INTRODUCTION

Skin grafts are required in numerous clinical procedures, such as reconstruction after skin removal, and correction of contracture or scarring because of burns, accidents, and trauma. The current standard for skin defect replacement procedures is the use of autologous skin grafts. However, donor-site tissue availability, which remains a major obstacle to the successful replacement of skin defects, often limits this option. Because of this limitation, other approaches are commonly employed to cover skin defects. These include commercially available skin products based on biomaterials and tissue engineering, allografts, and xenografts [1-3].

In addition, in vivo subcutaneous tissue expanders or meshed split thickness skin grafts are being used clinically to generate larger segments of autologous skin when donor-site tissue is limited. Subcutaneous tissue expanders are balloon implants that are sequentially filled with increasing amounts of saline to increase the amount of overlying skin. The mechanical stress of the tissue expander results in biologic creep, greater mitotic activity, and greater vascularity, which ultimately leads to expanded skin. Subsequently, the expanded skin can be used as a tissue flap or harvested for use as a skin graft. However, the shortcomings of this method lie in the requirement of additional surgical procedures, which may increase morbidity, and extensive waiting times to obtain appropriate sized tissue. Moreover, discom-
fort associated with the increasing expander volume and frequent tissue fibrosis remains as major limitations [4-14]. Currently, numerous reports supported that stem cells and their production have a great potential for accelerating wound healing and regeneration of damaged skin, however these approaches need further research to obtain healthy full skin for transplant [15-17].

In the present study, we investigated whether these difficulties could be overcome by developing an automated skin bioreactor which enables effective skin expansion ex vivo while maintaining tissue viability. In 2009, Ladd et al. [18] at the Wake Forest University in the United States reported the first full skin expansion using a computer-controlled bioreactor system, which resulted in skin expansion of 110.7% under culture conditions for 7 days. Following the work, Jeong et al. [19] in Korea published a paper in 2014 on a clinical trial with ten patients for investigation of whether tissue expansion using a bioreactor is effective and safe for reconstruction of large skin lesions and scars. The findings are average skin expansion rate of 10.54±6.25% for 10 days; take rate of 88.89±11.39%; and contraction rate of 4.2±2.28% after 6 months, which confirmed the safety and applicability of the ex vivo skin expansion.

We developed an advanced bioreactor system by adding functions of real time monitoring, remote-control, optimized grip structure, and creating pores to skin for efficient tissue expansion. We evaluated the morphological, ultra-structural, and mechanical properties of the expanded skin before and after expansion using histology, immunohistochemistry (IHC), and tensile testing. We further carried out grafting study in vivo using Yucatan pigs to investigate the feasibility of this method for clinical application.

MATERIALS AND METHODS

Bioreactor components

The system consists of three parts and additional components; the culture chamber, environment, and expansion control parts and components including skin grippers and expansion clamps. The skin is expanded in the chamber with culture medium and its temperature is maintained approximately 37°C by circulating water around the chamber using environment controller. The grippers are designed to hold the skin using micro-needles in order to avoid the skin necrosis due to excessive pressure. The grippers are connected to clamps which play a role for expanding skin under designated tension (Fig. 1).

Expansion study

Porcine full-thickness skins were obtained from local slaughterhouse (Farm pig, Yeongcheon, Korea). The skins were submerged in 70% EtOH for 10 sec, and washed with sterile phosphate buffered saline (PBS). The skin was expanded by 200 kPa of pneumatic pressure in Dulbecco’s modified eagle’s medium containing 10% fetal bovine serum and 2% penicillin-streptomycin for 4 hrs. Real time monitoring of the expanding skin for entire 4 hrs was carried out using IP camera. The area of the skin was calculated by Image J software (National Institute of Health, USA).

Grafting study

To evaluate the grafting efficiency of expanded skins, we used two female Yucatan pigs after getting approval from the Institutional Animal Care and Use Committee at DGMIF (Daegu-Gyeongbuk Medical Innovation Foundation, Daegu, Korea). For donor skin, the inguinal one was used. Full-thickness skin (5×5 cm) was obtained under anesthesia and subse-