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Advances and opportunities for stem cell research in skin tissue engineering

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Abstract. – Skin tissue engineering has made significant progress over recent years, but there are still many factors that hamper its further development; these include the critical choice of seed cells. Many researchers eager to develop new cell-based skin products have focused on the use of stem cells, which have demonstrated many prospects for being put into clinical application. In this paper, we review the recent studies investigating the use of different tissue-derived stem cell as seed cells for skin tissue engineering.

Key Words:
Stem cells, Tissue engineering, Skin substitute, Seed cell, Application.

Introduction

In recent years, different types of tissue-engineered skin substitutes have been developed[1], and these engineered skin substitutes have played a significant role in the repair of chronic wounds and in studies investigating skin cells and skin diseases *in vitro*[2]. However, tissue-engineered skin products are not yet fully perfected and cannot meet the demands of clinical use. One important limitation to the production of applicable engineered skin products is that there is no clearly appropriate source of seed cells. Therefore, research on seed cells for tissue engineering is being actively pursued. Their pluripotency and capacity for continuous self-renewal support the prospect of the broad application stem cells. In theory, complex skin tissues composed of multiple cellular components could be cloned from a single stem cell.

The Current State of Seed Cells Used in Skin Tissue Engineering

Tissue-engineered skin actually developed from epidermal sheet, i.e. from epidermal substitute to full-thickness tissue engineered skin containing stem cells. Previously, tissue-engineered skin constructs have been derived from keratinocytes and fibroblasts seed cells isolated from the skin. However, culture of these autologous cells takes time, which limits their usefulness, and donor site insufficiency is a problem for patients with large skin defects. Moreover, immune rejection becomes a major problem when an allogeneic source of cells is used. In addition, mature cells from an autologous or allogeneic source are mostly terminally differentiated and, thus, have lost the ability to form new tissues via proliferation and differentiation. Therefore, these cells are not the best choice for tissue engineering or regenerative medicine. As stem cells have the capacity for self-renewal, demonstrate pluripotency, are highly proliferative, and have low immunogenicity, numerous research endeavors have focused on the use of stem cells in recent years, aiming to develop new skin products for clinical application.

Progress in the Use of Stem Cells for Skin Tissue Engineering

Embryonic Stem Cells and Induced Pluripotent Stem Cells

Human embryonic stem cells are derivatives of the inner cell mass of fertilized embryos and have two significant features: self-renewal and pluripotency. *In vitro*, embryonic stem cells can differentiate into cells of all three germ layers. Guenou et al. showed that human embryonic stem cells growing in induction medium containing BMP4 (bone morphogenetic protein-4) and ascorbic acid can differentiate into basal keratinocytes, resulting in a mature epidermis com-

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posed of multiple layers of differentiated cells[3]. These tissues were also successfully transplanted into nude mice to facilitate wound repair. These studies provide a theoretical basis for the use of embryonic stem cells as seed cells for tissue-engineered skin. However, embryonic stem cell research is handicapped by moral and ethical restraints, and the source of embryonic stem cells is thus problematic.

In 2006, Yamanaka et al at Kyoto University in Japan observed when four genes related to the pluripotency of embryonic stem cells (*Oct4*, *Sox2*, *c-Myc*, and *Klf4*) were simultaneously transfected into cells from the mouse tail that these cells acquired properties similar to those of embryonic stem cells after unwanted cells were removed[4,5]. Skin cells can also be similarly reprogrammed to become induced pluripotent stem (iPS) cells, which can be further induced to form all tissue types[6,7]. These iPS cells have the same characteristics as those of embryonic stem cells[6] and can differentiate into different types of tissues. Because their use avoids moral and ethical controversies, iPS cells are a promising new source of seed cells for use in skin tissue engineering. Continuing development of iPS-cell reprogramming technology paves the way for a readily available source of specific stem cells for use in tissue substitutes[8]. iPS cells can be produced by the reprogramming of keratinocytes isolated from a single adult hair and then differentiated into a variety of skin and seed-cell types. Bilousova et al. induced iPS cells *in vitro* to differentiate into a skin-like cell line and to form multi-differentiated epidermis, hair follicles, and sebaceous glands[9].

