Full Thickness Skin Expansion *ex vivo* in a Newly Developed Reactor and Evaluation of Auto-Grafting Efficiency of the Expanded Skin Using Yucatan Pig Model

Man-Il Huh^{1,2} · Soo-Jin Yi^{1,2} · Kyung-Pil Lee^{1,2} · Hong Kyun Kim^{1,2} · Sang-Hyun An³ · Dan-Bi Kim³ · Rae-Hyung Ryu³ · Jun-Sik Kim³ · Jeong Ok Lim¹

Received: 6 July 2018 / Revised: 6 August 2018 / Accepted: 7 August 2018 / Published online: 1 September 2018

© The Korean Tissue Engineering and Regenerative Medicine Society and Springer Science+Business Media B.V., part of Springer Nature 2018

Abstract

BACKGROUND: Skin grafts are required in numerous clinical procedures, such as reconstruction after skin removal and correction of contracture or scarring after severe skin loss caused by burns, accidents, and trauma. The current standard for skin defect replacement procedures is the use of autologous skin grafts. However, donor-site tissue availability remains a major obstacle for the successful replacement of skin defects and often limits this option. The aim of this study is to effectively expand full thickness skin to clinically useful size using an automated skin reactor and evaluate auto grafting efficiency of the expanded skin using Yucatan female pigs.

METHODS: We developed an automated bioreactor system with the functions of real-time monitoring and remote-control, optimization of grip, and induction of skin porosity for effective tissue expansion. We evaluated the morphological, ultra-structural, and mechanical properties of the expanded skin before and after expansion using histology, immunohistochemistry, and tensile testing. We further carried out *in vivo* grafting study using Yucatan pigs to investigate the feasibility of this method in clinical application.

RESULTS: The results showed an average expansion rate of 180%. The histological findings indicated that external expansion stimulated cellular activity in the isolated skin and resulted in successful grafting to the transplanted site. Specifically, hyperplasia did not appear at the auto-grafted site, and grafted skin appeared similar to normal skin. Furthermore, mechanical stimuli resulted in an increase in COL1A2 expression in a suitable environment.

CONCLUSIONS: These findings provided insight on the potential of this expansion system in promoting dermal extracellular matrix synthesis *in vitro*. Conclusively, this newly developed smart skin bioreactor enabled effective skin expansion *ex vivo* and successful grafting *in vivo* in a pig model.

Keywords Animal model · Skin transplantation · Tissue expansion devices · Skin bioreactor

✉ Jeong Ok Lim
jolim@knu.ac.kr

¹ Biomedical Research Institute, Joint Institute for Regenerative Medicine, School of Medicine, Kyungpook National University, Kyungpook National University Hospital, 130 Dongdeok-ro, Jung-gu, Daegu 41944, Republic of Korea

² School of Medicine, Kyungpook National University, 680 Gukchaebosang-ro, Jung-gu, Daegu 41944, Republic of Korea

³ Daegu-Gyeongbuk Medical Innovation Foundation, 88 Dongnae-ro (360-4 Dongnae-dong), Dong-gu, Daegu 41061, Republic of Korea

1 Introduction

Skin functions as a protective barrier for the human body. When a large portion of skin is lost in accidents, human life is threatened. In spite of extensive efforts to produce substitutes that mimic human skin [1], it is still very difficult to cover large areas of skin in a relatively short period of time. Recent advancements in tissue engineering have enabled the production of skin for clinical applications [1–4] and promoted the healing of acute and chronic wounds. In skin tissue engineering, various biological and synthetic materials are combined with cells cultured *in vitro* to generate functional tissues [5–10]. A critical issue in skin tissue engineering is to obtain a sufficient number of cells while preserving the normal phenotype and functionality of the cells. Only then can these cells be used for either generation of skin substitutes that are suitable for transplantation or as *in vitro* test systems [8].

Another challenge is that most engineered skin products are partial skin, usually resulting in secondary contraction and leaving scar. Therefore, engineering full skin is necessary to completely cure the damaged skin without significant scarring. The current standard of obtaining full thickness skin in large scale is implantation of tissue expander into patient's own body for many months. These 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, however this method causes pain and discomfort to the patient [11].

