Biomedical Research 2017; 28 (5): 2290-2295 *ISSN 0970-938X*
www.biomedres.info

Advances in epidermis engineering: application of epidermal stem cells (ESCs) and tissue engineered skin (TES).

Wenbin Nan, Zhihao Xu, Hongli Chen*

Department of Life Sciences and Technology, Research Center of Stem Cell and Biotherapy Technology, Xinxiang Medical University, Xinxiang, PR China

Abstract

As specific stem cells of skin tissue, epidermal stem cells (ESCs) have been proved to be closely related to the wound repair. As a result, studying more targeted markers for a rapid acquisition of ESCs has become a hot issue for TES. For server burn wound or ulcer, the creation of ideal scaffold as matrix for cell proliferation and differentiation was needed for TES as useful ESCs being rare. In this review, the common use of the markers for the isolation and identification of ESCs are summarized. Furthermore, the research and development of tissue engineering skin scaffold during the past years were also discussed.

Keywords: Epidermal stem cells, Labeling, Tissue engineered skin, Tissue engineering scaffold.

Accepted on October 27, 2016

Introduction

It is well-known that the skin is composed of the epidermis and dermis, which play an important role in protecting the body from damage such as wounding. It has been proved that the skin acts as an anatomical barrier from pathogens and any damage between the internal and external environment in bodily defence. Generally, the epidermis is a thin layer with sufficient thickness, which is useful to provide vital barrier function and protection from ultraviolet radiation [1,2]. Epidermal stem cells (ESCs) have self-renewal ability and are the primary cells for keeping skin metabolism. Therefore, the ESCs are closely related to wound repair and considered as the basis of the regeneration and rebuilt of the skin. During the past decades, the researches on epidermal tissue engineering have made much progress due to its importance for the skin regeneration and rebuilt [3-5]. This article will review the recent advances on the epidermis engineering and the application of epidermal stem cells in skin tissue engineering.

Epidermal Stem Cells *Labelling*

ESCs, including hair follicles stem cells, interfollicular epidermal stem cells, sebaceous glands of somatic stem cells and isthmus stem cells, generally located in epidermal basal layer and hair follicles. The hair follicles stem cells were studied most and showed to be located in hair follicle bulge, which acted as the main location for skin stem cells and played a critical role in hair follicle metabolism and wound healing [6,7]. With the attention raised by tissue engineering, ESCs were more widely applied in many research areas such as skin tissue engineering, cell substitute therapy, gene engineering and so on. Thus, the isolation and identification of epidermal stem cells became a key issue to be solved.

Keratin family was proved to be related to the expression of structural proteins in epidermal cells. In fact, the different kinds of ESCs could be specifically labeled by different keratin markers. The ESCs were expressed by keratin 19 and hair follicle bulge stem cell was labeled by keratin 15 [8,9]. Ketin 5 and 14 were the markers of progenitor cells, and the keratins 1 and 10 were specifically expressed by the differentiated cells [10]. Among these markers, Keratins 15 has been studied mostly till recently. In basal keratinocytes, the expressions of keratin K5/K14 heterodimers were found to be associated with the expression of K15 [11]. In the bulge of hair follicles, K15 expression had been observed and a few studies implicated its potential as a stem cell marker [9]. Shulan previously used a keratin 15 promoter sequence to develop transgenic mouse. These mice enabled us to trace the lineage of cells derived from keratin 15 expressing cells during the hair growth cycle. Then, these mice were subsequently used to trace the lineage of keratin 15-expressing cells from the hair follicle into skin tumors through the multistage model of skin carcinogenesisouse [12].

