General Introduction

1.1 Introduction

Increasing ecological and environmental concerns has focussed attention towards commercialization of bio-based eco-friendly products. The biomedical, pharmaceutical and food packaging industries are showing a great interest in developing nanomaterials based biodegradable products with improved efficacy [1]. There are several such biodegradable materials which are available at a low price and have good mechanical properties. Recent scientific advancements in the field of biodegradable polymers have provided polymeric materials for various sectors that possess the critical property of biodegradability after they served their function. Polymeric materials with desired biological, chemical, physical and degradation profile can now be fabricated with precision to meet various challenges of the environment and biological systems [2]. So these materials take part in the natural cycle "from nature-to-nature" for environmental sustainability. Presently inadequate technologies, low-level production and high costs restrict their widespread applications.

1.2 Problems associated with non-biodegradable food packaging

Petroleum-based synthetic plastics are dominating the food packaging industries. They account for 37% of the total market share for food packaging materials due to their comparatively economical nature, lighter weight, high mechanical strength and rigidity, good barrier properties, shape versatility, heat sealing ability, and durability [3]. It is estimated that nearly 20 million tons of plastic usually enters the world oceans annually [4] and is all set to outweigh the fish population by 2050 due to its extremely slow degradation [5]. The enormous anthropogenic plastic debris, known as "white pollution" has become a critical environmental threat, especially to the marine ecological systems. Recent findings shows that plastic waste has endangered the coral reefs as well as more than 700 species of ocean animals [6] and has led to an increase in cases of plastics found in the stomach of seabirds, the death of the plastic entangled turtles, etc. Moreover, the detection of microplastics in marine foods and air samples has increased the concern of their potential influences on human health in future if it enters the food web [7].

1.3 Bio-based polymers used in food packaging applications

Natural bio-based polymers like agar, chitosan, cellulose and starch, which are extracted from collagen, whey protein, alginate, gluten, gelatin, and carbohydrate are used in bio-based food packaging. Recently, some synthetic bio-based polymers, which include polybutylene succinate, polyvinyl alcohol (PVA), polyglycolic acid, and polylactic acid has been developed as biodegradable substitutes (*Figure* 1.1). These synthetic polymers have high tensile strength, clarity, high gloss, flexibility and durability and are capable of sustaining the current industries [7–10].

1.3.1 Decomposition of bio-based polymers

Biodegradable plastics generally decompose into naturally occurring components such as water, carbon dioxide and biomass, and they are not retained in the environment for many years. Their decomposition follows the same processes as followed by the bio-degradable organic matter, so can be disposed of together with other degradable organic wastes [11] through aerobic and anaerobic routes:

1) Aerobic biodegradation of bioplastics (such as composting):

Biopolymer + O_2 + Aerobic microbes $\rightarrow$ CO_2 + H_2O

2) Anaerobic biodegradation of bioplastics (such as anaerobic digestion and landfill):