To overcome these difficulties, approximately 10 years ago, Ladd et al. [12] first attempted a novel approach by applying a bioreactor system that was able to expand full skin *ex vivo* by 110–120% in 2 weeks. Findings from this work opened a new path in obtaining high quality *ex vivo* full skin. However, the system is limited by its relatively low expansion rate in spite of long expansion time.

In order to improve the expansion efficiency, we developed a new skin bioreactor and skin was expanded to 150% of its original size in a very short time period of 4 hours. Grafting study using Yucatan female pigs demonstrated the success of the procedure [13]. Following this work, we further aimed to expand a larger area of full skin and develop an advanced type of closed skin expander, which is equipped with a novel grab and operates in an automated manner. The aim of this study is to effectively expand full thickness skin to clinically useful size using an automated skin reactor and evaluate auto grafting efficiency of the expanded skin using Yucatan female pigs.

2 Materials and Methods

2.1 Bioreactor components and expansion procedure

The bioreactor consists of an expansion chamber, a drive control, and an arm and grip as shown in Fig. 1A, D. In order to prevent skin rupture, a small individual motor (ME2U-MD20, Autonics, Busan, Korea) is used on each axis for skin extension. Additional details and structures include skin grippers, clamp extension shafts for attachment/detachment, and a computer for central control

Fig. 1 Skin bioreactor and grafting procedure. **A** Skin was expanded on the bioreactor. **B–D** Full-thickness porcine inguinal skin with an area of 4 cm × 4 cm (**B**) was removed and measured (**C**), and then

expanded and incubated in the bioreactor for 48 h (**D**). **E** Recipient dorsal skin with an area of 6 cm × 6 cm was removed. **F** The expanded skin was grafted on the dorsal skin

Table 1 Operating conditions

Frequency (times/hour)	150
Outbound interval (s)	10
Outbound distance (mm)	10
Inbound interval (s)	5
Inbound distance (mm)	5

functions such as program operation and data storage for device control. The harvested skin was extended to a tension of 80 N ($\approx$ 8.16 kgf).

Full-thickness porcine skins were obtained from Daegu-Gyeongbuk Medical Innovation Foundation (DGMIF, Daegu, Korea). The skins were sub-merged in 70% EtOH for 10 s and washed with sterile phos-phate-buffered saline (PBS). The skin was expanded in William's E medium containing 1% penicillin–streptomycin for 48 h. The operating conditions of the bioreactor are shown as below in Table 1. The area of the skin was calculated by Image J software (National Institute of Health, Bethesda, MD, USA).

2.2 Auto-grafting study

For evaluation of auto-grafting efficiency of expanded skin, we used two female Yucatan pigs after obtaining approval from the Institutional Animal Care and Use Committee at DGMIF (DGMIF-16100301-01, Daegu-Gyeongbuk Medical Innovation Foundation, Daegu, Korea). Inguinal skin was used as the donor skin. Full-thickness skin (4 cm $\times$ 4 cm) was harvested from the inguinal area under general anesthesia and subsequently expanded for 48 h using the bioreactor. The expanded skin was removed from the reactor and measured the size of the expansion. The expanded skin was removed from the reactor and measured the size. Subsequently the expanded skin was grafted on the back of the pig where the area of full-thickness skin matching the expanded size was removed. Per one individual pig, only two wounds were created sequentially. First wound was made when inguinal skin was harvested as a donor and the second wound at back site for grafting. To prevent infection and maintain a moist environment, the transplanted skin site was initially treated with Vaseline

gauze on a daily basis, followed by dry dressing after 4 weeks.

The animals were observed daily for 11 weeks and at the end of the period they were euthanized. Tissues including inguinal, back, and grafted skin tissues were harvested for histological and immunohistochemical analyses. All grafting experiments were conducted in the Laboratory Animal Center at DGMIF.

2.3 Measurement of mechanical properties

The changes in the mechanical properties of the expanded skin were evaluated by measuring the fracture stress. Young's module, maximum load, and tensile strength. Normal and expanded inguinal skins were measured by universal testing systems 5965 (Instron, Norwood, MA, USA). The values were evaluated by BLUEHILL[®] universal software (Instron). The experiments were replicated four times (n = 4).