Studies have also shown that by introducing three transcription factors (Ngn3, Pdx1, and Mafa) into exocrine pancreatic cells of adult mice that these cells transform into β cells, while avoiding the requirement to first generate an iPS cell intermediate[10]. It can be speculated that keratinocytes isolated from hair can be reprogramed into various types of skin stem cells without the need for iPS cells, and by way of composite tissue engineering they can produce complex skin tissues composed of many cell types.

Amniotic Fluid Stem Cells

Another more primitive source of stem cells is the amniotic fluid and placenta. These isolated cells are called amniotic fluid placenta stem cells (AFPSCs), express markers of embryonic and adult stem cells, and fully meet the requirements of pluripotent stem cells. AFPSCs also circumvent the ethical problems preventing the use of embryonic stem cells as well as the restrictions of reprogramming and low production efficiency of iPS cells. Studies have shown that AFPSCs can differentiate into adipocytes, osteocytes, myocytes, endothelial cells, cells of the nervous system, liver cells, and all germ layers[11]. This pluripotency makes AFPSCs the ideal source for tissue engineering and regenerative medicine. However, there has been no report on the use of AFPSCs as seed cells for tissue engineering of skin.

Epidermal Stem Cells

Epidermal stem cells are mainly located in the basal cell layer and the outer root sheath of the hair follicle bulge. As specific stem cells of the skin, they are the key cells involved in epidermogenesis and wound repair[12,13]. Epidermal stem cells are monopotent and are theoretically an ideal choice for tissue-engineered skin. However, due to the lack of specific surface antigens on epidermal stem cells, their purification is involved and there is no effective system for their culture or amplification. As a consequence, they are still not widely applied in skin tissue engineering.

Recent studies have also shown that hair follicle stem cells have the ability to form skin appendages. The dermal papilla isolated from the adult hair follicle near the epidermis can induce the formation of new hair follicles[14]. Thus, hair follicle stem cells could be used for constructing tissue-engineered skin containing hair follicles.

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are involved in early mesoderm development and are a class of cells capable of remarkable self-renewal and multilineage differentiation. These cells can be induced to differentiate into osteocytes, chondrocytes, adipocytes, myocytes, and cells of the nervous system *in vitro* and *in vivo*. As such, these cells have the prospect of broad clinical application. MSCs are abundant and present in various tissues, such as the bone marrow, periosteum, thymus, skin, adipose tissue, muscle, umbilical cord, and cord blood[15,16], and as seed cells they are commonly used in tissue engineering. Perng et al[17] inoculated Green Fluorescent Protein (GFP)-labeled human bone marrow MSCs in a pNIPAAm [poly(N-isopropylacrylamide)] polymer for repair of skin defects in nude mice and found that the expression of human pan-keratin

and cadherin, which are indicators of epithelial regeneration, was significantly increased after replantation. These observations indicated that bone marrow MSCs can differentiate into epidermal cells and repair skin defects. In addition, bone marrow- and umbilical cord-derived MSCs can be induced to differentiate *in vitro* into skin fibroblast cells[18-19]. Progress has also been made in the culture and differentiation of hair follicle, sweat gland, and sebaceous gland appendage cells from MSCs. Sheng et al[20] successfully induced the phenotypic transformation to sweat gland cells from bone marrow MSCs by direct coculturing of bone marrow MSCs with normal sweat gland cells. Duffy et al. showed that MSCs growing in scaffolds can be induced to differentiate into vascular endothelial cells *in vitro*[21].

Adipose-derived MSCs (AMSCs) can be isolated from liposuction aspirates and have a similar pluripotent differentiation capacity as bone marrow MSCs. Moreover, the adipose tissues from which AMSCs are derived are widely available and abundant in cells. Compared with the number of stem cells in 100 ml of bone marrow, 300 times as many AMSCs can be obtained from 100 g of fat tissue[22,23]. Brzoska et al[24] found that all-trans retinoic acid can induce AMSCs to differentiate into epithelioid cells, and immunofluorescent microscopy showed that these differentiated AMSCs were positive for keratin K18. Therefore, AMSCs may be ideal seed cells for future skin tissue engineering. However, the molecular events that takes place in seed cells during repair of bone and cartilage, have not yet been fully characterized. Additionally, AMSCs can be easily induced *in vitro* to differentiate into adipocytes, and the mechanical properties of the bone and cartilage tissues derived from AMSCs are poor and these tissues readily degenerate. In addition, there are differences in the pluripotency of AMSCs from different species and/or different tissue sources. As such, it remains to be determined from which tissue site AMSCs are most effective.

