Review Article

Cell Therapy in Burn Repair

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Abstract

Received: 16/Mar/2008, Accepted: 3/May/2008

Skin replacement has been a challenging task in wound healing resulted from burn. The application of laboratory based tissue expansion techniques is a potential solution to the problem of surface area cover. Fortunately, considerable progress has been made in approaches to allograft and autograft skin transplantation in order to replace skin temporarily or permanently. Despite of this progress, development of new treatments for burn victims are still a problem in cultured skin grafts. Hair follicles, sweat glands and other features of normal skin are absent in cultured skin. Scientists believe that Stem cells with unique characteristics including self renewal and differentiation potential offer a possible way for reconstruction of some structures within the wound. So, enhanced understanding of stem cell potentials may help develop novel therapies to overcome the problems in wound healing.

Keywords: Burn, Cell Therapy, Skin Transplantation, Stem Cells

Yakhteh Medical Journal, Vol 10, No 3, Autumn 2008, Pages: 167-178

Introduction

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality. A report of the National Center for injury Prevention and Control in the United States shows approximately 1.2 million people affected with burn injuries (1). Burn injuries in Iran, like other developing countries, are much more common than in the USA. Moreover, the mean age of Iranian patients is less than others, and the mortality rate is higher. In recent epidemiology study in Tokyo, the overall mortality rate was 15.4%, whereas this rate in shiraz was 32% (2).

The choice between tissue repair or replacement method in acute or chronic loss of skin barrier needs information about burn severity and involved skin layers determination (3). Several factors are used to determine the severity of a burn injury, including the patient's age, size and depth of burn, and the location of the burn. Burns are classified by depth and they may be first, second, or third degree which sometimes known as superficial, partial thickness, or full-thickness, respectively (4).

First degree burns (Superficial Burns) involve minimal tissue damage and they involve only the superficial layer of skin (epidermis). This type of burn usually heals in 5-6 days without any permanent scarring. Second-degree burns (Partial-Thickness Burns) affect both the outer-layer (epidermis) and the underlying layer of skin (dermis) causing redness, pain, swelling and blisters. This type of burn usually heals in 3-4 weeks, and scar formation may occur. Third-degree burns (Full-Thickness Burns) affect the epidermis, dermis and hypodermis; causing charring of skin, or translucent white coagulated vessels visible just below the skin surface. This type of burn can be extremely painful or relatively painless if the burn destroys the nerve endings. This burn is critical and requires immediate medical attention.

Loss of the functional skin barrier leads to increase susceptibility to infection, the major cause of morbidity and mortality following burns. Skin is the body's largest organ. Its structure has been designed in a way that functions as the first line of defense protecting against the invasion of foreign bodies and organisms. It has specific immune and metabolic functions and is important in regulating body temperature, fluid and electrolytes (3). Skin contains the three main layers of the most superficial epidermal barrier layer and the lower, much thicker, dermis and the deepest, hypodermis or fat layer. The epidermal barrier layer is relatively thin (0.1-0.2 mm in depth) and the most common cells in the epidermis are keratinocytes that form the surface barrier layer. The dermis varies in thickness depending on its site in the body; composing primarily of collagen I, dermal inclusions of hair shafts, and sweat glands; which are lined with epidermal keratinocytes. Fibroblasts form the lower dermal layer and provide strength and resilience (5).

A number of approaches were taken in burn repair. One way is skin grafting. Advantages of graft-take to wound healing include an immediate barrier to microorganism

invasion and minimal new tissue synthesis required to close the defect. The take of a skin graft requires minimal new tissue synthesis (6). Other approaches are the developing of skin substitutes such as an acellular matrix complex that would guide the migration of fibroblasts into a pattern that had dermal-like qualities (7). Another way is to expand a small piece of epidermis into a very large transplantable viable autologous-epidermal cell layer through tissue culturing (8). The new method is to develop a skin equivalent composed of both a collagen matrix populated with viable fibroblasts and a dermal equivalent layer that is covered with viable keratinocytes (epidermal layer) (9). This skin equivalent have both a connective tissue component and viable cells (6).

However, methods for handling burn wounds have changed in recent decades like: transplantation, tissue engineering and now, stem cells therapies. But, questions related to optimal cell type for culture, culture techniques, transplantation of confluent sheets or non-confluent cells, immediate and late final take, carrier and transfer modality, as well as final outcome, ability to generate an epithelium after transplantation, and scar quality are still not fully answered. In this review article, we are going to mention the current and promising cell therapy methods to burn repair.

Skin grafts

There are a variety of skin grafts, some that provide temporary cover and others that are for permanent wound coverage.