Biopolymer + Anaerobic microbes → CO_2 + CH_4 + H_2O

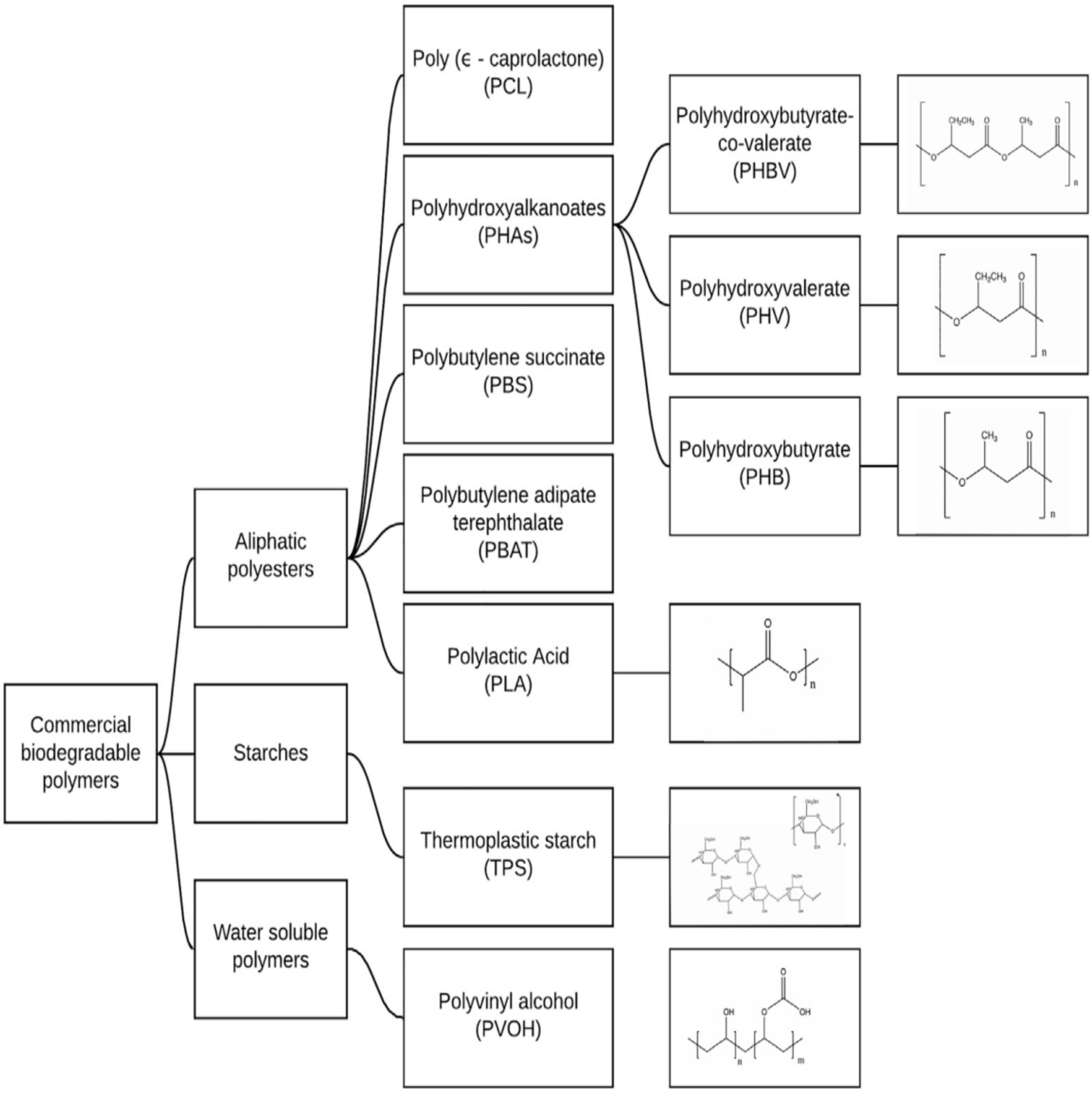

Figure 1.1: Commercial biodegradable thermoplastics [5]

1.4 Nano-composites

A nano-composite is the hybrid material consisting of a polymeric matrix reinforced with a fiber, platelet, or particle having one dimension on the nanometer (nm) scale. The desirable properties like barrier property, mechanical strength etc. of degradable polymers can be improved through nanoparticle reinforcements. Nano-composites are considered as the next generation of smart-materials [2].

1.5 Nanofibers by electrospinning

Electrospinning is the method of the choice for the development of composite nanofibrous mats due to its simplicity, cost efficiency, and versatility. It can be used for the development of antibacterial bio-polymeric nanofibrous composites [12]. The electrospinning process is a simple and effective strategy for fabricating nanofibers directly, continuously and even in a large scale. It provides the advantages of mild experimental conditions, ease of operation and function and use of a wide range of raw materials, etc. [1]. The spinning process is controllable, and the parameters can be adjusted according to the different requirements in various fields. The electrospinning technology is an effective method to prepare nanofibers with various nanostructure and surface characteristics and can meet the functional requirements for active food packaging materials and wound dressings. The versatility depends on the different design of device and a wide range of raw materials for selection, especially polymers. Pure and composite degradable polymeric packaging membranes can be obtained by blending polymer, designing the spinneret, such as multiple-jet and coaxial electrospinning [13]. Moreover, different inorganic fillers can be incorporated into the fibers to enhance their functionality for broader applications. Resulting electrospun films are post-treated by the thermal treatments, surface modifications or dip-coating to obtain superior satisfactory performance [14].

1.6 Impact of bio-based biodegradable polymers on various sectors

For sustainable growth, the idea of a bio-based economy is steadily gaining attention from industries, various organization, and common people. There are several areas in which the bio-based biodegradable polymers can reduce the negative effect of conventional non-biodegradable polymers on the environment and the eco-system. Bio-based biodegradable polymers not only reduce greenhouse gas emissions but also combat mismanaged waste that find way into the environment. Depending on the end-of-life, there is significant employment generation potential for value-added compost [15,16].

