CHAPTER V

DISCUSSION

Decellularization method has been identified as a potential method to produce acellular scaffold. Several detergents and enzymes were used to remove cell and cellular debris from natural animal tissue while leaving intact structural protein scaffold. This decellularization procedure has been shown to reduce major histocompatability complex Class I and Class II molecules to undetectable levels. The efficacy of acellular dermial matrix in the treatment of full-thickness skin injuries as a dermal substitute depends on three important properties: its low antigenicity, the capacity for rapid vascularization, and stability as a dermal template. Earlier studies employed several methods for producing ADM from skin including treatment with trypsin, freeze-thawing, and enzymes act upon the dermis [5]. In this study, trypsin was used in acellularization process and Sodium dodecyl sufate (SDS), which has detergent property, was used in the process of cells debris removal in hADM.

In H&E staining before implantation, epidermis layer was absent in both hDED and hADM. We found remnant nucleus stained of fibroblast cells integrated between old-collagen fibers in hDED but fibroblast cells were rarely found in hADM. This indicated that enzymatic treatment could partly wash out attached fibroblast cells in collagen bundles. hADM collagen fibers were shorter and thinner than in hDED which still retained the dense and large collagen fibers associated with the result of confocal microscopic pictures that revealed more retocilated collagen bundles in hADM than in hDED.

As the evident shown in *in vivo* study at 1st week of implantation, we found a number of fibroblast cells infiltration around the specimen, neo-capillaries with red blood cells distribution, new-extracellular matrix formation in dermal matrix of. In contrast to, hADM which had a large amount of round clumped lymphocyte cells between collagen bundles. None of new-extracellular matrix was formed between collagen bundles. New-extracellular matrix was reported to have

a major effect on the healing process. Additional extracellular matrix components were documented to enhance wound healing [24]. They also serve many functions including the provision of structural support and tensile strength, attachment sites for cell surface receptors, and as a reservoir for signaling factors that modulate such diverse host processes as angiogenesis and vascularization, cell migration, cell proliferation and orientation, inflammation, immune responsiveness and wound healing [25]. Also in confocal microscopic pictures of 1st week, the stained collagen fibers of hDED specimen seemed larger and thicker but in hADM collagen fibers still liked fibrous structure

Angiogenesis and vascularization, which were obviously found in the gross- specimens of the 1st week, were an evident of provision phase in the 1st week of wound healing process as shown in Figure 4.5 a-c. Provision phase, which major contained of fibronectin, played an important role in wound healing to provide adhesion ligand promoting fibroblast infiltration into the wound [23].

In H&E staining of 2nd and 4th week, as shown in Figure 4.12 and 4.17 we found a numbers of migrated and differentiated fibroblast cells obviously increased. New extracellular matrix and new collagen were clearer. In contrast with in hADM, there were only numerous lymphocytes which migrated into the specimen. Most of lymphocytes were interstitial between collagen bundles. Severe foreign body reaction occurred in the 1st week and prolonged in 2nd and 4th week.

As illustrated in Figure 4.14 and 4.19, the architecture of collagen in hDED were more reticulated network with porous than hADM, which were dense and compacted structure.

In conclusion, hDED could induce new dermis regeneration and better in promoting infiltration, migration, and differentiation of fibroblast cells. New extracellular matrix deposit and neo-collagen formation were observed. The orientation of neo-collagen networks were recticular network. hADM had severe foreign body reaction and did not have new extracellular matrix and neo-collagen formation. hDED had low foreign body reaction and retained biological properties

for cell migration and adhesion better than hADM which were lost biological properties by decllularization process. Fibroblast cells which left inside collagen bundle of hDED didn't affect a potential to form neo-collagen. This study proofed that biological of collagen fibers was more important than de-cellularization process in producing of human de-epidermized dermis and human acellular dermis.