Allogenic skin graft (Temporary Wound Covering)

Allogeneic or alloplastic skin substitute coverage as a temporary solution is necessary until definitive cover can be achieved (10-18). The clinical use of allograft skin in the modern era was popularized by James Barrett Brown, who described its use in 1942. Skin grafting, which consists of excision or the surgical removal of burn injured tissue; choosing a donor site or an area from which healthy skin is removed to be used as cover for the cleaned burned area; and harvesting, where the graft is removed from the donor site by a dermatome that shaves a piece of skin, about 10/1000 of an inch thick, off the unburned area. Finally, the surgeon places and secures the skin graft over the surgically cleaned wound so that it can heal. To help the graft heal and become secure, the area of the graft is not moved for five days following each surgery (immobilization period). During this immobilization period, blood vessels begin to grow from the tissue below into the donor skin, bonding the two layers together. Five days after grafting, exercise therapy programs, tub baths and other normal daily activities resume (19). Allogenic skin grafts may be completely integrated into the healing wound initially and bridge the critical time gap in the early phase of burn treatment.

However, the need to provide skin cover in a situation of inadequate donor sites lead to the interest in culturing elements of the uninjured allograft skin which is associated with accelerated wound healing. It seems, the resulted wound healing by cultured epidermal allografts is attributable to cytokine contents in the cultured epidermis, e.g. TGF-α, IL-1α, IL-1β, IL-6, IL-8, GM-CSF and keratinocyte derived T-cell growth factors, which they could reconstruct damaged areas quicker (20-25). Allografts, however, ultimately provoke rejection through the expression of immunological crucial HLA-DR antigen by the Langerhans cells (11, 16, 26-28). Clinicians have responded to this problem of rejection with the increased use of immunosuppressive therapies, but the harmful consequences have limited the widespread clinical application of this approach.

The other limitation in application of uncultured skin allograft is for children. Burn treatments in children compared to adults are associated with several difficulties, e.g. limitation of available skin replacement, expansion of donor area, increase in subsequent hypertrophic scar and contracture due to their physical growth. The principal targets in the treatment of burns in pediatric patients are (i) early closure of wounds; (ii) minimize scar size; and (iii) minimize donor area. Yanaga et.al (29) have applied cryopreserved cultured epidermal allografts to pediatrics (Fig 1).

Autogenic skin graft (Permanent Wound Covering)

They believe cryopreserved cultured epidermal allografts have several advantages: (i) it is frozen stored, and can be used anytime when necessary; (ii) it brings about early closure of wounds; (iii) it can be applied repeatedly; and (iv) a donor is not required, but the disadvantage is that it is not taken permanently (29).

Autograft is skin taken from the person burned, which is used to cover wounds permanently. There are two types of uncultured autografts used for permanent wound coverage: sheet grafts and meshed grafts. It is notable that uncultured skin autograft is used for limited burned area and for widespread burns; the cultured autograft skin is needed.

Uncultured skin autograft

Sheet Graft

Sheet Graft is piece of donor skin, removed from an unburned area of the body, a process called «harvesting the donor». The size of the donor skin that is used to patch a burned area is about the same size as the burn size. The donor sheet is laid over the excised wound and stapled in place. The disadvantages of sheet grafts are that small areas of graft might be lost from build up of fluid (hematoma) under the sheet right after surgery. Sheet grafts also need a larger donor site than meshed skin. A sheet graft is usually more durability and scars less (19).

Meshed skin graft

It is difficult to cover when there is very large areas of open wounds because of not enough unburned donor skin availability. So, it is necessary to enlarge donor

skin to cover a larger body surface area. Meshing is a mean to enlarge donor skin. Meshing involves running the donor skin through a machine that makes small slits, which allows expansion similar to that in fish netting. In a meshed skin graft, the skin from the donor site is stretched to allow it to cover an area larger than itself. Most donor skin is meshed at a 1:1 or 1:2 ratio because the larger the size mesh the more fragile the graft. No matter what size meshing is used; healing occurs as the spaces between the mesh; called the intricities, fill in with new epithelial skin growth. The disadvantages of meshing are to be a less durable graft than a sheet graft. Meshing serves two purposes: it allows blood and body fluids to drain from under the skin grafts, preventing graft loss, and it allows the donor skin to cover a greater burned area because it is expanded (19).