Ideal seed cells should have the following characteristics: First, they must be highly proliferative and pluripotent. Second, they should be readily obtainable using less invasive methods. Finally, they must be expandable in large quantities *ex vivo*. Compared with other tissue sources of MSCs, umbilical cords have the advantages in that they are readily obtainable non-invasively, without ethical and moral constraints, and can be used extensively for research. Many studies have demonstrated that MSCs derived from the umbilical cord are similar to bone marrow MSCs in biological characteristics and that these cells can be further differentiated into myocardiocytes, adipocytes, and nerve cells[25-27]. Some investigators have compared the proliferative and pluripotent potentials between human MSCs derived from the umbilical cord and bone marrow and found that human umbilical cord MSCs (HUCMSCs) are more applicable for research and development[28]. In view of this, HUCMSCs are an ideal cell choice for skin tissue engineering, as they are a class of self-renewable, proliferating, and pluripotent stem cells and have clear advantages over MSCs from other sources, as shown in Table I. In the future, umbilical cord banks can be established, from which an individual's own HUCMSCs can be provided to him or her when needed. Therefore, HUCMSCs are an important source of stem cells for tissue regeneration and repair.

Recent studies on the plasticity of stem cells have made the reconstruction of skin appendages, including the sweat glands, possible. Our preliminary work shows that MSCs differentiation into sweat gland cells by simulating the genesis and development of the sweat glands, which may be one of the pathways for the reconstruction of the sweat glands, and it is expected that tissue-engineered full-thickness skin containing sweat gland structures can be generated. Therefore, *ex vivo* culture of epithelial cells of the sweat glands has potentially important biological and clinical significance, as this not only provides an excellent experimental system for

Table I. Comparison of the biological characteristics of mesenchymal stem cells from different sources.

Source	Acquisition	Cell content	Proliferation and differentiation	Immunogenicity	Ethical dispute
Marrow	Invasive, painful	Low	Low, affected by age	High	Exist
Adipose	Invasive	Low	Low, affected by age	High	Exist
Cord blood	Non-invasive	Extremely low	High	Low	No
Cord tissue	Non-invasive	Abundant	High	Low	No

further study of pathology and pathogenesis of sweat gland diseases, it also provides guidance for the development of tissue-engineered skin with appendages[29]. In short, HUCMSCs are likely to become an important source of stem cells for cellular transplantation and tissue engineering of skin in the future[30].

Problems Associated with the Use of Stem Cells

Research and development into the use of stem cells has made great progress over recent years, but there are still some problems associated with their application. First, a universally accepted and reliable stem cell surface marker has not yet been identified, which renders the screening and isolation of stem cells difficult. Second, the process of *ex vivo* culture of autologous stem cells is complex and expensive, and it takes considerable time to generate stem cells using current *ex vivo* culture systems for the repair of large acute skin defects (such as extensive burns). Finally, the precise induction mechanisms under different conditions are not fully characterized. Therefore, the regulation of stem cell differentiation still requires further study.

The ultimate goal of skin tissue engineering is the construction of skin substitutes for the rapid and complete reconstruction of skin function. This tissue-engineered skin should have all of the skin appendages (hair follicles, sebaceous glands, sweat glands, and receptors), good layering, rapid revascularization and reconstruction of nerve function, and scar-free healing with the adjacent tissue. Although many mature tissue-engineered skin products are being used clinically and many types of artificial skin have been developed, most of these products are only structurally similar to human skin, providing only a barrier function. Due to their lack of skin appendages, these tissues do not have the full function of the skin and do not represent true reconstructions of the skin.