1.6.1 Food packaging

Bio-based packaging materials derived from renewable resources have several environmental benefits such as biodegradability and nontoxicity. Nano-composite packaging materials are being fabricated with enhanced characteristics like gas barrier, strength and antimicrobial property for food packaging applications [10]. Antimicrobial biodegradable nano-composite packaging film of natural origin can be potentially used as the replacement for plastics. The antimicrobial packaging may enhance the shelf-life of food products preventing microbial infection and degradation [8].

1.6.2 Biomedical and pharmaceutical applications of biodegradable polymers

Biomedical and pharmaceutical industries are also focusing on safe and effective solutions to the day-to-day medical waste related problems. Natural polymers like collagen have been used biomedically for a long time, but the application of synthetic degradable polymers is relatively new and started in 1960s [17]. Selection of biomaterials for tissue engineering and drug delivery devices depends on the biological, chemical and physical properties. It is based on the host response to the biomaterials. For biodegradable biomaterials, an additional issue of continuing changes in the material properties induced by degradation over time during application is to be taken care of. Host responses to these biomaterials in the long term may be greatly different from the initial response. So, in the design of biodegradable biomaterials, many important parameters must be considered. The biomaterials must: (1) not evoke a sustained inflammatory response; (2) possess sufficient degradation time as per their application; (3) possess appropriate mechanical strength for their intended application; (4) produce non-toxic, readily resorbable or excretable degradation products; (5) include appropriate permeability and processability for designed application; and (6) be biocompatible. These properties greatly depend on but not limited to material chemistry, molecular weight, hydrophobicity, surface charge, water adsorption, degradation and erosion mechanism [18].

1.6.2.1 Wound dressings

Patients with topical wounds normally face the problem of wound dressing adhesion to the wound, causing pain, discomfort and scar when removed in case of traditional dressings as well as most of the market available dressings. This has been the stimulus for the development of new advanced biocompatible, bio-absorbable wound dressings to protect the wound from environmental threats and microbial penetration. Few low adherence dressings techniques such as adding a polyamide contact layer, coating viscose fibre with polypropylene, and impregnating paraffin onto the gauze have been developed. Still, they provide partial relief for the complex wound healing applications [19].

Fibrous electrospun dressings could promote the process of tissue regeneration due to its biomimetic nature. Electrospinning can produce fibrous scaffolds at ambient condition providing leverage of safely utilizing heat-sensitive biomolecules without denaturation for rapid healing [12,20,21]. Nanofibrous dressings have been found to show great potential for both acute and chronic wound healing. It can be obtained from synthetic, natural or blend of synthetic and natural polymers with active moieties.

Currently, bio-based natural polymers such as polysaccharides and derivatives (e.g., carboxymethlycellulose, alginates, chitosan and heparin), proteoglycans and proteins (e.g., collagen, gelatine, fibrin, and keratin) are widely employed to develop modern wound dressings due to their suitable biodegradability, biocompatibility and similarity to macromolecules that are usually recognized by the human body are ineffective in dealing with delayed healing of complex wounds [22]. Nanofibrous based dressings provided an alternative therapy and possess several advantageous characteristics, such as (1) adaptability to the irregular contour of the wound, thus enhancing suppleness and resilience of the scaffold; (2) encapsulation and controlled drug delivery of therapeutics (antimicrobials and nanoparticles); (3) gas exchange to avoid dehydration; (4) fluid absorption, eliminating the excess of wound exudate typical of chronic wounds and preventing the growth of microorganisms; (5) surface functionalization with molecules such as collagen or fibronectin, which increases biocompatibility and further promotes wound healing; and (6) porosity and high surface area to enhance further the biological activity of the dressing [14]. The conventional wound dressing materials lack in most of these requirements.

Biohybrid nanofibrous wound dressings can outperform classical drug delivery systems as these can be tailored for controlled drug delivery [23]. Nanofibrous dressings are mostly biodegradable and mimic the natural extracellular matrix (ECM) structure. This has been the inspiration for the development of new advanced multifunctional biocompatible, bio-absorbable fibrous nano-composite wound dressings with the anti-scar property. The main advantage of using biodegradable and bio-absorbable fibrous dressings is that no further removal of dressing required because they slowly degrade and ultimately absorbed by the tissues in the course of healing, preventing any trauma to the patient [22].