2.4 Indocyanine green fluorescence angiography by the SPY fluorescent imaging system

To evaluate the vascularity or blood circulation at the recipient site after skin grafting, laser-assisted indocyanine green (ICG) angiography using the SPY imaging system was performed. The SPY imaging system (Novadaq Technologies Inc., Toronto, Canada) provides real-time assessment of tissue perfusion and viability and serves as a tool for non-invasive perfusion assessment with a high degree of sensitivity and specificity. The ICG molecule binds strongly to plasma proteins, causing it to remain in the intravascular space and enabling fluorescent detection. Intraoperative laser angiography using ICG is a desirable option for tissue evaluation. The use of SPY fluorescent imaging allows the visual assessment of superficial blood flow and tissue perfusion of transplanted skin. Evaluation of blood flow assessment was performed at 1, 3, 6, and 10 weeks.

Table 2 Reverse transcription PCR primers

Gene	Sequence	Annealing °C	Cycles
Type I collagen	5'-CAAAGAAGGCAGCAAAGGTC-3'	60 °C	30
	5'-GGAAGCCTCTTTCTCCTCGT-3'		
Type III collagen	5'-GTCCCGATGGTTCTCGTAAA-3'	60 °C	30
	5'-CCACCATTTCATGGATTCTCC-3'		
GAPDH	5'-GTCGGTTGTGGATCTGACCT-3'	60 °C	20
	5'-CCCTGTTGCTGTAGCCAAAT-3'		

2.5 Reverse transcription polymerase chain reaction (RT-PCR)

To evaluate gene expression of the skin, total RNA from each skin of the experimental groups was extracted using RNeasy Mini (GE Healthcare Bio-Sciences AB, Little Chalfont, UK) according to the manufacturer's protocol. cDNA was prepared with 1 µg of total RNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). The cDNAs were used for RT-PCR. The PCR primers and annealing temperatures for target genes were designed based on published porcine gene sequences (Table 2). The products from RT-PCR were visualized by 1% agarose gel electrophoresis and ethidium bromide staining. GAPDH was used as an internal loading control. Images were obtained from an ImageQuant LAS 4000 (GE Healthcare Bio-Sciences AB, Little Chalfont, UK). The ratio of expression levels to GAPDH was quantitated by ImageJ (National Institute of Health, Bethesda, MD, USA).

2.6 Histology and immunohistochemistry

Retrieved skin tissues were fixed with 4% paraformaldehyde in PBS (pH 7.4) at 4 °C overnight. Tissues were dehydrated using a graded ethanol series, cleared with xylene, and mounted in paraffin. Slides were made with 2-µm sections, and hematoxylin and eosin (H&E), Masson's trichrome, and May-Grünwald Giemsa stains were performed routinely. For immunohistochemistry (IHC), sections were de-waxed, rehydrated, and labeled with proliferating cell nuclear antigen (PCNA), E-cadherin, and biotin-conjugated secondary antibody. For detection, sections were incubated with ABC complex kit and then visualized using the VectorNovaRED substrate kit. Nuclear counterstaining was performed with hematoxylin. Images were taken using an Eclipse 80i (Nikon, Tokyo, Japan).

2.7 Terminal deoxynucleotidyl transferase dUTP nick end labeling assay

To determine whether skins underwent apoptosis during expansion on the reactor, we used TACS[®] 2 TdT Fluorescein Kit (Trevigen, Gaithersburg, MD, USA). The procedure was performed according to the manufacturer's protocol and counterstained with DAPI (1 µg/mL). As a positive control, sections were treated with TACS-NucleaseTM.

2.8 Statistical analysis

Data in this study are presented as mean ± standard error of the mean. As all data were shown to be not normally distributed, the non-parametric Mann–Whitney U test was used to determine significant differences between samples. $p < 0.05$ indicates statistical significance.

3 Results

3.1 Expansion study

Full thickness porcine inguinal skin of initial size of 4 × 4 cm was expanded to 5.5 cm × 6 cm in the bioreactor in 48 h. The expansion rate was calculated to 182.68 ± 29.72% (Fig. 2).