Integrin are transmembrane receptors which acts as the bridges for cell-cell and cell-extracellular matrix (ECM) interactions. Besides, integrin are obligate heterodimers, meaning that they have two different chains: α and β subunits. β1 and α4β6 integrins were part of the ECM architecture and play regulatory roles in various cell types, including the epidermis [13]. Because of integrin adhesions to the cytoskeleton playing a critical role in the control of ECM deposition and remodeling, integrin-linked kinase (ILK) was found to be a

possible key regulator of the bulge extracellular matrix micro-environment and stem cell activation and thereby skin homeostasis [14]. Gene marker P63 was firstly discovered by Yang [15] and considered as an important transcription factor with pivotal functions in epidermal cell lineage commitment. However, the function of P63 to the ESCs was still a debate [16]. Additionally, Lgr6 as a new gene marker for the epidermal stem cells was showed to be expressed in the earliest embryonic hair placodes and marked the most primitive ESCs [17]. Lim [18] also discovered Wnt target gene Axin2, which was able to mark interfollicular epidermal stem cells.

Cadherins were critical components of adheren junctions (AJ) [19]. The studies of Tinkle and colleagues showed that the levels rather than cadherin subtypes were key factors to keratinocyte stability and thus epidermal integrity [19]. Antibody-specific fluorescent staining was an important method to determine keratinocyte populations in the basal layer of the epidermis. The resultant fluorescent intensities were then used to distinguish the population subtypes in the basal layer of the epidermis. Less intensity of staining was observed in cells with decreased proliferation capacities [20]. Antibody specific staining of AJ complex subunits revealed higher staining intensities for E-cadherin, β-catenin, and γ-catenin [21]. Thus, the levels of cadherin with integrin may provide biomarkers for the epidermal stem cell. So far, although there have been many advances in biological marker of ESCs, the specificity of current marker for ESCs has still being problems. Therefore, the further work on identification of ESCs will mainly focus on developing highly specific surface marker to identify and separate ESCs rapidly.

Tissue Engineered Skin (TES)

Traditional strategies for TES

Tissue engineering is emerging as a newly and much concerned treatment method for replacing diseased or damaged tissues. Extracellular matrix provides an environment for cell adhesion, growth, proliferation and metabolism, which acts as a template for supporting and thus leads to tissue regeneration and regulation. So, tissue engineering scaffold is usually designed to be as artificial ECM. An ideal tissue engineering scaffold has to meet following requirements. For materials properties: (1) allowing the cell to adhere to its surface, promote cell proliferation, and retain the function of the differentiated cells; (2) being capable of degradation, and degradation products are non-cellular toxicity and do not cause inflammation; (3) good biocompatibility. For materials structure: (1) being with high porosity to provide enough space for cell adhesion, extracellular matrix regeneration and cell distribution; (2) providing the appropriate 3D location for growth factor storage and releasing and the cell anchorage.

Natural materials

The natural materials had the advantages of low toxicity and low chronic inflammatory response included polypeptides, hyaluronan, glycosaminoglycans (GAGs), fibronectin, collagen, chitosan and alginates [22-24], and collagen had been developed as the most mature product. Artificial skin, called Apllgraf, was reported as the first active skin substitute developed by Organogenes Ct. Lot [25]. This product was made by collagen-gel scaffold, which was created by firstly seeded with fibroblasts to form cellular collagen gel and then incubated with epidermal cells. Compared to traditional gel, Apllgraf showed faster healing rate for bullous epidermolysis and no side effects during healing, reducing more pain from wound at the same time.

As a newly tissue engineering materials, acellular dermal matrix (ADM) was derived from full-thickness skin by removing cells and cellular components rather than native dermal structure and extracellular proteins. ADM was proved to be capable for maintaining the skin basement membrane, normal structure of collagen fibers and components of dermal extracellular matrix after a series of treatments and thus can significantly reduce the immunogenicity and accelerate vascularization [26]. Such biological benefits indicate the greater applications in skin wound healing, tissue filling, reconstruction, cosmetic surgery and so on. However, its clinical safety and long-term efficacy are still not clear. So how to further improve the preparation process and stability of acellular dermal matrix is still to be explored. Alloderm produced by Life Cell was a composite scaffold made by intact basement membrane and dermal extracellular matrix after removing epidermis and cells. The previous research work demonstrated application of laser drilling technology into the whole drill process of acellular dermal matrix can improve the interstitial fluid penetration and the regeneration of the micrangium [27].

