to advanced wound healing and management. Cromwell Press, UK; 2005.

3. Sen C, Roy S, Gordillo G. Wound Healing (Neligan Plastic Surgery: Volume One). Amsterdam, Netherlands: Elsevier; 2017.

4. Wild SH, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030: response to Rathman and Giani. Diabetes care. 2004;27(10):2569-.

5. Järbrink K, Ni G, Sönnergren H, Schmidtchen A, Pang C, Bajpai R, et al. The humanistic and economic burden of chronic wounds: a protocol for a systematic review. Systematic reviews. 2017;6(1):1-7.

6. Sen CK. Human wounds and its burden: an updated compendium of estimates. Mary Ann Liebert, Inc., publishers 140 Huguenot Street, 3rd Floor New …; 2019.

7. Amin N, Doupis J. Diabetic foot disease: from the evaluation of the "foot at risk" to the novel diabetic ulcer treatment modalities. World journal of diabetes. 2016;7(7):153.

8. Peck MD. Epidemiology of burns throughout the world. Part I: Distribution and risk factors. Burns. 2011;37(7):1087-100.

9. Singh AK. Burns management in India: The way ahead. Indian Journal of Burns. 2018;26(1):3.

10. Porter C, Tompkins RG, Finnerty CC, Sidossis LS, Suman OE, Herndon DN. The metabolic stress response to burn trauma: current understanding and therapies. The Lancet. 2016;388(10052):1417-26.

11. Mortal G. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;385(9963):117-71.

12. Peck MD, Toppi JT. Epidemiology and Prevention of Burns Throughout the World. Handbook of Burns Volume 1: Springer; 2020. p. 17-57.

13. Liu H-F, Zhang F, Lineaweaver WC. History and advancement of burn treatments. Annals of plastic surgery. 2017;78(2):S2-S8.

14. Gonzalez ACdO, Costa TF, Andrade ZdA, Medrado ARAP. Wound healing-A literature review. Anais brasileiros de dermatologia. 2016;91(5):614-20.

15. Dhivya S, Padma VV, Santhini E. Wound dressings–a review. BioMedicine. 2015;5(4).

16. Shi C, Wang C, Liu H, Li Q, Li R, Zhang Y, et al. Selection of Appropriate Wound Dressing for Various Wounds. Frontiers in bioengineering and biotechnology. 2020;8:182-. PubMed PMID: 32266224. eng.

17. Brett D. A review of collagen and collagen-based wound dressings. Wounds. 2008;20(12):347-56.

18. Boateng J, Catanzano O. Advanced therapeutic dressings for effective wound healing—a review. Journal of pharmaceutical sciences. 2015;104(11):3653-80.

19. Zitterl-Eglseer K, Sosa S, Jurenitsch J, Schubert-Zsilavecz M, Della Loggia R, Tubaro A, et al. Anti-oedematous activities of the main triterpendiol esters of marigold (Calendula officinalis L.). Journal of ethnopharmacology. 1997;57(2):139-44.

20. Parente LML, Lino Júnior RdS, Tresvenzol LMF, Vinaud MC, de Paula JR, Paulo NM. Wound healing and anti-inflammatory effect in animal models of Calendula officinalis L. growing in Brazil. Evidence-based complementary and alternative medicine. 2012;2012.

21. Preethi KC, Kuttan R. Wound healing activity of flower extract of Calendula officinalis. J Basic Clin Physiol Pharmacol. 2009;20(1):73-9.

22. Herold A, Cremer L, Calugăru A, Tamaş V, Ionescu F, Manea S, et al. Antioxidant properties of some hydroalcoholic plant extracts with antiinflammatory activity. Roumanian archives of microbiology and immunology. 2003;62(3-4):217-27.

23. Della Loggia R, Becker H, Issac O, Tubaro A. Topical anti-inflammatory activity of Calendula officinalis extracts. Planta Médica. 1990;56(06):658-.

24. Della Loggia R, Tubaro A, Sosa S, Becker H, Isaac O. The role of triterpenoids in the topical anti-inflammatory activity of Calendula officinalis flowers. Planta medica. 1994;60(06):516-20.

25. Zitterl-Eglseer K, Sosa S, Jurenitsch J, Schubert-Zsilavecz M, Della Loggia R, Tubaro A, et al. Anti-oedematous activities of the main triterpendiol esters of marigold (Calendula officinalis L.). J Ethnopharmacol. 1997 Jul;57(2):139-44. PubMed PMID: 9254116. Epub 1997/07/01. eng.

26. Neukirch H, D'Ambrosio M, Sosa S, Altinier G, Della Loggia R, Guerriero A. Improved Anti-Inflammatory Activity of Three New Terpenoids Derived, by Systematic Chemical Modifications, from the Abundant Triterpenes of the Flowery Plant Calendula officinalis. Chemistry & biodiversity. 2005;2(5):657-71.

27. Rao S, Udupa A, Udupa S, Rao P, Rao G, Kulkarni D. Calendula and Hypericum: two homeopathic drugs promoting wound healing in rats. Fitoterapia. 1991;62(6):508-10.

28. Mir M, Ali MN, Barakullah A, Gulzar A, Arshad M, Fatima S, et al. Synthetic polymeric biomaterials for wound healing: a review. Progress in biomaterials. 2018;7(1):1-21. PubMed PMID: 29446015. Epub 2018/02/14. eng.

29. Gonzalez JS, Ludueña LN, Ponce A, Alvarez VA. Poly(vinyl alcohol)/cellulose nanowhiskers nanocomposite hydrogels for potential wound dressings. Mater Sci Eng C Mater Biol Appl. 2014 Jan 1;34:54-61. PubMed PMID: 24268233. Epub 2013/11/26. eng.

30. Miguel SP, Ribeiro MP, Brancal H, Coutinho P, Correia IJ. Thermoresponsive chitosan-agarose hydrogel for skin regeneration. Carbohydr Polym. 2014 Oct 13;111:366-73. PubMed PMID: 25037363. Epub 2014/07/20. eng.

31. Madaghiele M, Demitri C, Sannino A, Ambrosio L. Polymeric hydrogels for burn wound care: Advanced skin wound dressings and regenerative templates. Burns Trauma. 2014;2(4):153-61. PubMed PMID: 27602378. Pubmed Central PMCID: PMC5012024. Epub 2014/01/01. eng.

32. Waghmare VS, Wadke PR, Dyawanapelly S, Deshpande A, Jain R, Dandekar P. Starch based nanofibrous scaffolds for wound healing applications. Bioactive materials. 2018;3(3):255-66.

33. Chaudhari AA, Vig K, Baganizi DR, Sahu R, Dixit S, Dennis V, et al. Future prospects for scaffolding methods and biomaterials in skin tissue engineering: a review. International journal of molecular sciences. 2016;17(12):1974.

34. Ajay K, Jashanjit S. Drug loaded porous scaffold films: novel method in wound healing. Indian Journal of Novel Drug Delivery. 2016;8(1):1-8.