Table 3 Mechanical properties of the initial and expanded inguinal skin

Mechanical property	Initial	Expanded
Maximum load (N)	75.00 ± 14.31	142.09 ± 16.10
Young's module (MPa)	9.17 ± 1.00	27.90 ± 7.76
Tensile extension (mm)	33.26 ± 4.10	20.86 ± 3.26

Fig. 2 Skin expansion rate. Full-thickness porcine inguinal skin was expanded on the bioreactor for 48 h. **A** The expanded area was calculated by ImageJ. **B** The mean expansion rate was 182.68 ± 29.72%. The measurement was replicated four times. Statistical significance: * $p < 0.05$ versus pre-expanded skin

Fig. 3 Physical properties. **A** Maximum load, **B** tensile strength, and **C** Young's module of pre- and post-expanded skin were evaluated using a universal testing system 5965 (Instron). Maximum load and

tensile strength were increased but Young's module was decreased in the expanded skin ($n = 3$). * $p < 0.05$

Fig. 4 Blood flow assessment during wound healing. Bright-field images showed skin wound healing after autografting. Blood plasma distribution was visualized with indocyanine green (ICG) fluorescence by Spy system. Red intensity indicates concentration of blood

albumin tagged with ICG. At 3 weeks after grafting, blood flow was detected in the grafted site and then remained during wound healing. (Color figure online)

3.2 Biomechanical properties

As shown in Table 3 and Fig. 3, the expanded skin showed a higher maximum load score (142.09 ± 16.10 N) and Young's module (27.90 ± 7.76 MPa) as compared with normal skin. However, the maximum extension which is the displacement at the point of skin rupture decreased after expansion (Fig. 3).

3.3 ICG fluorescence angiography by the SPY fluorescent imaging system

As shown in Fig. 4, the colored spy image identifies perfusion zones in red. The results demonstrated a significant increase in the blood flow of expanded skin during wound healing at 10 weeks after transplantation.

3.4 Collagen gene expression

To determine changes in the expression of collagen, which is a major component of skin, we examined gene

expression by RT-PCR. The expression of COL1A2 was elevated dramatically after *ex vivo* expansion and its level was retained during wound healing at 5 weeks after transplantation. After 11 weeks, the elevated expression was attenuated. However, normal inguinal and back skins did not express COL1A2. The expression of COL3A1 decreased slightly after the expansion, but it increased during wound healing over 11 weeks (Fig. 5).

3.5 Histology

Histological examinations were performed pre- and post-expansion on inguinal, dorsal, and transplanted skins. The histological analysis demonstrated a significant increase in the porosity of the dermal layers and a uniaxial arrangement of the collagen fibers. In particular, post-expanded skin showed thinner epidermis and straightened arrangement (Fig. 6A, B). Collagen colorized by Masson's trichrome stain showed the weakest intensity at 5 weeks after transplantation (Fig. 6B). In the elastin stains, short elastin

Fig. 5 RT-PCR. mRNA levels of the skin were analyzed by RT-PCR. **A** COL1A2 expression was elevated by expansion and transplantation (at 5 and 11 weeks), and COL3A1 was upregulated at 5 weeks after transplantation. **B** The ratio of expression levels to

GAPDH was quantitated using ImageJ. The measurements were replicated four times. Statistical significance: *** $p < 0.001$ vs. Normal inguinal skins. Abbreviations: Col. I; COL1A2, Col. III; COL3A1

Fig. 6 Hematoxylin and eosin (H&E), Masson's trichrome, and elastin staining. **A–E** H&E, **F–J** Mason's trichrome, and **K–O** elastin staining were performed with pre- (**A**, **F**, and **K**) and postexpanded

inguinal (**B**, **G**, and **L**), dorsal (**C**, **H**, and **M**), transplanted skin at 5 (**D**, **I**, and **N**) and 11 weeks (**E**, **J**, and **O**) after surgery. Scale bar is 100 μ m

Fig. 7 TUNEL assay. TUNEL assay was performed with **A** pre- and **B** post-expanded inguinal, **C** dorsal, transplanted skin at **D** 5 and **E** 11 weeks after surgery. **F** Sections digested using DNase I were used as a positive control. Scale bar is 100 μ m

fibers were observed in post-expanded, transplanted skin at 5 and 11 weeks after surgery (Fig. 6C).

3.6 Terminal deoxynucleotidyl transferase dUTP nick end labeling assay

To detect apoptosis, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was performed. The results revealed no positive signal in all skins. Nuclease-treated skin was used as a positive control (Fig. 7). *Ex vivo* skin expansion for 48 h did not cause significant damage to cells and tissues present in the skin.