1.6.2.2 Nanoparticle-mediated drug delivery system

Biofilm-associated iatrogenic and open wound infections are very common. Nearly 80% of human bacterial infections are biofilm-associated. *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Staphylococcus aureus* are among the most prominent causative agents. Lack of well-defined treatment protocol for biofilm-associated infections, contribute significantly to patient morbidity and healthcare costs. Few possible strategies, such as targeting the regulators and eradicating biofilms, have already been investigated [24], but nanoparticle-mediated targeted drug delivery systems have the potential to provide an effective solution to the control of biofilm-related infections. Biofilm-related infections need to be handled with utmost care as negligence, and excessive use of broad-spectrum antibiotics may lead to the emergence of tolerant bacterial species with multi-drug resistance biofilm and excessive antibiotic load on patients' body. These muti-drug resistant biofilms may cause severe health issues and may prove multiple time fatal than recent Coronavirus disease (COVID-19) to humans.

1.7 Research gap

From the above discussion, it is clear that there exists a research gap in the following fields, and it needs to be addressed properly in a scientific way. In particular following aspects need immediate attention:

1. Lack of cost effective biodegradable packaging materials.
2. Lack of antimicrobial packaging to enhance the shelf-life of food products.
3. Lack of bio-absorbable medical dressings to eliminate the need for undue painful dressing removal and discomfort to patients.
4. Lack of dressings for rapid full-thickness anti-scar wounds healing.
5. Lack of a novel solution to treat multi-drug resistance biofilms related infections and minimize antibiotic load on to patient’s body.

The present work was planned with the objective of synthesizing nanofiber-based eco-friendly solution to the above issues utilizing natural biodegradable polymeric nano-composites with antimicrobial characteristics. Nanomaterials of natural origin, curcumin and green synthesized silver nanoparticles (AgNPs) have been blended to enhance the desirable features.

1.8 Objectives

In view of the above following objectives are set for this work and the relevant details pertaining to each objective is presented in different sections of the thesis.

1. To green synthesize AgNPs using medicinal herb *Ocimum tenuiflorum* (Tulsi) and incorporate it into biodegradable polymeric films for active packaging application.

2. To fabricate biodegradable AgNPs impregnated antimicrobial nanofibrous film by electrospinning and assess its suitability for antimicrobial food packaging.

3. To develop bio-absorbable curcumin incorporated biomimetic electrospun nanofibrous scaffold for topical wounds dressing.

4. To carry-out *in vitro* antimicrobial and anti-oxidant evaluations of developed nanofibrous dressing for assessing its suitability for topical wound dressing.

5. To assess the *in vivo* wound healing efficacy of developed nanofibrous scaffold in a full-thickness wound (FTW) for the anti-scar property.

6. To develop a novel biocompatible nanoparticle-mediated drug delivery system for eradication of biofilm-associated infections.

7. To utilize the least amount of antibiotics for bacterial biofilm treatment to avoid the occurrence of multi-drug resistance biofilm-related infections.

To address to these problems and achieve the viable eco-friendly solutions the work was divided into three broad sections, each one is discussed separately in three different chapters of this thesis.

Part I deals with the development of active electrospun composite antimicrobial nano-layers for packaging to inhibit microbial degradation of packaged food and extend its shelf-life in an eco-friendly manner. The fibrous nano-layers can release the active constituents and show antimicrobial activity. The nano-layered packed meat displayed extended shelf-life by one week with better organoleptic quality. The biodegradability of composite packaging makes it a suitable replacement for plastic packaging film.

In Part II, the details pertaining to development of a composite biomimetic, bio-absorbable, nanofibrous wound dressing material loaded with curcumin and cerium ion dual anti-oxidants to help in anti-scar wound healing by protecting the injured tissues from the reactive oxygen species (ROS) and results of its evaluation are presented. It has the potential of preventing microbial infiltration, reducing the moisture and gaseous exchange rates, providing high surface area with a microporous skeletal framework for rapid cell proliferation and granulation. The biocompatibility of nanofibrous scaffold tested *in vivo* in model Wistar rat's wounds by direct application on circular excision F. The dressing got bio-absorbed with healing, thus eliminated its removal and resultant discomfort to the patient during removal.

Part III deals with the problems caused by microbial biofilm such as iatrogenic infections and infection at the open wounded site. A novel Eudragit RL100 encapsulated gentamicin sulfate (E-G-S) nanoparticle-mediated drug delivery system has been developed for effective eradication of biofilm-associated infections in an economical manner. The E-G-S nanoparticle-mediated drug delivery system was found 10-20 times more effective against biofilm-related infections. A lower dose of the drug is needed without exceeding the systemic toxicity value of the drug and preventing possible side effects. It has been shown that Eudragit RL100 nanoparticle-mediated drug delivery system provides a promising way to reduce the cost of treatment.

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