CHAPTER 1

INTRODUCTION

1.1 Project Background

Over the past decades, experts have recognized that 2D cell cultures do not represent in vivo structures due to their architecture and functional differentiation (Simian & Bissell., 2017). With the advancing technology and modern knowledge, many new 3D in vitro models have been developed. Organoids are 3D multicellular in vitro tissue that provides the possibility to perform mechanistic studies of the human system with justifiable ethical concerns. This new in vitro culture technology mimics the corresponding in vivo organs and allows the generation of cultured cells under controlled conditions in 3D structures (Lehhmann et al., 2019). These in vitro 3D cellular cells are obtained from many sources such as primary tissue, embryonic stem cells, or induced pluripotent stem cells that has the ability to undergo self-renewal, self-organization, and manifesting similar organ functionality from the derived tissue (Fatehullah et al., 2016).

The development of organoids has significantly contributed to the study of human disease and development since these are challenging when studied using animal models. Human organoids have the potency to provide a more inexpensive and precise preclinical setting for drug discovery in the future (Simian & Bissell., 2017). Lately, the growing interest in functional biomaterials, especially hydrogels, in constructing physiologically relevant 3D tissues with distinguished properties has caused a major scientific breakthrough. Due to the highly hydrated nature of hydrogels, organoids are often cultured in 3D hydrogel systems (Giobbe et al., 2019). High water content in hydrogels can imitate the native Extracellular Matrix (ECM microenvironment because of their high biocompatibility and flexible properties (Liu et al., 2019).

In the recent past, keratin has received increased interest due to its ability in self-assembly hence making it possible to make a three-dimensional matrix. In addition, keratin can assist in cell attachment. Keratin was shown to be able to support fibroblast growth, proliferation, and ECM production. Specifically, human hair keratin also possesses LDV (Leu-Asp-Val) cell-binding motifs that could support cell attachment through integrin α4β1 (Hartrianti et al., 2015). Another thing that makes keratin such an outstanding biomaterial is that it is stable, insoluble, and highly disulfide-bonded that is normally disregarded by common proteases. So far, only keratinases are able to split the peptide bonds in keratin (Navone & Speight., 2018). The high disulfide bonds in keratin are due to abundant cysteine residues which make the inter and intramolecular disulfide bonds when oxidized. This results in a 3D-linked network of keratin fiber and this distinct structure are the reason for its relatively poor mechanical properties (Tran & Mututuvari., 2016). On account of the high cysteine content, the dissolution and extraction of keratin have become a challenge. Harsh chemical conditions are necessary for its extraction. These would cause the disruption of the strong α-helix structure, thus resulting in poor mechanical properties (Donato et al., 2019). There are some common methods used for keratin extraction - oxidation, reduction, seam explosion, microbial method, microwave irradiation, and the use of ionic liquids (Feroz et al., 2020).

Experts have been conducting experiments to further improve the mechanical properties of keratin. There are many ways to improve its mechanical properties. Keratin is sometimes compressed into films, made into a gel, into patches, nanofibers, or hydrogels. Despite all the methods, they usually crosslink with chemicals. For example; they found that when keratin films are crosslinked with glutaraldehyde, it produces keratin films with the smoothest surface (Thonpho et al., 2016). Until the present time, the best way to enhance keratin's mechanical properties and also preserve its biological properties is by incorporating various natural and/or synthetic polymers. Incorporating keratin with other materials is by far the

best way in improving its mechanical strength. This way, keratin-based materials are one of the most familiar biodegradable natural polymers due to their many unique properties.

In order to tackle the limitations of keratin, several materials have been incorporated into keratin-based biomaterials so its physical strength can be optimized. There are two types of polymers that are commonly used - natural and synthetic. Primarily, the addition of chitosan into keratin-based biomaterials has been seen to increase its mechanical strength and the upside of chitosan is that it has antibacterial activity, high biocompatibility, and biological function that is suitable for wound healing (Lin et al., 2018). The next common natural polymer used is silk fibroin. Keratin scaffolds with silk fibroin, gelatin, and calcium peroxide were able to produce a scaffold that can release a high level of oxygen over to weeks in vitro and fasten the repair of urethral defect animal models (Lv et al., 2016). Another common natural polymer used is alginate; keratin-alginate sponges are not only physically better but are porous, flexible, well accommodating in vitro, and biocompatible enough in vivo (Hartrianti et al., 2016).