3.7 Immunohistochemistry (IHC)

PCNA staining showed that PCNA (+) cells were predominantly located at the basal layer of the epidermis, just above the basement membrane, in both pre- and post-expanded skin. The post-expanded skin showed more proliferating epithelial cells than did the pre-expanded skin. Especially, numerous dermal fibroblasts were stained at 5 weeks, but decreased at 11 weeks, which was similarly observed in the epidermis (Fig. 8).

To determine epidermal integration, E-cadherin distribution was visualized with VectorNovaRED. E-cadherin expression was observed in the intercellular regions of the entire epidermis area except in the superficial layer. Despite physical expansion, cell–cell adhesion was well

preserved. Especially, E-cadherin showed weak staining at 5 weeks compared to pre-expanded inguinal skin (Fig. 9).

To investigate the participation of immune cells during wound healing, May-Grünwald Giemsa staining was performed. The dermis of pre-expanded inguinal skins, post-expanded inguinal skin, and dorsal skin showed few cells stained in dark blue. However, numerous dark blue-stained cells were observed in the dermis at 5 and 11 weeks after surgery (Fig. 10). The cells in the dermis were supposedly macrophages.

4 Discussion

After damage, skin recovery undergoes a complicated process that results in incomplete recovery due to shrinkage and fibrosis of the dermis. In addition, restoration of damage requires a substantial period of time because it involves processes such as cell migration, remodeling of the extracellular matrix (ECM), and formation of blood vessels [14, 15]. There have been attempts to improve the curing process and enhance the quality of the restored skin tissues [16, 17]. Changes in skin tissues and cells due to mechanical tension have been studied for a long time. Proliferation of skin epidermal cells was observed by stretching or friction in living rats [17, 18], and tissue enlargement in guinea pigs was reported to increase cell division in many cells [16]. *In vitro* stretching of fibroblasts increases mitotic rate [19], and mechanical stress can

Fig. 8 Immunohistochemistry (PCNA). Immunohistochemistry of PCNA was performed with **A** pre- and **B** post-expanded inguinal, **C** dorsal, transplanted skin at **D** 5 and **E** 11 weeks after surgery. **F** A section labeled with IgG only was used as a negative control. Scale bar is 50 μm

Fig. 9 Immunohistochemistry (E-cadherin). Immunohistochemistry of E-cadherin was performed with **A** pre- and **B** post-expanded inguinal, **C** dorsal, transplanted skin at **D** 5 and **E** 11 weeks after

surgery. **F** A section labeled with IgG only was used as a negative control. Scale bar is 50 μm

induce the release of growth factors [20]. Mechanical expansion results in a low density metric of the dermis, thereby facilitating entry of peripheral cells [21]. Elongation of cells by mechanical tension shows growth instead of cell death and increases cell proliferation [22, 23]. *In vivo*,

tensile strength affects the heterogeneity of caliber and curvature in stimulated vessels and induces vascular remodeling [23].

Currently, various clinical methods are applied in the treatment of skin loss such as epidermal grafting, artificial

Fig. 10 May-Grünwald stain. To visualize immune cells, May-Grünwald staining was performed with **A** pre- and **B** post-expanded inguinal, **C** dorsal, transplanted skin at **D** 5 weeks and **E** 11 weeks after surgery. Inlets show magnified image of each *. **D, E** The cells

stained in dark blue were presented in the dermal matrix at 5 and 11 weeks after transplantation. **E** Stained cells decreased at 11 weeks after grafting. Scale bar is 200 μ m

skin based on biomaterials, and *in vivo* skin expander. However, these methods have limitations such as discomfort, high cost, inflammation, and long-term regulation. Various attempts have been made to overcome these limitations, but *ex vivo* expansions only showed an expansion rate of approximately 110% [12, 24]. Our previous study showed an expansion rate of 156.7% and successful autologous skin transplantation using pneumatic pressure, but the expression of COL1A2 was not confirmed in the *ex vivo* expansion [13]. It is speculated to be due to limitations of the machine based on the pneumatic pressure and short culture period. In this study, we tried to overcome these problems using various approaches, and we developed a new method of skin expansion using a screw driver motor-based skin expander, yielding an expansion rate of up to 200% and inducing the expression of COL1A2.