Synthetic materials

Due to good biocompatibility, controlling degradation rate and designable mechanical properties, synthetic polymers were widely applied in skin tissue engineering. Currently, researches mostly focused on improving cell adhesion and hydrophilicity of synthetic materials via surface modification. Polylactic acid (PLA) was one of the earliest biodegradable materials approved by Food and Drug Administration (FDA) due to its non-toxicity and good biocompatibility. PLA was able to degrade into lactate in human body and metabolized in forms of water and carbon dioxide [28]. Dermagraft, as a mature artificial skin substitute, was developed by Advanced Tissue Sciences in 2000 [29]. This product was designed by seeding fibroblasts on degradable PLA fiber nets to form an artificial dermis with three parts (fibroblasts, extracellular matrix and degradable biomaterials). Compared to traditional therapy, Dermagraft showed faster cure on ulcers and lower corresponding complications.

Hybrid scaffold

Was not limited to be made of one kind of natural materials or synthetic materials. One applicable hybrid scaffold was the gelatin and polycaprolactone (GT/ PCL) electrospun membrane fabricated by Duan [30]. The presence of GT

enhanced the biodegradability and biocompatibility of the membranes. PCL improved the mechanical properties of the sheets. This hybrid material was potential of being used in both skin and nerve engineering. Monteiro [31] created a single three-dimensional epidermal-dermal scaffold by taking advantage of the properties of haluronic acid (HA) and Poly-L-Lysine (PLL) to efficiently produce a stable membrane (the epidermal component), which was adsorbed on top of a porous HA scaffold (the dermal component). Another applicable hybrid scaffold was the chitin membrane modified by cross linked with type I collagen isolated from the rat tail [32]. This scaffold was investigated to be with small pore size of 2-10 mm, and high adhesion to hair follicle ESCs. The rat skin defect test showed that collagen-chitin biomimetic membrane (C-CBM) was sufficient to achieve morphological, structural and functional reconstruction of the full-thickness skin defects.

Nanofibrous scaffold

The scaffolds with more accurate structure and defined pore size were needed to match surrounding tissues and provided a better support for cell adhesion and growth. Thus, new technologies were developed for meeting more strict requirements, such as electrospinning [33]. Electrospinning of nanofibers showed a novel approach to a completely new dimension of biomaterials, which provided greater possibility to match cell proliferation and differentiation. Jin [34] investigated the potential of human BM-derived MSC for epidermal cell differentiation *in vitro* on electrospun collagen/poly(l-lactic acid)-co-poly(3-caprolactone) (Coll/PLLCL) nanofibrous scaffolds with 209 ± 26 nm fiber diameters and suggested such scaffold could mimic the native skin extracellular matrix environment and can be used as the promising substrates for advanced skin tissue engineering. The previous studies showed the effects of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) nanofibers on the support dermal fibroblasts, keratinocytes, Schwann cells and mesenchymal stem cells adherence and proliferation [35-37], and based on previous findings, Sundaramurthi [38] demonstrated the full-thickness wound healing efficiency and biocompatibility of PHBV nanofibers fabricated by electrospinning and found PHBV captured with bone marrow-derived mesenchymal stem cells (BM-MSCs) can provide an instantaneous coverage to deep wounds, burns and post-traumatic wounds.

Advanced strategies for TES

Actually, due to the complexity *in vivo* micro-environment, the traditional designs of TES are far from satisfying the needs in skin damage treatment. Further research need to be extended in TES to increase more possibilities in successful skin treatment.