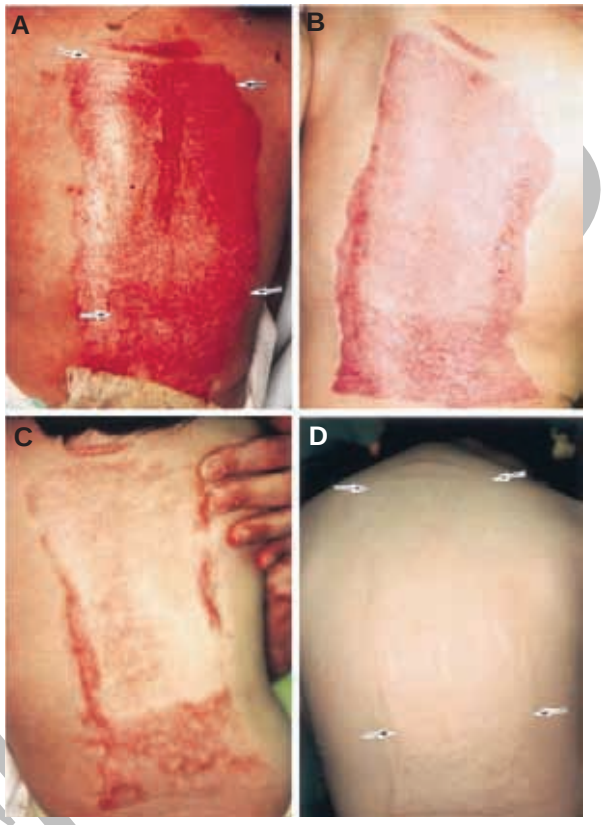

***Fig 1:** A 10-month-old baby girl. A) Seven days after grafting cryopreserved cultured epidermal allografts on the recipient site of the back. Inside the arrow heads shows the area where the grafts were taken. B) One month after grafting. The grafted area has milder redness compared to the non-grafted area. C) Seven months after grafting. The grafted area has less scar formation compared to the non-grafted area. D) Thirty-nine months after grafting. Scar formation was clearly suppressed on the grafted area. Inside the arrow heads shows the grafted area. Adopted from (29).*

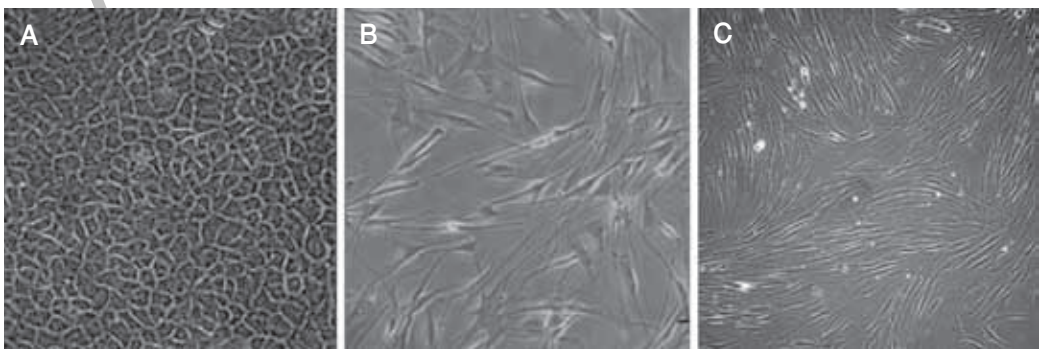

***Fig 2:** The main cells of skin. A) Keratinocytes; are the most common cell type in the epidermis and form the surface barrier layer. B) Melanocytes; are found in the lower layer of the epidermis and provide skin color, C) Fibroblasts form the lower dermal layer and provide strength and resilience*

Cultured skin autograft

In massive burns, however, the available skin donor sites for autografting may be very limited. This has fostered the development of alternative methods such as autologous cultured skin graft and allograft skin substitutes as mentioned before. Two main techniques in autogenous graft for burn treatment include "cultured epithelial autografts; CEA" and "cell suspension".

Cell cultured epithelial autograft (CEA)

In 1975, Reinwald and Green demonstrated that disaggregated epidermal cells (Fig 2) could be isolated and serially sub-cultured in vitro (shown in Fig 3) (30). Shortly afterwards, viable epithelial sheets, suitable for grafting were produced. From 1981, clinical case reports describing the use of cultured keratinocytes as permanent autografts in burn wound management were published (31).

Cultured sheets of human autologous epithelium (CEA = cultured epithelial autografts) still represent the "gold standard" to resurface large wounds (8). So, cultured epidermal sheet autografts became available to complement autologous split thickness skin grafts in treating major burns or other large wounds (8).

Despite of more laboratory skills in producing confluent grafts of keratinocytes, the epidermal sheet grafts have several shortcomings (13, 33, 34). First, harvesting the cell sheets from the culture dishes by trypsin treatment could damage the anchoring proteins of the cells (35-37). This could be one of the reasons of a mechanical instability of epidermal sheet grafts and insufficient dermal–epidermal reconstitution that lowers the uptake ratio of the grafts for a long time after transplantation (36, 38, 39). Second, epidermal sheet grafts usually require a long fabrication period (40). Third, cultured epidermal sheet grafts composed of fully differentiated keratinocytes might not exhibit further proliferation of keratinocytes after transplantation (35). Fourth, epidermal sheets are only 8–10 cells thick, which make them fragile and difficult to handle (8, 36) and have high costs of production (37).

The attention to, and understanding of, these shortcomings have led to a progressive development of skin culture techniques and an increased use of suspensions of keratinocytes single cells being transplanted instead of sheet grafts.

Cell suspensions

Surprisingly good clinical results using the technique of "epithelial cell seeding" had been published by von Mangoldt as early as in 1895 to treat chronic wounds and wound cavities (41). In his original description he harvested epithelial cells or cell clusters by scraping off superficial epithelium from a patient´s forearm with a surgical blade until fibrin was exudated from the wound. This mixture was then applied to wounds. He claimed reduced donor site morbidity and a more regular aspect of the resurfaced wounds when compared to the method of Reverdin, which was the common method at that time. One of his key observations was the fact that single cells or cell clusters would better attach to the wound bed than conventional pieces of skin.

