CHAPTER 1: INTRODUCTIONS

1.1. Research Background

Diabetes is a chronic non-communicable disease with a growing prevalence in nearly all regions of the world in the past three decades (WHO, 2017; Harding et al., 2018). Approximately 15% to 25% of diabetic patients will develop a diabetic foot ulcer (Alexiadou & Doupis, 2012) due to many symptoms associated with diabetes, such as cardiovascular diseases, nephropathy, retinopathy, neuropathy, and ischemia (WHO, 2017). This number is more prominent in older and type 2 diabetes patients (Zhang et al., 2016; WHO, 2017). Even though most cases (60-80%) will heal, diabetic wounds exhibit delayed wound closure, possible infections, prolonged inflammation, and the formation of scar tissues (Alexiadou & Doupis, 2012; Carrejo et al., 2018). A more severe case will lead to a lower extremity amputation or death.

The currently available treatment for chronic wounds such as diabetic foot ulcers consists of managing infected areas, wound debridement and cleaning, drug therapy, offloading the ulcer, and applying appropriate dressing materials (Alexiadou & Doupis, 2012; Qin et al., 2018). Modern wound dressing materials have started to gain popularity for chronic wound therapy because they can reduce scar formation, maintain a sterile wound environment, and facilitate a favorable microenvironment for wound healing. One such example of a modern wound dressing would be hydrogels, which are often used in many other biomedical applications (Aswathy et al., 2020). Hydrogels are hydrophilic polymers with a large pore size that can form many 3D architectures resembling native tissues to create a favorable environment for cell attachment and growth (Qin et al., 2018; Li et al., 2020). Furthermore, due to their hydrophilic moieties, hydrogels can swell and store a large volume of water that provides fluid and good hydration, ideal for chronic wounds (Amin & Doupis, 2016; Aswathy et al., 2020). In the experiment, the wound healing properties of hydrogels made from keratin, bacterial cellulose (BC), and tamanu oil are tested in the diabetic mice model.

Keratins are proteins present in the skin, hair, wool, horns, nails, hooves, scales, and many others. For example, keratin derived from human hair has been extensively researched and used as an alternative biomaterial for tissue engineering due to its excellent biocompatibility (Guo et al., 2018; Kim et al., 2019). A study conducted in 2012 by Than and colleagues shows that keratin-based biomaterials were reported to have accelerated wound healing in patients. Furthermore, in vitro and in vivo study shows that keratin hydrogels promote wound closure and migration of keratinocytes (Kim et al., 2019) at the expense of lesser mechanical durability (Azarniya et al., 2019). Thus, keratin hydrogels are often mixed with other polymers to improve their physicomechanical durability and antimicrobial properties.

Bacterial cellulose (BC) is a polysaccharide polymer produced by bacteria that is widely used for hydrogel wound dressing (Gupta et al., 2020). The recent discovery by Azarniya and colleagues in 2019 found that bacterial cellulose improved the elasticity and tensile strength of keratin hydrogels significantly. It also enhances the hydrogel's hydrophilicity and enhances cell adhesion and proliferation in vitro (Azarniya et al., 2019). Furthermore, due to its high purity, BC is considered to have exceptional biocompatibility resembling a natural extracellular matrix, which helps regenerate tissue (Portela et al., 2020).

Tamanu oil is extracted from the seeds of *Calophyllum inophyllum*, which are native to tropical Asia with many coastal regions like Indonesia. The oil is traditionally used as medicine, especially for dermal problems (Raharivelomanana et al., 2018; Urbánková et al., 2019). Bioassays and multiple assessments show that tamanu oil has an excellent antimicrobial activity along with anti-inflammatory and antioxidant properties (Urbánková et al., 2019; Koşarsoy Ağçeli et al., 2021).

Considering these excellent biomaterial properties proposed here, the novel hydrogel scaffold consisting of keratin, BC, and tamanu oil has a promising potential as a modern wound dressing candidate for diabetic patients. Thus, this paper aims to test the effectiveness of the matrix in vivo to evaluate the healing outcome in diabetic mice.

1.2. Research Objectives

The objectives of the research are:

- To evaluate antimicrobial activities of KBC with black tamanu oil
- To produce KBC hydrogels with sufficient physicomechanical properties to be used as a dressing
- To investigate the wound healing activities of dressing in diabetic-induced mice

1.3. Scope of the Research

The scope of work of this research is:

- Keratin extraction and characterization (BCA Assay)
- Synthesis of bacterial cellulose
- Characterization of black tamanu oil (antimicrobial test)
- Fabrication and characterization of the KBC dressing matrix (FT-IR, swelling, and erosion)
- Wound reduction analysis
- Histopathological observation of the skin biopsy

1.4. Research Hypothesis

- Treatment with keratin BC can reduce the wound size significantly and improve the tissue remodeling stage of wound healing.
- The addition of tamanu oil can prevent infection and reduce bacterial growth at the wound site.