Wound healing of a full-thickness skin defects requires a large amount of ECM remodeling. Collagens are the most abundant proteins in the dermis that have a triple helical fibril structure. They not only give structural support to resident cells but also regulate their functions [25]. In particular, type I collagen is a major component of the ECM-composed dermis [26, 27]. As shown in Fig. 5, COL1A2 was not expressed in normal inguinal skin, but was upregulated by *ex vivo* expansion using the bioreactor. This is thought to be the first report whereby COL1A2 increases in *ex vivo* expansion.

During wound healing of the dermis, the porous space induced by the expansion is recovered like normal skin after transplantation. The change in the intensity of collagen by Masson's trichrome staining is likewise similar. Elastin fibers are known to play an important role in the elasticity of the skin, and short elastin fibers show low elasticity [28–30]. As shown in Fig. 6, the stained pattern of the shortened elastin fibers after expansion is consistent with the physical properties of the expanded skin (Fig. 3).

In the present study, all tissues showed non-positive TUNEL staining, and the conditions of the *ex vivo* expansion did not appear to damage cells (Fig. 7). In the epithelial cells, cell proliferation increased after expansion and transplantation and decreased at 11 weeks. Especially in the dermis, fibroblast proliferation was increased markedly at 5 weeks after transplantation (Fig. 8). Epithelial cell integrity was examined by E-cadherin staining and appeared well stained on the intercellular interface except in skin tissue after 5 weeks (Fig. 9). Numerous cells were observed in the dermis at 5 weeks, which appeared to be macrophages. They were then observed to decrease at 11 weeks after wound healing (Fig. 10). Histological analysis indicated that external expansion caused cellular activity without the toxicity of isolated skin and led to a post-transplant repair phase. Specifically, hyperplasia did not appear at the auto-grafted site, and the expanded skin resembled normal skin.

All experimental data demonstrated that approximately 200% of the transplanted area recovered without adverse effects through auto-grafting with *ex vivo* expansion using the bioreactor. Furthermore, it is worthy to note that mechanical stimulus can increase the expression of COL1A2 in a suitable environment. These results supported the potential of this technology in promoting dermal ECM synthesis *in vitro*.

Acknowledgements This research was supported by 2017 Medical Cluster R&D Support Project through Daegu Gyeongbuk Medical Innovation Foundation funded by the Ministry of Health and Welfare, the Republic of Korea (H116C2505), and ETRI R&D Program [15ZC3110, Local-based medical device/robot development and medical IT convergence for small and medium enterprises revitalization project].

Compliance with ethical standards

Conflicts of interest The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical statement Animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee at DGMIF (DGMIF-16100301-01, Daegu-Gyeongbuk Medical Innovation Foundation, Daegu, Korea).

