CHAPTER I

INTRODUCTION

1.1 Project Background

Wounds are a rising health problem that affects 7.5% (2012) to 8.2% (2013) in Indonesia. Fall-related injuries account for up to 40.9%, followed by 40.6% due to traffic injuries, which can affect any age group (Fauziah & Soniya, 2020). All wounds are expected to heal within 6 weeks, but all acute wounds have potential to become a non-healing wound which may never heal (Sood *et al.*, 2014). Approximately, 2.21 up to 10 people in a thousand of general population developed chronic wound from acute wound. Lanau-Roig *et al.* (2017) mentioned 95% of them are mostly venous leg ulcers, ischemic, or pressure ulcers and it accounts for increased morbidity, mortality, and decreased quality of life which may persist for months to years (Martinengo *et al.*, 2018).

Wounds, especially non-healing ulcers, require intensive management to alleviate the wound burden and optimize wound re-epithelialization (Moura *et al.*, 2013). However, the development of wound care dressing in Indonesia is limited because it is not covered by national health insurance. The limited wound dressing products available in Indonesia leaving an excellent opportunity for the development of wound management, primarily because of the high wound dressing market that reaches 7.1 billion USD and is projected to reach 11.3 billion USD by 2025 (Research and Markets, 2020; Wibisono *et al.*, 2018). There are currently many types of wound dressing available in the market, but each type has its own unique and there is no single ideal dressing for all wound types. However, an ideal wound dressing should promote moist wound environment, granulation, autolytic processes, angiogenesis, and reduce the risk of infection (Everett & Mathioudakis, 2018; Hilton *et al.*, 2004).

Recently, several studies have utilized various formulations to form novel wound dressing, such as biocellulose (BC). BC is a product of Gram-negative bacteria in a sugar-rich environment. It can be obtained from Kombucha fermentation on molasses, which is less expensive compared to plant cellulose. Other than that, the BC is also pure and possesses finer network structure, that makes the BC has better physicochemical properties and biocompatibility compared to plant cellulose (Lin *et al.*, 2013). Based on a previous study by Kucińska-Lipka *et al.* (2015), BC provides a constantly moist environment due to its swelling behavior and water retention capacity. These properties are needed for cell growth and wound healing. However, this material is biologically inactive thus exhibiting insufficient wound healing properties and antimicrobial activity. Hence, further improvement of this material is needed. Fortunately, due to the highly porous structure and water-absorbing property of BC, it is possible to incorporate and entrap compounds and materials that may improve wound healing properties and antimicrobial activity.

Several protein-based materials have been studied for the purpose of assisting wound healing and regeneration. Among those, keratin has received increased interest recently due to their abundance and biocompatibility. Keratin-based biomaterial can be easily found on hair. This material is biocompatible and able to form many applications, including enhanced wound healing and regeneration. Keratin from human hair possesses leucine-aspartic acid-valine (LDV), the cell-binding motif, which can bind $\alpha_4\beta_1$ that is expressed in cells such as fibroblast and macrophages in response to injury. This interaction promotes thrombus formation, as well as cellular attachment, infiltration, and scar maturation (Amersi *et al.*, 2003; Bachman *et al.*, 2015; Fujita *et al.*, 2020; Rouse & Van Dyke, 2010). These activities are suitable for accelerating wound healing.

As mentioned earlier, the BC lacks antimicrobial properties, the addition of tamanu extract might enhance the dressing. Tamanu oil is a local natural product obtained from nuts

of *Calophyllum inophyllum*. This ingredient can be potentially used for wound healing because it was proven to have callophyllolide, which is able to regulate cytokine production, increase keratinocytes and dermal fibroblast production, as well as wound healing activity. The callophyllolide has shown the ability to reduce myeloperoxidase (MPO), negatively affects pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α), yet upregulates anti-inflammatory cytokine (IL-10), and induce macrophage phenotype switching from M1 to M2. Moreover, tamanu oil contains calophyllolide, inophyllum C, and inophyllum E, which possess antimicrobial activity including antibacterial and antifungal properties, especially towards skin pathogen (Ansel *et al.*, 2016; Léguillier *et al.*, 2015; Raharivelomanana *et al.*, 2018). By utilizing the moist wound environment provided by BC, faster wound healing properties of keratin, and antimicrobial activity of tamanu, this project is aimed to evaluate wound healing capabilities of keratin incorporated on BC hydrogels and tamanu oil.

1.2 Research Objectives

Objectives of this research are:

- To develop biocellulose (BC) hydrogels with the incorporation of keratin extract
- To investigate the effect of keratin-BC (KBC) hydrogels and tamanu oil as wound dressing by assessing full-thickness wound reduction, as well as evaluate tissue remodeling using histological study

1.3 Research Hypotheses

Hypotheses of this research are:

- A wound dressing with acceptable physicomechanical properties observed from swelling and erosion activity, as well as chemical properties from fourier transform-infrared (FTIR) spectra were obtained

- A significant difference of wound healing results will be observed on mice treated with KBC with tamanu oil. These results will be observed based on increase of wound closure, tissue remodeling on histology result, and antimicrobiological study

1.4 Research Scope

The scopes of this research include:

- Keratin extraction from human hair
- Fabrication of BC with keratin and characterization of KBC hydrogel and tamanu oil
- *In vivo* wound healing study by assessing full-thickness microbial wound swab analysis, wound closure, and histological study in mice model