Epidermal growth factor (EGF)

In human body, cells are exposed to a complex pattern of molecular cues and signals, which trigger a series of events that participated in control cell proliferation, differentiation and death. Since these cues or signals are considered as the critical components of early developmental pathways involved in cell specification, incorporating them into TES matrix could lead to a great advancement in skin regeneration. Epidermal growth factor (EGF) has been reported to be effective in wound healing and homeostasis in a number of tissues including colon, skin, mammary gland and liver [39]. In 1999, the high concentration of EGF was studied to have possibility of accelerating healing of skin lesions in animal [40]. Within the last few years, the novel strategies of skin regenerative treatment were aimed at the development of biologically responsive nanofibrous scaffolds which were capable of delivering multiple bioactive agents and cells to the target tissues. Mohammad [41] fabricated and evaluated the bioactive hybrid nanofibrous scaffolds of gelatin and PLGA, encapsulating recombinant human EGF, and such hybrid scaffold exhibited excellent blood clotting and platelet adhesion in comparison to the commercial wound dressing. Moreover, human fibroblast showed an increased cell proliferation on the PLGA/EGF/gelatin scaffold. Thus, the scaffolds with capability of encapsulation and controlled release of the protein exhibited their potential applications in skin tissue engineering and wound dressing. In another study, EGF was conjugated on the surface of electrospun poly (E-caprolactone) nanofibers functionalized with amine groups and it was reported that the EGF nanofibers improved the in vivo wound healing processes significantly compared to the direct application of EGF solutions [42].

Cultured epidermal autograft (CEA)

Many massive burns patients were usually bothered with limited available donor site, which have driven the search for alternative means to develop ideal split-thickness skin autograft. Cultured epidermal autograft is a product applied with basic method of tissue engineering, where the high concentration of vitro cultured keratinocytes are cultured on the artificial extracellular matrix with acceptable degradation rate, to achieve cell expansion, then the artificial skin was constructed and transplanted. Rheinwald and Green developed a technology for producing graftable epidermal cells and such method has become a widely-accepted technology applied in CEA preparation [43]. The first commercialized cultured epidermal autograft was Epicel that had been currently used as a cover for full-thickness burns in the US and in Europe [44]. The advantages of CEA are as follow: (1) only a small number of autologous epidermal cells can obtain a large area of wound dressing; (2) donor site morbidity can be minimized; (3) the cells can be frozen for storage; (4) the barrier function of skin can be quickly recovered and aesthetic effect can be improved. In 2009, Japan Tissue Engineering Co., Ltd. (Gamagori, Aichi, Japan) marketed a CEA called 'JACE'. JACE adopted a procedure that the CEA was placed on the reconstructed dermal layer using allograft or artificial skin, and this procedure had been used for over 150 severe burn patients thus far [45]. However, deficiencies still existed in current CEA products: (1) the collection cycle of the epidermal cells was relatively long (at least 3 weeks from biopsy to the layer); (2) permanent survival was not satisfactory; (3) the cultured layer

was too fragile to operate. (4) poor elasticity and poor wear resistance of epidermal tissue was studied after healing. Because of those limitations, more and more studies focused on the recovery observation of patients that accepted CAE treatment.

Bio-printing

Bio-printing is based on the existing technologies. It is layers of biological structures, rather than that of plastic, being used to create real living tissue in the 3D bio-printing, which is still in the preliminary stage. In the last decade, printing techniques have drastically been developed and resulted in TES moving from 2D to 3D. The emergence of 3D bio-printing technology solves the most stubborn problem faced in tissue engineering, because 3D bio-printer can generate a complex 3D microenvironment where the cells are integrated into hydrogel to mimic natural ECM of a particular tissue [46]. The "ink" for this printer is nothing but targeted cell medium and materials. In order to develop more uniform and precise size for printed products, the print head was constantly updated. Binder designed a small-scale system which had two different cartridges for fibroblast and keratinocytes, with spatial resolution set up at 1.57 μm and pressure at 6.89 kPa. Cells were embedded into the matrix and then implanted at the site of the wound [47,48].

Gene therapy

Advances in understanding the biological responses at molecular or cellular level involved in skin repair and regeneration has led to creative development of gene therapy for wound healing. Peng [49] designed a highly efficient gene system for the transfection of ESCs. The gelatin/β-TCP scaffold with CYD-PEI mediated 3D transfection system was prepared to improve the transfection of ESCs. Such advanced scaffold provided a basis for the transfected ESCs (TESCs) as a promising therapeutic agent and gene delivery reservoir for wound therapy.