References

- Cubo N, Garcia M, Del Cañizo JF, Velasco D, Jorcano JL. 3D bioprinting of functional human skin: production and *in vivo* analysis. *Biofabrication*. 2017;9:015006.
- Andreassi A, Bilenchi R, Biagioli M, D'Aniello C. Classification and pathophysiology of skin grafts. *Clin Dermatol*. 2005;23:332–7.
- Loss M, Wedler V, Künzi W, Meuli-Simmen C, Meyer VE. Artificial skin, split-thickness autograft and cultured autologous keratinocytes combined to treat a severe burn injury of 93% of TBSA. *Burns*. 2000;26:644–52.
- Sheridan R. Closure of the excised burn wound: autografts, semipermanent skin substitutes, and permanent skin substitutes. *Clin Plast Surg*. 2009;36:643–51.
- Cai SJ, Li CW, Weihs D, Wang GJ. Control of cell proliferation by a porous chitosan scaffold with multiple releasing capabilities. *Sci Technol Adv Mater*. 2017;18:987–96.
- Mandal BB, Kundu SC. Cell proliferation and migration in silk fibroin 3D scaffolds. *Biomaterials*. 2009;30:2956–65.
- Sadeghi-Avalshahr A, Nokhasteh S, Molavi AM, Khorsand-Ghayeni M, Mahdavi-Shahri M. Synthesis and characterization of collagen/PLGA biodegradable skin scaffold fibers. *Regen Biomater*. 2017;4:309–14.
- Vig K, Chaudhari A, Tripathi S, Dixit S, Sahu R, Pillai S, et al. Advances in skin regeneration using tissue engineering. *Int J Mol Sci*. 2017;18:E789.
- Law JX, Liao LL, Saim A, Yang Y, Idrus R. Electrospun collagen nanofibers and their applications in skin tissue engineering. *Tissue Eng Regen Med*. 2017;14:699–718.
- Park TH, Choi WY, Lee JH, Lee WJ. Micronized cross-linked human acellular dermal matrices: an effective scaffold for collagen synthesis and promising material for tissue augmentation. *Tissue Eng Regen Med*. 2017;14:517–23.
- Spence RJ. Clinical use of a tissue expander-enhanced transposition flap for face and neck reconstruction. *Ann Plast Surg*. 1988;21:58–64.
- Ladd MR, Lee SJ, Atala A, Yoo JJ. Bioreactor maintained living skin matrix. *Tissue Eng Part A*. 2009;15:861–8.
- Huh MI, An SH, Kim HG, Song YJ, Choi EC, An SH, et al. Rapid expansion and auto-grafting efficiency of porcine full skin expanded by a skin bioreactor *ex vivo*. *Tissue Eng Regen Med*. 2016;13:31–8.
- Vidmar J, Chingwaru C, Chingwaru W. Mammalian cell models to advance our understanding of wound healing: a review. *J Surg Res*. 2017;210:269–80.
- Guo S, Dipietro LA. Factors affecting wound healing. *J Dent Res*. 2010;89:219–29.
- Bullough WS, Laurence EB. The control of epidermal mitotic activity in the mouse. *Proc R Soc Lond B Biol Sci*. 1960;151:517–36.
- Lorber M, Milobsky SA. Stretching of the skin *in vivo*. A method of influencing cell division and migration in the rat epidermis. *J Invest Dermatol*. 1968;51:395–402.
- Mackenzie IC. The effects of frictional stimulation on mouse ear epidermis. II. Histologic appearance and cell counts. *J Invest Dermatol*. 1974;63:194–8.
- Curtis AS, Seehar GM. The control of cell division by tension or diffusion. *Nature*. 1978;274:52–3.
- Vandemburgh HH. Mechanical forces and their second messengers in stimulating cell growth *in vitro*. *Am J Physiol*. 1992;262:R350–5.
- Lancerotto L, Orgill DP. Mechanoregulation of angiogenesis in wound healing. *Adv Wound Care (New Rochelle)*. 2014;3:626–34.
- Ingber DE. Tensegrity II. How structural networks influence cellular information processing networks. *J Cell Sci*. 2003;116:1397–408.
- Pietramaggiore G, Liu P, Scherer SS, Kaipainen A, Prsa MJ, Mayer H, et al. Tensile forces stimulate vascular remodeling and epidermal cell proliferation in living skin. *Ann Surg*. 2007;246:896–902.
- Jeong C, Chung HY, Lim HJ, Lee JW, Choi KY, Yang JD, et al. Applicability and safety of *in vitro* skin expansion using a skin bioreactor: a clinical trial. *Arch Plast Surg*. 2014;41:661–7.
- Xue M, Jackson CJ. Extracellular Matrix reorganization during wound healing and its impact on abnormal scarring. *Adv Wound Care (New Rochelle)*. 2015;4:119–36.
- Ghaffari A, Kilani RT, Ghahary A. Keratinocyte-conditioned media regulate collagen expression in dermal fibroblasts. *J Invest Dermatol*. 2009;129:340–7.
- Callaghan TM, Wilhelm KP. A review of ageing and an examination of clinical methods in the assessment of ageing skin. Part I: cellular and molecular perspectives of skin ageing. *Int J Cosmet Sci*. 2008;30:313–22.
- Uehara E, Hokazono H, Hida M, Sasaki T, Yoshioka H, Matsuo N. GABA promotes elastin synthesis and elastin fiber formation in normal human dermal fibroblasts (HDFs). *Biosci Biotechnol Biochem*. 2017;81:1198–205.
- Mithieux SM, Weiss AS. Design of an elastin-layered dermal regeneration template. *Acta Biomater*. 2017;52:33–40.
- Kielty CM, Sherratt MJ, Shuttleworth CA. Elastic fibres. *J Cell Sci*. 2002;115:2817–28.