One problem associated with pipetting keratinocytes in suspension is to prevente spillage of cells from the wound and achieve an even delivery (42). Fraulin et al. (43), in 1998, described a novel technique in which they used an aerosol device to spray epithelial cells on wounds in pigs. They noted that re-epithelialisation, re-growth of epithelial tissue over a denuded surface, was quicker than in unsprayed controls. Further advantages of suspension transplantation are to reduce time needed for culture and the fact that suspended keratinocytes can be transported from laboratory to patient in small vials, thus reducing the costs involved and storing frozen in clinic for transplantation (44). Because the cells, in culturing and transplanting, are as a suspension rather than a sheet; the use of enzymes like, Dispase1 can be avoided (45). Navarro et al. (46) developed this technique further by combining it with meshed split thickness skin grafts. They reported faster healing and a better quality of cells when they were sprayed.

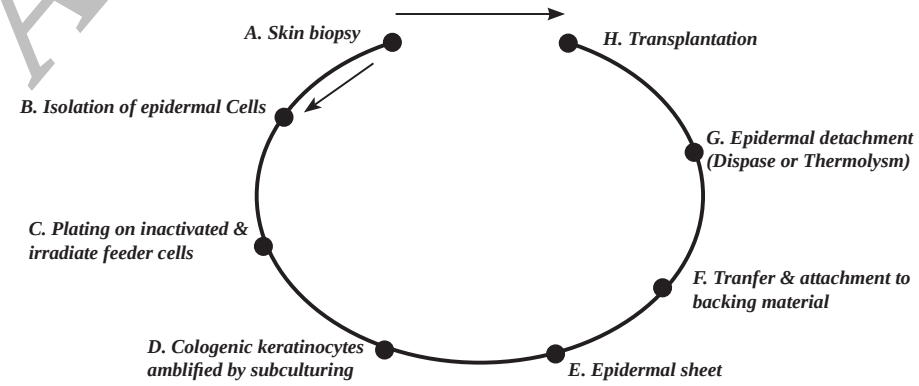

Fig 3: Diagram showing the various steps of the classical keratinocyte culture technique described by Rheinwald and Green. Adopted by modification from (32).

A comparative in vitro study has been done by Fredriksson and et al. (44), considering commonly used application techniques. Although, it has not been compared in vivo with in vitro condition, it did provide valuable information about different measures in transplantation of autologous keratinocytes as a single cell suspension. There is hope that, with further studies, advances in this field will lead to the development of an equipment that is fairly cheap and easy to operate (44).
However, an alternative approach to facilitate the delivery of keratinocytes in suspension is to use a matrix material such as fibrin glue to fix the cells (47).

Membrane delivery systems
To transfer preconfluent keratinocytes to a wound, a delivery system is required. Various methods have been described. Cells can be grown in a culture vessel, trypsinized, and applied directly in suspension or grown directly onto a delivery membrane that is then removed from the dish, inverted, and applied to the wound bed.
A number of delivery systems based on biological tissue including Collagen I (48-51), Fibrin Glue, a human plasma protein concentrate that contains fibrinogen, factor XIII, and fibronectin that have undergone viral inactivation (52, 53), Hyaluronic Acid (54), Acellular Porcine Dermis (55) or based on synthetic polymers such as Polyurethane (56), Polymeric Film (57), Teflon® Film (58), Poly(hydroxyethyl Methacrylate) (59), Celltran (60), Spherical Microcarriers (61) have been developed (review in (56).
Membrane delivery systems have the advantage of easy handling and ensuring contact of cells with the wound. The potential disadvantages are that a proportion of keratinocytes may not attach to the membrane, and of those that do attach, not all will transfer to the wound bed (56). These potential inefficiencies need to be assessed for each delivery method. However, it is difficult to compare the efficacy of the delivery systems because of variations in used keratinocyte seeding density and studied types of wound.
Moreover, these delivery methods only transfer keratinocytes and are only part of the solution to wound coverage after full-thickness skin loss in burns patients. It is widely appreciated that the addition of a dermal substitute to such a wound is important for stable wound healing (62-65). This may also require the transplantation of fibroblasts to enhance healing further and improve the mechanical properties of the graft (66-68). The role of delivery of preconfluent keratinocytes in conjunction with methods of dermal delivery should also be assessed.

Table 1: Current commercially available or marketed matrices and products for tissue engineered skin substitutes. Adopted from (41).