Conclusion

Seeding cells is one of the critical parts of skin regeneration. Although the exploration of biological markers of ESCs has achieved great progress, the highly specificity is still a problem in the research of markers for ESCs. To create full-thickness skin products for improve patients' survival rate and life quality after injuries, many novel technologies were developed in skin tissue engineering including the designed 3D printing. But the current developed TES products or technologies are limited to temporary substitutions. In order to develop permanent and more desired TES, how to ensure adequate nutrients supply and provide the blood circulation as the same as the vivo environment are two urgent problems need to be solved.

Acknowledgment

We are grateful to the financial support from Natural Science Foundation of China (81401519) and Scientific Research Fund of Xinxiang Medical University (2014QN137).

References

1. Yuko MH, Koji YA, Ozeki R. Angiotensin-converting enzyme inhibitor (enalapril maleate) accelerates recovery of mouse skin from UVB-induced wrinkles. Biochem Bioph Res Co 2013; 442: 38-43.
2. Watt FM. Role of integrins in regulating epidermal adhesion, growth and differentiation. EMBO J 2002; 21: 3919-3926.
3. Pikula M. Flow cytometric sorting and analysis of human epidermal stem cell candidates. Cell Biol Int 2010; 34: 911-915.
4. Vagnozzi AN, Reiter JF, Wong SY. Hair follicle and interfollicular epidermao stem cells make varying contributions to wound regeneration. Cell Cycle 2015; 14: 3408-3417.
5. Liang L, Bickenbach JR. Somatic epidermal stem cells can produce multiple cell lineages during development. Stem Cells 2002; 20: 21-31.
6. Jayarama Reddy, Radhakrishnan S, Ravichandran R, Mukherjee S, Balamurugan R, Sundarrajan S, Ramakrishna S. Nanofibrous structured biomimetic strategies for skin tissue regeneration. Wound Rep Reg 2013; 21: 1-16.
7. Balañá ME, Charreau HE, Leirós GJ. Epidermal stem cells and skin tissue engineering in hair follicle regeneration. World J Stem Cells 2015; 7: 711-727.
8. Michel M, Török N, Godbout MJ, Lussier M, Gaudreau P, Royal A, Germain L. Keratin 19 as a biochemical marker of skin stem cells in vivo and in vitro: keratin 19 expressing cells are differentially localized in function of anatomic sites, and their number varies with donor age and culture stage. J Cell Sci 1996; 109: 1017-1028.
9. Bose A, Teh MT, Mackenzie IC, Waseem A. Keratin K15 as a Biomarker of Epidermal Stem Cells. Int J Mol Sci 2013; 14: 19385-19398.
10. Fei L, Jun Y. Hair follicle stem cells and its application in tissue engineering. Chinese Journal of Aesthetic Medicine 2008; 17: 943-945.
11. Bose A, Teh MT, Hutchison IL, Wan H, Leigh IM, Waseem A. Two mechanisms regulate keratin k15 expression in keratinocytes: Role of PKC/AP-1 and FOXM1 mediated signalling. PLoS ONE 2012; 7: e38599.
12. Li S, Park H, Trempus CS, Gordon D, Liu Y, Cotsarelis G, Morris RJ. A Keratin 15 containing stem cell population from hair follicle contributes to squamous papilloma development in the mouse. Mol Carcinog 2013; 52: 751-759.
13. Simpson CL, Patel DM, Green KJ. Deconstructing the skin: Cytoarchitectural determinants of epidermal morphogenesis. Nat Rev Mol Cell Biol 2011; 12, 565-580.

