CHAPTER I

INTRODUCTION

1. Background

Recently, natural polymers have been studied as biotechnological and biomedical resources due to their unique properties including nontoxicity, biodegradability, and biocompatibility. Various natural polymers have been utilized as cell support scaffolds for the creation of a dermal replacement [1]. Generally, there are three types of dermal replacement reported in the literature. The first one is the use of cryopreserved cadaver skin of which the epidermis is removed. However, immunological reactions with the epithelial components of these dermal allografts and possible inflection from donor skin have not been resolved [2]. The second type of dermal substitute, first reported by Burke et al. [3], is a synthetic dermal matrix consisting of a cross-linked bovine collagen matrix covered with a silicone membrane. Neo-vascularization of this dermal substitute has been observed, but the resultant tissue resembles granulation tissue rather than normal dermis and reconstituted collagen matrices do not posses the exact three dimensional structures. The third type employs an acellular dermal matrix (ADM) or acellular dermis derived from full thickness skin treated to remove epithelial and dermal components [4-6].

Acellular dermis is a natural scaffold, which has been widely used as dermal replacement. It is produced from cadaver skin or animal skin such as porcine, rat, and bovine skin. Due to limited supply of cadaver skin, porcine skin is widely used to produce ADM for biomedical applications. The similarity to human skin, the mature collagen bundles, and the porous nature of porcine dermis are all favorable features for a potential dermal substitute [7].

Biologic scaffolds derived from decellularized tissues and organs have been successfully used in both pre-clinical animal studies and human clinical applications. The efficiency of cell removal from tissues is dependent on the origin of tissues and specific methods used. Each treatment affects the biochemical composition, tissue ultra structure, and mechanical behavior of remaining extracellular matrix (ECM) scaffold [8]. Various methods have been reported in order to remove the immunogenic cellular constituents. The physical treatments such as freeze-thawing method can be used to remove cellular component, but these treatments are generally insufficient to achieve complete decellularization [9]. Enzymatic technique of decellularization such as trypsin is one of the most commonly used. Oliver et al. [10] reported that trypsin-treated xenogeneic dermis was not rejected upon transplantation. Their method, however, required a lengthy incubation with trypsin (2-28 days at room temperature) resulting in collagen disruption. Walter et al. [11] reported that dispase-Triton treatment was more efficient to remove cell than NaCl-SDS treatment. However, ADM components are generally more abundant in NaCl-SDS ADM than Dispase-Triton ADM. The former may more readily support cell attachment and proliferation. A cell removal process on xenogeneic such as porcine skin was reported by Chen et al. [12]. Trypsin, dispase II, and SDS solution were utilized in a decellularized method. They claimed that cells could be completely removed, qualitatively observed from hematoxylin and eosin (H&E). However, their method was complex and a lengthy incubation of both enzymes was required.

In this study, the protocol of decellularized was developed to produce ADM from porcine skin. The decellularized process by enzyme was employed incorporation with a periodic pressurized technique in order to enhance the efficiency of cell removal and reduce time of enzyme incubation. The effects of enzyme type (trypsin and dispase II) and pressurized period conditions on the efficiency of cell removal and characteristics of ADM were investigated.

2. Objectives

To develop a decellularized protocol for producing acellular dermis from porcine skin and characterize the obtained acellular dermis as a dermal substitute.

3. Scope of works

1. Preparation of acellular porcine dermis by enzymatic treatment. Parameter to be studied are:
   1.1 Conditions for incorporating periodic pressurized technique to enzymatic treatment: period time, number of periods
   1.2 Type of enzyme: trypsin and dispase II
2. Characterization of acellular porcine dermis including:
   2.1 Fat content determination
   2.2 Cell removal by DNA assay
   2.3 Surface morphology by SEM
   2.4 Histological examination
   2.5 Fourier Transform Raman (FT-Raman) spectroscopy
3. Animal study of acellular porcine dermis.
   Biocompatibility (immunological response) and cell infiltration into ADM are observed.