At present, some researchers are attempting to reconstruct hair follicles *ex vivo*[31,32], but there has been no reports on the *ex vivo* reconstruction of sweat glands. Tissue-engineered skin products currently lack skin appendages and vascular structures, and there is also the problem of immune rejection of allogeneic seed cells. Therefore, these products suffer from a short period of viability and poor clinical function.

Mature skin cells such as fibroblasts can be reprogrammed genetically *in vitro* to be induced to form pluripotent iPS cells. Currently, this technology is in its infancy, but it is certain that this will be a new direction for future study of seed cells[33].

Outlook for the Engineering of Skin Tissues

Progress in tissue engineering for reconstruction of large skin defects is of great importance, but how to choose the best seed cells still requires further exploration. Stem cells, in this regard, show the most substantial promise. Stem cells can be induced to differentiate into various types of skin cells, and tissue engineered skin from such stem cells, in combination with proper scaffolds, can match the true structure of human skin and achieve excellent wound healing. We believe that with the development and technological progress of stem cell biology, engineering and materials science, and other disciplines, multi-functional full-thickness composite skin tissues containing normal blood vessels, sweat glands, sebaceous glands, nerves, and hair follicles can be generated. Through combined efforts and developments in these aspects, someday we will be able to fully realize tissue-engineered skin for proper wound repair.

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References

1) Auger FA, Lacroix D, Germain L. Skin substitutes and wound healing, Skin Pharmacol Physiol 2009; 22: 94-102.
2) MacNeil S. Progress and opportunities for tissue-engineered skin. Nature 2007; 445: 874-880.
3) Guenou H, Nissan X, Larcher F, Feteira J, Lemaitre G, Saidani M, Del Rio M, Barrault CC, Bernard FX, Peschanski M, Baldeschi C, Waksman G. Human embryonic stem-cell derivatives for full reconstruction of the pluristratified epidermis: a preclinical study. Lancet 2009; 374: 1745-1753.
4) Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors cell. Cell 2006; 126: 652-655.
5) Qi H, Pei D. The magic of four: induction of pluripotent stem cellsfrom somatic cells by Oct4, Sox2, Myc and Klf4. Cell 2007; 17: 578-580.

6) Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007; 448: 313-317.

7) Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, Bernstein BE, Jaenisch R. *In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature 2007; 448: 318-324.

8) Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, Gonzalez F, Vassena R, J, Pekarik V, Tiscornia G, Edel M, Boué S, lzpisua Belmonte JC. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotechnol 2008; 6: 276-1284.

9) Bilousova G, Chen J, Roop DR. Differentiation of mouse induced pluripotent stem cells into a multipotent keratinocyte lineage. J Invest Dermatol 2011; 131: 956-964.

10) Zhou Q, Brown J, Kanarek A, Rajagopal J, Melton DA. In vivo reprogramming of adult pancreatic exocrine cells to beta-cells. Nature 2008; 455: 627-632.

11) De Coppi P, Bartsch G Jr, Siddiqui MM, Xu T, Santos CC, Perin L, Mostoslavsky G, Serre AC, Snyder EY, Yoo JJ, Furth ME, Soker S, Atala A. Isolation of amniotic stem cell lines with potential for therapy. Nat Biotechnol 2007; 25: 100-106.

12) Blanpain C. Stem cells skin regeneration and repair. Nature 2010; 464: 686-687.

13) Abbas O, Mahalingam M. Epidermal stem cells: practical perspectives and potential uses. Br J Dermatol 2009; 16: 228-236.

14) Stenn KS, Cotsarelis G. Bioengineering the hair follicle: fringe benefits of stem cell technology. Curr Opin Biotechnol 2005; 16: 493-497.

15) Schneider RK, Püllen A, Kramann R, Bornemann J, Knüchel R, Neuss S, Perez-Bouza A. Long-term survival and characterisation of human umbilical cord-derived mesenchymal stem cells on dermal equivalents. Differentiation 2010; 79: 182-193.

16) Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 2007; 25: 2739-2749.

17) Perng CK, Kao CL, Yang YP, Lin HT, Lin WB, Chu YR, Wang HJ, Ma H, Ku HH, Chiou SH. Culturing adult human bone marrow stem cells on gelatin scafold with pNIPAAm as trmasplanted grafts for skin regeneration. J Biomed Mater Res A 2008; 84: 622-630.