14. Morgner J, Ghatak S, Jakobi T, Dieterich C, Aumailley M, Wickström SA. Integrin-linked kinase regulates the niche of quiescent epidermal stem cells. Nat Commun 2015; 6: 1-15.
15. Yang A, Kaghad M, Wang Y, Gillett E, Fleming MD, Dötsch V, Andrews NC, Caput D, McKeon F. p63, p53 homoIog at 3q27-29,encodes multiple products with transactivating death-inducing and dominant-negative activities. Med Cell 1998; 2: 305-316.
16. Chen XD, Tian-zeng L, Shao-hai QI. P63 and β1 integrin expression in epidermal stem cells in normal skins at different body parts. Chin J Exp Surg 2004; 21: 1376-1378.
17. Snippert HJ, Haegebarth A, Kasper M, Jaks V, van Es JH, Barker N, van de Wetering M, van den Born M, Begthel H, Vries RG. Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the Skin. Science 2010; 327: 1385-1389.
18. Lim X, Tan SH, Koh WL, Chau RM, Yan KS, Kuo CJ, van Amerongen R, Klein AM, Nusse R. Interfollicular Epidermal Stem Cells Self-Renew via Autocrine Wnt Signaling. Science 2013; 342: 1226-1230.
19. Tinkle CL, Pasolli HA, Stokes N, Fuchs E. New insights into cadherin function in epidermal sheet formation and maintenance of tissue integrity. Proc Natl Acad Sci 2008; 105: 15405-15410.
20. Staunstrup NH, Madsen J, Primo MN, Li J, Liu Y, Kragh PM, Li R, Schmidt M, Purup S, Dagnæs-Hansen F. Development of transgenic cloned pig models of skin inflammation by DNA transposon-directed ectopic expression of human β1 and β2 integrin. PLos ONE 2012; 7: e36658.
21. Garrod D, Chidgey M. Desmosome structure, composition and function. Biochim Biophys Acta 2008; 1778: 572-587.
22. Rémi PB, Robert G, Francois B. Collagen-based biomaterials for tissue engineering applications. Materials 2010; 3:1863-1887.
23. Patricia BM, Gabriela AS Rui LR. Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. Adv Drug Deliver Rev 2007; 59: 207-233.
24. MacNeil S. Progress and opportunities for tissue-engineered skin. Nature 2007; 445: 874-880.
25. Angelo SM and David JM. Regenerative medicine: Current therapies and future directions. PNAS 2015; 112: 14452-14459.
26. Shukla A, Nandi P, Ranjan M. Acellular Dermis as a Dermal Matrix of Tissue Engineered Skin Substitute for Burns Treatment. Ann Public Health Res 2015; 2: 1023.
27. Chai JK, Liang LM, Yang HM, Feng R, Yin HN, Li FY, Sheng ZY. Preparation of laser micropore porcine acellular dermal matrix for skin graft: An experimental study. Burns 2014; 33: 719-725.
28. Marin E, Briceño MI, Caballero-George C. Critical evaluation of biodegradable polymers used in nanodrugs. Int J Nanomedicine 2013; 8: 3071-3091.
29. Marston WA. Dermagraft, a bioengineered human dermal equivalent for the treatment of chronic nonhealing diabetic foot ulcer. Expert Rev Med Devices 2004; 1: 21-31.
30. Duan H, Feng B, Guo X, Wang J, Zhao L, Zhou G, Liu W, Cao Y, Zhang WJ. Engineering of epidermis skin grafts using electrospun nanofibrous gelatin polycaprolactone membranes. Int J Nanomedicine 2013; 8: 2077-2084.
31. Monteiro IP, Shukla A, Marques AP, Reis RL, Hammond PT. Spray-assisted layer-by-layer assembly on hyaluronic acid scaffolds for skin tissue engineering. J Biomed Mater Res A 2015; 103: 330-340.
32. Shen Y, Dai L, Li X, Liang R, Guan G, Zhang Z, Cao W, Liu Z, Mei S, Liang W. Epidermal Stem Cells Cultured on collagen-Modified Chitin Membrane Induce In Situ Tissue Regeneration of Full-Thickness Skin Defects in Mice. PLoS One 2014; 9: e87557.
33. Li M, Mondrinos MJ, Gandhi MR, Ko FK, Weiss AS, Lelkes PI. Electrospun protein fibers as matrices for tissue engineering. Biomaterials 2005; 26: 5999-6008.
34. Jin G, Prabhakaran MP, Ramakrishna S. Stem cell differentiation to epidermal lineages on electrospun nanofibrous substrates for skin tissue engineering. Acta Biomater 2011; 7: 3113-3122.
35. Biazar E, Keshel SH. Chitosan-cross-linked nanofibrous PHBV nerve guide for rat sciatic nerve regeneration across a defect bridge. ASAIO J 2013; 59: 651-659.
36. Kuppan P, Vasanthan KS, Sundaramurthi D, Krishnan UM, Sethuraman S. Development of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) fibers for skin tissue engineering: effects of topography, mechanical, and chemical stimuli. Biomacromolecules 2011, 12: 3156-3165.
37. Paşcu EI, Stokes J, McGuinness GB. Electrospun composites of PHBV, silk fibroin and nano-hydroxyapatite for bone tissue engineering. Mater Sci Eng C Mater Biol Appl 2013; 33: 4905-4916.
38. Sundaramurthi D, Krishnan UM, Sethuraman S. Epidermal Differentiation of Stem Cells on Poly (3-hydroxybutyrateco-3-hydroxyvalerate) (PHBV) Nanofibers. Ann Biomed Eng 2014; 42: 2589-2599.
39. Wilcox JN, Derynck R. Developmental expression of transforming growth factors alpha and beta in mouse fetus. Mol Cell Biol. 1988; 8: 3415-3422.
40. Chettibi S, Ferguson MWJ. Wound repair: an overview. In: Gallin J.I, Snyderman R, editors. Inflammation: basic principles and clinical correlates. 3rd ed. Lippincott Williams & Wilkins; Philadelphia, PA; 1999.
41. Norouzi M, Shabani I, Ahvaz HH, Soleimani M. PLGA/gelatin hybrid nanofibrous scaffolds encapsulating EGF for skin regeneration. J Biomed Mater Res A 2014; 103: 2225-2235.
42. Choi JS, Leong KW, Yoo HS. In vivo wound healing of diabetic ulcers using electrospun nanofibers immobilized with human epidermal growth factor (EGF). Biomaterials 2008; 29: 587-596.
43. Ronfard V, Rives JM, Neveux Y, Carsin H, Barrandon Y. Long-term regeneration of human epidermis on third

