Chapter 1

Introduction

1.1 Background

Sharp objects, changes in temperature, chemicals, radiation, or animal bite trauma could cause tissue damage. This condition is called a wound (Ariani et al., 2014). Wounds could appear on both the exterior and interior parts of the body. On the external surface, the damage might be located in the epithelial tissue and connective tissue within the skin, causing the loss of the epithelial layer which is normally referred to as a 'wound'. On the other hand, a bruise is an example of a wound that is located inside the body (Dunnill et al., 2015; Prasetyono, 2009).

Wounds require some time to be healed. Generally, it will take about 30 days to completely heal acute wounds, although the wound conditions might alter the rapidity of the healing. In contrast, chronic wounds do not have a specific healing time frame, as the patient's underlying conditions might disrupt the process of healing (Dunnill et al., 2015). Nonetheless, it is important that proper wound care is applied to both conditions. Individuals could experience a minimal to large impact on their quality of life (QoL) due to the presence of wounds on or in their bodies. A patient might face disturbances in everyday activities such as sleeping or limited mobility due to pain, and avoid social interaction and contact with others (International Consensus, 2012). Additionally, infection might take place in wounds, causing impairment and delay in the wound healing process, and eventually mortality if it worsens significantly. Hence, good and effective care for wounds is needed to improve the patient's condition and QoL (Dunnill et al., 2015; Lindholm & Searle, 2016; Teixeira et al., 2020).

One of the ways to manage wounds is by using wound dressing. Wound dressing could provide protection and optimum healing conditions for the wound, especially a moist condition that is ideal for wound healing (Teixeira et al., 2020; Dwiyana et al., 2019). A number of materials could be used as wound dressing, including biocellulose (BC), carbopol dressing, hydrogel dressing, and many more

(Chvapil et al., 1991). Among those, BC, a polymer product from bacteria in the form of biofilm, showed great promise to be further utilized and studied as a wound dressing. It provides advantageous properties as a wound dressing to protect the tissue, and is able to promote tissue regeneration due to the presence of moisture (Portela et al., 2019; Dwiyana et al., 2019). Additionally, BC possesses suitable characteristics of a good wound dressing and is relatively inert (Portela et al., 2019; Rauchfuß et al., 2019). However, due to its inert nature, BC has limited activity in promoting tissue remodeling. Hence, reinforcement of BC with other bioactive materials that provide support for tissue remodelling and regeneration would be advantageous. This mechanism has been confirmed by a previous study (Chvapil et al., 1991).

One of the beneficial bioactive materials for wound healing is keratin. Keratin is a crucial structural protein for preserving the structural tissues' durability and is mentioned to play a major part in the wound healing process (Konop et al., 2021; Than et al., 2012). A previous *in vivo* study mentioned that mice treated with keratin-hydrogel experienced accelerated epithelialization and remodeling rate compared to the negative control by two folds (Konop et al., 2021). Hence, keratin might help to speed up the wound healing process when combined with BC.

Addition of an antibacterial agent into the wound dressing could increase the overall wound healing activity. Antibacterial agents could prevent the penetration of bacteria into the wound, and subsequently hinder microbial growth (Simões et al., 2018). Both synthetic antibiotics and medicinal plants containing antibacterial properties could be utilized for this function. However, due to the extensive use of synthetic antibiotics in the past years, the latter is more preferred as an alternative to prevent cross-resistance (Zheng et al., 2020). Black tamanu (BT), *Calophyllum inophyllum* L., was proven to exert antibacterial properties, as well as other pharmacological activities, including antioxidant and anti-inflammatory, which together are able to help with wound healing. A previous study mentioned that tamanu oil was able to produce bactericidal effects against several skin

bacterias and boost the wound-healing process in both fibroblast cells (HMDF) and keratinocyte cells (HaCaT) via *in vitro* study (Raharivelomanana et al., 2018).

To understand how these combinations would perform, an *in vivo* study was performed. The current study is a continuation from a previous study using mice models. Prior to planning clinical trials in humans, interspecies study using other animals are preferred to ensure the safety and efficacy of the treatment (Nowak-Imialek et al., 2020). Hence, the study was continued using a rabbit model. Rabbit possesses desirable characteristics for laboratory study, namely good temperaments, ease of handling, longer life span compared to several laboratory animals, convenient size, and has more similar characteristics to humans. Additionally, previous studies successfully used rabbits for wound healing study, increasing the probability of success to observe the healing activity in the present study (Ravishankar et al., 2019; Ariani et al., 2014; Al-Ahmad et al., 2020; Colby et al., 2019). In the current study, the rabbits were wounded and treated using BT and keratin-biocellulose (kBc) combinations. Following that, the wound healing and antibacterial activity in the treated rabbit were assessed.

1.2 Objectives

This study aimed to assess the wound healing and antibacterial activity of black tamanu keratin biocellulose through an *in vivo* study on rabbit model.

1.3 Hypothesis

The hypothesis of this research includes:

1. Keratin biocellulose dressing with tamanu oil would provide an improved wound healing period through the measurement of wound size reduction and histology observation of regenerated skin tissue.
2. Wounds treated with black tamanu oil would have the lowest bacterial count compared to the other treatments.