18) He L, Nan X, Wang Y, Guan L, Bai C, Shi S, Yuan H, Chen L, Liu D, Pei X. Full-thickness tissue engineered skin constructed with autogenic bone marrow mesenchymal stem cells. Sci China C Life Sci 2007; 50: 429-437.

19) Sheng Z, Fu X, Cai S, Lei Y, Sun T, Bai X, Chen M. Regeneration of functional sweat gland-like structures by transplanted differentiated bone marrow mesenchymal stem cells. Wound Repair Regen 2009; 17: 427-435.

20) Han Y, Chai J, Sun T, Li D, Tao R. Differentiation of human umbilical cord mesenchymal stem cells into fibroblasts in vitro. Biochem Biophys Res Commun 2011; 413: 561-565.

21) Duffy GP, McFadden TM, Byrne EM, Gill SL, Farrell E, O'Brien FJ. Towards in vitro vascularisation of collagen-GAG scaffolds. Eur Cell Mater 2011; 21: 15-30.

22) Danisovic L, Varga I, Polák S, Ulicná M, Hlavackova L, Böhmer D, Vojtassák J. Comparison of in vitro chondrogenic potential of human mesenchymal stem cells derived from bone marrow and adipose tissue. Gen Physiol Biophys 2009; 28: 56-62.

23) Oedayrajsingh-Varma MJ, van Ham SM, Knippenberg M, Helder MN, Klein-Nulend J, Schouten TE, Ritt MJ, van Milligen FJ. Adipose tissue-derived mesenchymal stem cell yield and growth characteristics are affected by the tissue-harvesting procedure. Cytotherapy 2006; 8: 166-177.

24) Brzoska M, Geiger H, Gauer S, Baer P. Epithelial differentiation of human adipose tissue.derived adult stem cells. Biochem Biophys Res Commun 2005; 330: 142-150.

25) Friedman R, Betancur M, Boissel L, Tuncer H, Cetrulo C, Klingemann H. Umbilical cord mesenchymal stem cells: adjuvants for human cell transplantation. Biol Blood Marrow Transplant 2007; 13: 1477-1486.

26) Secco M, Zucconi E, Vieira NM, Fogaca LL, Cerqueira A, Carvalho MD, Jazedje T, Okamoto OK, Muotri AR, Zatz M. Multipotent stem cells from umbilical cord: cord is richer than blood! Stem Cells 2008; 26: 146-150.

27 Hardy SA, Maltman DJ, Przyborski SA. Mesenchymal stem cells as mediators of neural differentiation. Curr Stem Cell Res Ther 2008; 3: 43-52.

28) Baksh D, Yao R, Tuan R. Comparison of proliferative and multilineage differentiation potential of human mesenchymal stem cells derived from umbilical cord and bone marrow. Stem Cells 2007; 25: 1384-1392.

29) Tao R, Han Y, Chai J, Li D, Sun T. Isolation, culture, and verification of human sweat gland epithelial cells. Cytotechnology 2010; 62: 489-495.

30) Wu KH, Zhou B, Mo XM, Cui B, Yu CT, Lu SH, Han ZC, Liu YL. Therapeutic potential of human umbilical cord-derived stem cells in ischemic diseases. Transplant Proc 2007; 39: 1620-1622.

31) Qiao J, Turetsky A, Kemp P, Teumer J. Hair morphogenesis in vitro: formation of hair structures suitable for implantation. Regen Med 2008; 3: 683-692.

32) Nakao K, Morita R, Saji Y, Ishida K, Tomita Y, Ogawa M, Saitoh M, Tomooka Y, Tsuji T. The development of a bioengineered organ germ method. Nature Methods 2007; 4: 227-230.

33) Zhu XQ, Pan XH, Wang W, Chen Q, Pang RQ, Cai XM, Hoffman AR, Hu JF. Transient in vitro epigenetic reprogramming of skin fibroblasts into multipotent cells. Biomaterials 2010; 31: 2779-2787.