Material	Brand Name	Manufacture
Collagen gel + cult. Allog. HuK + allog. HuFi	Apligraf™ (earlier name: Graftskin™)	Organogenesis, Canton, MA
cult. Autol HuK	Epicell™	Genzyme Biosurgery, Cambridge, MAq
PGA/PLA + ECMP DAHF	Transcyte™	Advanced Tissue LaJolla, CA
Collagen GAGsilicone foil	Integra™	Integra LifeScience, Plainsborough, NJ
Acellular dermis	AlloDerm™	Lifecell Corporation, Branchberg, NJ
HAM + cult. HuK	Laserskin™	Fidia Advanced Biopolymers, Padua, Italy
PGA/PLA + allog. HuFi	Dermagraft™	Advanced Tissue Sciences, LaJolla, CA
Collagen + allog HuFi +allog HuK	Orcel™	Ortec International, Inc., New York, NY
Fibrin sealant + cult. Autol HuK	Bioseed ™	BioTissue Technologies, Freiburg, Germany
PEO/PBT + autol. HuFi +cult autol HuK	Polyactive ™	HC Implants
HAM + HuFi	Hyalograft 3D™	Fidia Advanced Biopolymers, Padua, Italy
Silicone + nylon mesh + collagen	Biobrane™	Dow Hickham/Bertek Pharmac., Sugar Land, Tx

ECMP = extracellular matrixproteins, DAHF= derived from allog. HuFi, GAG=glycosaminoglycan, PGA = polyglycolic acid (Dexon™), PLA = polylactic acid(Vicryl™), PEO = polyethylen oxide, PBT = polybutyliterephthalate, cult. = cultured;autol.= autologous, allog. = allogeneic, HuFi = human fibroblasts, HuK= human keratinocytes, HAM = microperforated Hyaluronic Acid Membrane (benzilic esters of hyaluronic acid =HYAFF-11®)

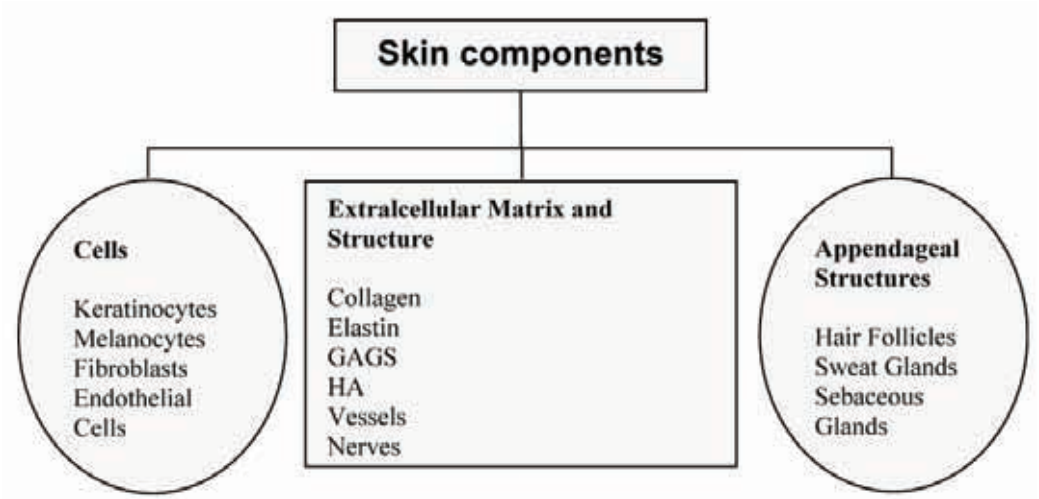

Fig 4: Skin components in tissue engineering.

At the moment, scientific principles and practical approaches to replace skin temporarily or permanently are advancing at a rapid rate. However, there is definitely need further progress to optimize skin substitute performance by tissue engineering procedures.

Skin tissue engineering

The skin is indeed a complex structure incorporating a fusion of several different cell types, integrated within a three dimensional matrix containing both fibrillar and nonfibrillar elements. To synthesize such a complex structure by identifying the component parts and to put them together is neither practical nor realistic. It must be observed, however, that this integrative strategy has been the major one used in skin tissue engineering during its less productive phase (69).

Three factors should be considered in the development of tissue-engineered materials: the safety of the patient, clinical efficacy and convenience of use. Any cultured cell material carries the risk of transmitting viral or bacterial infection, and some support materials (such as bovine collagen and murine feeder cells) may also have a disease risk. There must be clear evidence that tissue-engineered materials provide benefit to the patient. Essential characteristics are that it heals well and has the physical properties of normal skin. To achieve effective healing, the tissue-engineered products must attach well to the wound bed, be supported by new vasculature, not be rejected by the immune system and be capable of self repair throughout a patient's life. Finally, materials need to be convenient to use or they will not achieve clinical uptake (5).

Most tissue-engineered skin is created by expanding skin cells in the laboratory (at a rate much greater than would be achieved on the patient) and used to restore barrier function (the primary objective for burns patients) or to initiate wound healing (for chronic non-healing ulcers). Currently, commercially available or marketed matrices and products for tissue engineered skin substitutes are shown in Table 1. There are those that replace the epidermal layer only, those that provide a dermal substitute, and a small number that provide both. In some clinical conditions (such as non-healing ulcers and superficial burns) simply transferring laboratory-expanded cells can benefit patients, but the treatment of major full-thickness burns requires the replacement of both dermis and epidermis. There are four major challenges in this field: improving safety, finding a substitute for split-thickness grafts, improving angiogenesis in replacement tissue once it has been grafted to the wound bed, and improving ease of use (5). Fig 4 shows the 'biological' as opposed to the 'engineering' concepts of the skin structure.

