CHAPTER I

INTRODUCTION

1.1 Problem and Background

Every year, there are several severe burned patients who suffer from the injuries. From the report of National Institute of General Medical Science, USA has to spend 1.25 million dollar for medical instruments to cure burned victims. Third-degree burns or an extensive full-thickness burn (>50% TBSA) by definition destroy of both epidermis and dermis, necessitating skin replacement [1]. Estimates for hospitalizations from burns range from 60,000 to 80,000 annually and costs for recovery from burns range from acute injuries range from US$ 36,000 to 117,000 per patient [2]. Traditionally, we can use 3 skin replacement sources; cadaver skin (allograft), animal skin (xenograft), and owner skin (autograft).

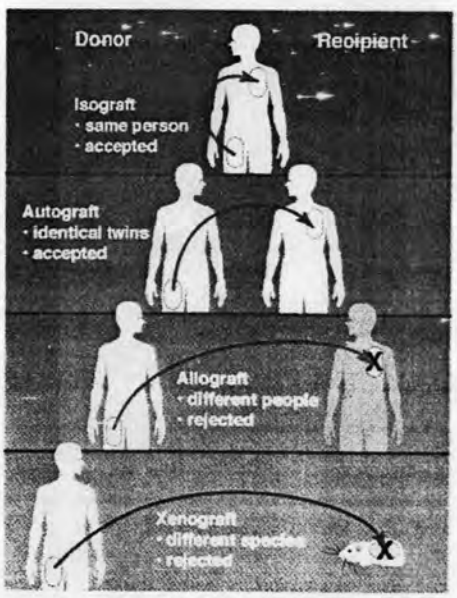

Figure 1.1: Sources of skin replacement [20]

These sources have been studied due to their properties including nontoxicity, biodegradability and biocompatibility. Because of the high mortality among patients who lose a large amount of skin, skin substitute is needed. Synthetic skin substitute constructed by biocompatible polymer which cells can migrate and establish their

extracelluar matrix (ECM), has been developed. These synthetic polymer scaffolds are strong, can be fabricated to degrade at predetermined rate and can be designed to mimic the material properties of the native tissue they are designed to replace. While these characteristics are appealing, several clinical complications are often encounters. But natural source of scaffold include small intestine submucosa, acellular dermis, cadaveric fascia or amniotic membrane also offered alternatives to synthetically engineered polymeric scaffold for tissue regeneration. With the naturally-occurring framework scaffold retain a structure and composition nearly identical to their native state. The host cells are removed and the scaffolds are used acellularly to replace damaged tissues [3].

Tissue engineering is a new emerging interdisciplinary field of study which intended to assist the regeneration of body tissue defects too large to self-repair as well as to substitute for the biological functions of damaged and injured organs by using cells with proliferated and differentiated potential. In addition to basic research on such cells, it is undoubtedly indispensable for successful tissue engineering to create an artificial environment enabling cells to induce tissue regeneration. Such an environment can be achieved by making use of a scaffold [4]. The artificial three-dimension scaffold, which made of biodegradable synthetic or natural polymer, was transported into the wound site where the cell can replicate, reorganize and form new tissue. At the same time, the artificial polymers gradually break down, leaving only a completely natural final product in the body. Scaffold for tissue regeneration has to be non-toxic, sterilizable, reproducibly manufactured and have a long shelf life.

Skin tissue engineering is one of the most popular for studying because skin is the largest organ in the body and can be easy to investigate the result. Full-thickness skin lose can cause skin defection such as scar formation, skin contraction which make the whole body out of work. The aims of skin substitution are to regenerate the novel skin without scar formation and to accelerate the wound healing process without any infections. The main function of skin substitution is to mimic as an extracelluar matrix (ECM) in dermis to reduce the novel formation of extracelluar matrix by migrated cell surrounding the wound. By using skin substitution, migrated cells can perform their biological roles suddenly when they migrate into the naturally

scaffold. They will not try to create new extracellular matrix or new tissue structure in the wrong direction which can cause the scar formation. The migrated cells can secrete the cytokines or growth factor to induce the tissue regeneration. There are a lot of commercial skin substituted products including that made of synthetic or naturally biodegradable polymer, and naturally-occurring scaffolds.

Engineered skin substitutes*

Trademark name	Source	"Dermis"	"Epidermis"
EpiCel™	Genzyme Tissue Repair	Allodermis	Cultured auto HK
Integra™	Integra Life Sciences	Collagen-GAG & silicone	Thin autograft
AlloDerm™	LifeCell Corporation	Acellular dermal matrix	Thin autograft
DermaGraft™	Advanced Tissue Sciences	PGA/PLA+allo HF	Thin autograft
n/a	Univ Cincinnati/Shriners	Collagen-GAG+auto HF	Cultured auto HK
LaserSkin™	Fidia Biopolymers (Italy)	Hayluronic acid	Cultured auto HK
PolyActive™	HC Implants (The Netherlands)	PEO PBT+auto HF	Cultured auto HK
ApliGraf™	Organogenesis, Inc.	Collagen gel+allo HF	Cultured allo HK
ORCEL™	Ortec International, Inc.	Collagen + allo HF	Cultured allo HK
TransCyte™	Advanced Tissue Sciences	Allo HF	BioBrane™

* The list of products is presented as neither all-inclusive, exclusive, or an endorsement.

Figure 1.2: Engineered skin substitutes in commercial products [2]

Acellular dermis is considered to be one of the basic tissue engineering products. The efficacy of acellular dermal matrix in the treatment of full-thickness skin injuries as a dermal substitute depends on its low antigenicity, capacity for rapid vascularization and stability as a dermal template [5]. There are several sources of dermis to be fabricated into acellular dermis such as porcine skin, small intestine submucosa, amniotic membrane tissue, and cadaver skin. These scaffolds will play role as cell growth supports to provide a conductive environment for normal cellular growth, differentiation, and angiogenesis.

Human cadaver skin is biological scaffold derived from deacellularized human skin have been successfully used in both pre-clinical animal studies and human clinical applications. The efficiency of cell removal from tissue is dependent on the origin of tissues and specific method used. Each treatment affects the biochemical composition, tissue ultra structure and mechanical behavior of remaining extracellular matrix [6]. Many researchers have explored the uses of human acellular dermis. They have demonstrated that acellular dermis retains many of its structural elements after processing, is durable, and can be lyophilized and stored at room temperature [7].

In this study, we studied human acellular dermis (hADM) which was prepared by protocol of Ghosh MM. (1997) compared with human de-epidermalized dermis (hDEM) which prepared by protocol of Ghosh MM. (1997) without de-cellularization process [8-10]. We want to examine the effect of enzymatic and chemical treatment to micro-construction of collagen framework in dermis.

1.2 Objective

To study human acellular dermis (hADM) and compare with human de-epidermized dermis (hDED) *in vivo*

1.3 Scope of works

1.3.1 Prepare human acellular dermis (hADM) by standard protocol and human de-epidermized dermis (hDED) without de-cellularalization process

1.3.2 Characterization of human acellular dermis (hADM) and human de-epidermized dermis (hDED) by

1.3.2.1 Histology of raw material: H&E staining

1.3.2.2 Confocal study: collagen type I

1.3.3. *In vivo* study of human acellular dermis (hADM) and human de-epidermized dermis (hDED) by

1.3.3.1 Subcutaneous implantation on the back of Wistar rat

1.3.3.2 Histology: H&E staining

1.3.3.3 Confocal study: collagen type I