degree burns transplanted with autologous cultured epithelium grown on a fibrin matrix. Transplantation 2000; 70: 1588-1598.

44. Wright KA, Nadire KB, Busto P, Tubo R, McPherson JM, Wentworth BM. Alternative delivery of keratinocytes using a polyurethane membrane and the implications for its use in the treatment of full-thickness burn injury. Burns 1998; 24: 7-17.
45. Matsumura H, Gondo M, Imai R, Shibata D, Watanabe K. Chronological histological findings of cultured epidermal autograft over bilayer artificial dermis. Burns 2013; 39: 705-713.
46. Nair K, Gandhi M, Khalil S, Yan KC, Marcolongo M, Barbee K, Sun W. Characterization of cell viability during bioprinting processes. Biotechnol. J 2009; 4: 1168-1177.
47. Singh DT, Singh D, Han SS. 3D Printing of Scaffold for Cells Delivery: Advances in Skin Tissue Engineering. Polymers 2016; 8: 19.
48. Binder KW, Allen AJ, Yoo JJ, Atala A. Drop-on-demand inkjet bioprinting: A primer. Gene Ther Regul 2011; 11: 1-19.
49. Peng LH, Wei W, Qi XT, Shan YH, Zhang FJ, Chen X, Zhu QY, Yu L, Liang WQ, Gao JQ. Epidermal Stem Cells Manipulated by pDNA-VEGF165/CYD-PEI Nanoparticles Loaded Gelatin/β-TCP Matrix as a Therapeutic Agent and Gene Delivery Vehicle for Wound Healing. Mol Pharmaceutics 2013; 10: 3090-3102.

***Correspondence to**

Hongli Chen

Department of Life Sciences and Technology

Research Center of Stem Cell and Biotherapy Technology

Xinxiang Medical University

PR China