Although progress has been made in developing new treatments for burn victims, including skin grafting and artificial skin technologies; these cultured skin grafts do not have hair follicles, sweat glands and other features of normal skin. The result is thin, inflexible skin (which hampers mobility of joints), and skin that dramatically differs from the remaining healthy skin. A promising alternative to these techniques is stem cell-based therapy. Scientists believe that results of stem cell research will help identify those cells responsible for differentiating into the various elements that comprise the dermis, and eventually produce skin that will help patients heal quicker with less scarring and more flexibility, and perhaps, even produce a skin that literally matches that of the rest of the body.

Stem cell strategies in burn care

Stem cells are characterized by their prolonged self renewal capacity and their asymmetric replication (70) (Fig 5). Asymmetric replication describes a special property of stem cells: with every cell division, one of the cells retain its self-renewing capacity, whereas the other enter a differentiation pathway and join a mature non-dividing population (71). Stem cells were first identified as pluripotent cells in embryos, and these were called embryonic stem (ES) cells which are defined by

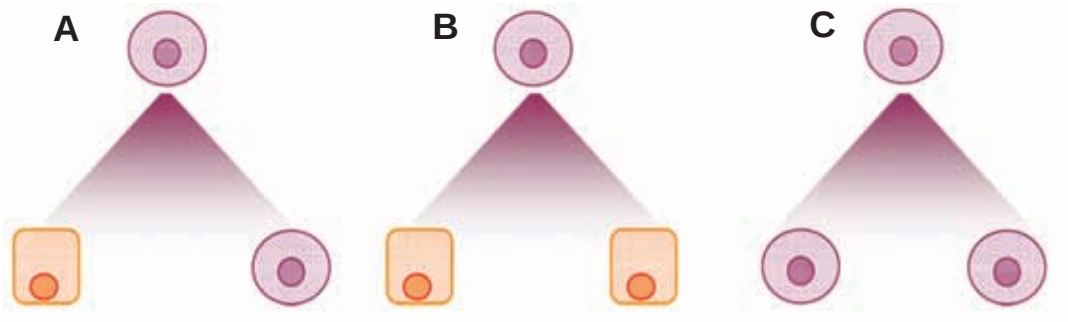

Fig 5. Asymmetric and Symmetric division of Stem cells. A) If stem cell (SC) replication gives rise to one daughter cell that retains SC capabilities and the other differentiates, the preceding mitotic event is considered to be asymmetric. B, C)) If stem cell (SC) replication gives rise to daughter cells that share the same fate (become committed to differentiate or SCs), the preceding division is considered to be symmetric. (This figure has also been printed in full-color at the end of the issue)

their origin (the inner cell mass of the blastocysts) (72). It is now clear that stem cells are also present in many, if not all, tissues in adult animals and contribute to the maintenance of tissue renewal and homeostasis. Currently, the challenge is to define the optimum source; processing and method of application of stem cells as well as defining their role.

It has been known for several decades that the epidermis of the skin contains a subpopulation of basal cells that exhibit the properties expected of somatic stem cells: slow cell cycle, high proliferative potential, location in a protected niche, capacity to maintain and repair the tissue in which they reside, and long life span (Fig 6) (73-77). Slowly, cycling epidermal stem cells have been identified by long-term nuclear retention of tritiated thymidine or bromodeoxyuridine label (74, 78). These undifferentiated label-retaining stem cells have been shown to reside in the bulge area of the hair follicle, (76, 79, 80) and in the interfollicular basal layer of the epidermis (74, 78, 81). They are self-renewing and able to produce daughter transient amplifying cells that undergo a finite number of cell divisions before they differentiate and leave the proliferative basal compartment, a property similar to stem cells in other continuously renewing tissues (82). Scientists have found that skin progenitor stem cells (keratinocyte progenitors) in adult human skin have a significant capacity for growth and tissue regeneration.

Stem cells can be induced to differentiate into cells with specialized functions, such as skin keratinocytes (84). It is for this reason that stem cells show such potential for treating burns. But what type of stem cell shows the most applicable? The clinical application of embryonic stem cells is likely to beset with numerous ethical but also safety concerns. Fetal tissue, likewise, will be associated with ethical issues. The reality of widespread applications of stem cells devoid of complex ethical dimensions really begins with human umbilical cord blood and Bone-marrow derived stem cells. This has been used in a number of clinical 'haematopoetic' applications as a 'transplant' which underlines the safety and efficacy of these stem cell sources (85-87).

There are two main branches of stem cells in the bone marrow (BM), hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) (Fig 7). Adult bone marrow-derived HSCs have long been recognized to give rise to all blood cell lineages and some non-blood cells such as hepatocytes, (88) endothelial cells (EC), smooth muscle cells, and cardiac myocytes (89).