Pectin is another of the most frequently explored natural material that is also commonly used in combination with synthetic polymers and cellulose as a wound dressing (Mir et al., 2018). Pectin is non-toxic, has excellent biodegradability and biocompatibility properties. Pectin, like alginate and chitosan, is widely studied and used in various industries. Due to its antimicrobial, antiviral, water solubility, and good mechanical properties, It is commonly used in food or drug packaging, antimicrobial films, and coatings. Unlike chitosan and alginate, it does dissolute or shrink in low pH, for example, the gastric environment but instead pectin has a quite good pH sensitivity that can be formulated and optimized as needed (Martău et al., 2019). Pectin is a polysaccharide largely composed of galacturonic and it is the component of the plant cell wall (Espinoza et al., 2018). This natural polysaccharide carries a linear chain of (1-4)-linked- α-D-galacturonic acid residues with

carboxyl groups (Chatterjee et al., 2017). Due to the limitations of each polymer, the wound dressing matrix of pectin and human hair keratin mixture will be fabricated with the hope of increasing the mixture's mechanical strength while making it more bioactive.

To confirm the strength and efficacy of the keratin-pectin hydrogels, 3D culture will be done. Even though testing the compatibility of the matrix on a 2D-coated surface is simpler, low-cost maintenance and can increase the understanding of cells and matrix interaction, enhance the understanding of its mechanism of action, 2D cell culture is not highly preferred because it does not mimic the natural structure of the tissue, lacks cell-cell, and cell-extracellular environment interactions and it tends to change the morphology of cells causing the loss of various phenotypes and polarity (Kapałczyńska et al., 2018). On the other hand, with a similar principle, 3D cell culture allows cells to grow on a scaffold; pectin-keratin hydrogel matrix. Scaffold/matrix-based 3D culture can be initiated by normal cell seeding or by dispersing cells in a liquid-form matrix and accompanied by solidification or polymerization. This causes the proliferation rate in the 3D cell culture to show a reduced proliferation rate from that of the 2D cell culture (Edmondson et al., 2014). Tedious as it seems, 3D cell culture has advantages that outweigh the 2D cell culture method. The pronounced advantage would be the expression of ECM and cell-cell and cell-matrix interactions. Furthermore, the utilization of ECM material by default promotes cells to form 3D spheroids. These spheroids give an important characteristic which resembles that of in vivo cells (Chaicharoenaudomrung et al., 2019). Organoids made from hydrogels can be used in many usage one of them being as a wound dressing. In this study, keratin-based hydrogel would be incorporated with pectin polymer and results would be compared to the pectin-only hydrogel and collagen-pectin hydrogel. These groups of hydrogels would act as the negative and positive control groups respectively.

1.2 Research Objective

The objectives of this project are listed as follows:

- To extract and characterize keratin from human hair through various characterization methods
- To fabricate keratin-based hydrogels and evaluate the physicochemical properties of fabricated hydrogels made up of keratin-based material combined with pectin
- To study the cell compatibility and histopathology of the in vitro three-dimensional cell culture of HaCaT and NIH3T3 in the fabricated hydrogels

1.3 Research Hypothesis

The Hypothesis is listed as follows:

- Keratin can successfully be extracted from human hair using Shindai Method
- Fabricated keratin-based hydrogel shows good mechanical characteristics
- Immortalized cells such as HaCaT and NIH3T3 Fibroblast cells show better growth and penetration in the keratin-based hydrogel when compared to the (Collagen-Pectin) positive control and (pectin-only) negative control.

1.4 Research Scope

The research will focus on the cell culture where the interaction between the cells and the fabricated 3D environment occurs. The specific scope of work in the experiment is listed as follows:

- Extraction of keratin from human hair through a method called the Shindai Method
- Characterization of extracted keratin from human hair
    - Protein separation and analysis by SDS-PAGE method
    - Protein quantification through Bradford assay
    - Compound identification through Fourier transform infrared (FTIR)
- Fabrication and characterization of hydrogels
- Cell compatibility and histopathology

- In vitro three-dimensional cell culture of HaCaT and NIH/3T3 in hydrogels
- Histology study