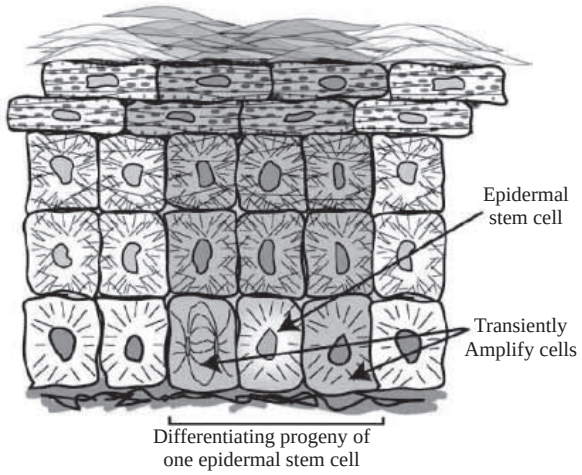

Fig 6: The epidermal stem cell. Adopted from (83)

However; much controversy exists over HSC plasticity. In contrast, BM-MSCs are self-renewing, clonal precursors of non-hematopoietic tissues. Although they are present as a rare population of cells in bone marrow, representing perhaps 0.001–0.01% of the nucleated cells and about 10-fold less abundant than HSCs; they are expandable in culture, multipotent, and capable of differentiating into osteoblasts, chondrocytes, astrocytes, pneumocytes, hepatocytes, neurons, and cardiac myocytes (89-95). As Bone Marrow derived Cells (BMDCs) have been found in skin epidermis in several studies (96-100),

it is assumed that Bone Marrow Stem Cells (BMSCs) may be involved in skin repair and regeneration.
The most studied progenitor cell type is the hematopoietic stem cell (HSC) from the bone marrow. By creating chimeric mice that express green fluorescent protein (GFP) only in their bone marrow cells; Hocking and et al (96) have found that HSCs migrate to sites of dermal injury, differentiate into several cell phenotypes, and incorporate into the cutaneous wound for the long term. The majority of these bone marrow derived cells resemble undifferentiated dermal fibroblasts with occasional dendritic type cells and endothelial cells. These findings suggest that bone marrow derived cells in the wound, not only participate in the inflammatory response, but are an important source of cells for reconstituting the dermis.

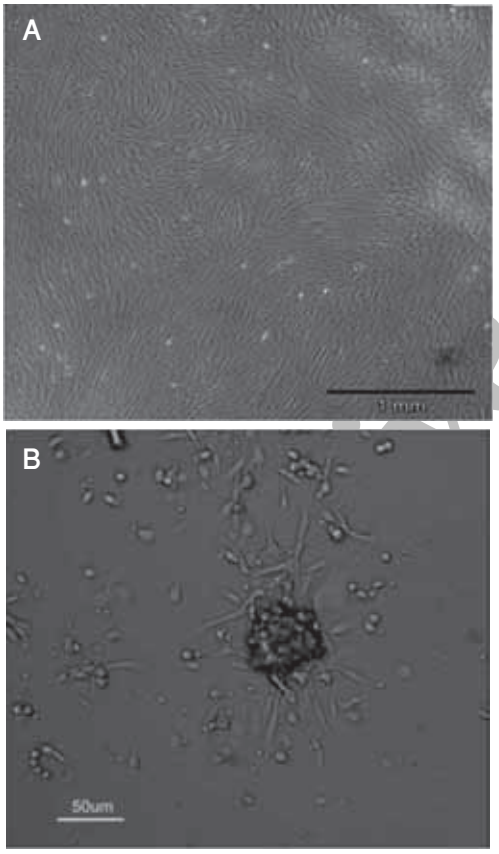

Fig 7: Bone Marrow derived stem cells. A) Mesenchymal stem cell, B) Heamatopoietic stem cell colony

MSCs seem to be non immunogenic and maybe ''universal''(101). They can confer a state of immune tolerance to the recipient (97-100). If this is true, a new era of understanding will be started in Transplantation. But, why should a cell have evolved in such a way to be involved both in regeneration and tolerance? Burn patients have transient states of immune suppression and acceptance of allografts in the acute phase in parallel with their increased pool of circulating MSCs (101, 102). Direct injection of bone marrow derived mesenchymal stem cells or endothelial progenitor cells into injured tissues shows improved repair through mechanisms of differentiation and/or release of paracrine factors (70). Previous studies have shown that cultured Epithelial Cells(EPCs) release growth factors, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor, G-CSF, GM-CSF, and platelet-derived growth factor-B61 that could exert a protective effect on endogenous EC and other myocardial cells. Cultured BM-MSCs have been found to release VEGF, basic fibroblast growth factor (bFGF), IL-6, placental growth factor (PlGF), and monocyte chemoattractant protein-1 (103).
Could MSCs be the link between this state of tolerance and the capacity to regenerate? If it is to be proved, the new field of Regenerative Medicine, Transplantation and Burns beside many other disciplines will profoundly benefit from these discoveries without any doubt. In fact, Han et al (104), have been shown that Burn rat serum has a stronger chemotactic effect on MSCs and the migration ability of MSC derived from burn rat is stronger than that of MSC derived from normal rat.
The prospect of being able to replace damaged tissue by the process of regeneration would dramatically and irrevocably change the impact, management and outcome of burns. The current understanding of stem cell-based modulation and therapy together with their potential developments bring this prospect ever closer to a clinical reality. Despite of the potential surrounding the stem cell field, we remain a long way from translating the research now being conducted in laboratories to therapies for patient.

Conclusion & Future outlook

Burns are one of the most harmful and complex physical injuries. They often happen unexpectedly and have the potential to cause death, lifelong disfigurement and dysfunction. The challenge of surviving a major burn depends on skin repair. Recently, skin grafting has evolved from the initial autograft and allograft preparations to biosynthetic and tissue-engineered living skin replacements. Tissue engineering now provides the clinician with more therapeutic options and more challenges. Consequently, it is essential to critically analyze the clinical needs of skin repair and understand skin replacement in terms of the availability, compatibility, safety and durability.
However both through basic and clinical research, there will be major improvements in the understanding and ability to effectively deal with the problems of wound healing and replace a truly functional skin with dermal appendages. Research on stem cells may lead to improve skin reconstitution, while overcoming current limits of donor sites and donor site morbidity in afflicted patients.

References

1. Http://www.americanburn.org.
2. Rajabian MH, Aghaei S, Fouladi V. Analysis of survival and hospitalization time for 2057 burn patients in Shiraz, southwestern Iran. Med Sci Monit, 2007; 13(8): CR353-355
3. Slavin J. The role of cytokines in wound healing. J Pathol, 1996; 178(1): 5-10
4. Mousa HA. Burn and scald injuries. East Mediterr Health J, 2005; 11(5-6): 1099-1109
5. MacNeil S. Progress and opportunities for tissue-engineered skin. Nature, 2007; 445(7130): 874-880
6. Ehrlich HP. Understanding experimental biology of skin equivalent: from laboratory to clinical use in patients with burns and chronic wounds. Am J Surg, 2004; 187(5A): 29S-33S
7. Burke JF, Yannas IV, Quinby WC, Jr., Bondoc CC, Jung WK. Successful use of a physiologically acceptable artificial skin in the treatment of extensive burn injury. Ann Surg, 1981; 194(4): 413-428
8. Gallico GG, 3rd, O'Connor NE, Compton CC, Kehinde O, Green H. Permanent coverage of large burn wounds with autologous cultured human epithelium. N Engl J Med, 1984; 311(7): 448-451
9. Bell E, Ehrlich HP, Buttle DJ, Nakatsuji T. Living tissue formed in vitro and accepted as skin-equivalent tissue of full thickness. Science, 1981; 211(4486): 1052-1054
10. Gallico GG, 3rd. Biologic skin substitutes. Clin Plast Surg, 1990; 17(3): 519-526
11. Horch R, Stark GB, Kopp J, Spilker G. Cologne Burn Centre experiences with glycerol-preserved allogeneic skin: Part I: Clinical experiences and histological findings (overgraft and sandwich technique). Burns, 1994; 20 Suppl 1: S23-26
12. Horch RE, Corbei O, Formanek-Corbei B, Brand-Saberi B, Vanscheidt W, Stark GB. Reconstitution of basement membrane after 'sandwich-technique' skin grafting for severe burns demonstrated by immunohistochemistry. J Burn Care Rehabil, 1998; 19(3): 189-202
13. Nanchahal J, Dover R, Otto WR. Allogeneic skin substitutes applied to burns patients. Burns, 2002; 28(3): 254-257
14. Phillips TJ. Biologic skin substitutes. J Dermatol Surg Oncol, 1993; 19(8): 794-800
15. Rouabhia M. In vitro production and transplantation of immunologically active skin equivalents. Lab Invest, 1996; 75(4): 503-517
16. Rouabhia M, Germain L, Bergeron J, Auger FA. Allogeneic-syngeneic cultured epithelia. A successful therapeutic option for skin regeneration. Transplantation, 1995; 59(9): 1229-1235
17. Shakespeare P. Burn wound healing and skin substitutes. Burns, 2001; 27(5): 517-522
18. Van Luyn MJ, Verheul J, van Wachem PB. Regeneration of full-thickness wounds using collagen split grafts. J Biomed Mater Res, 1995; 29(11): 1425-1436
19. http://www.phpsc.com/burn_care.htm
20. Coffey RJ, Jr., Derynck R, Wilcox JN, Bringman TS, Goustin AS, Moses HL, et al. Production and auto-induction of transforming growth factor-alpha in human keratinocytes. Nature, 1987; 328(6133): 817-820
21. Kupper TS, Ballard DW, Chua AO, McGuire JS, Flood PM, Horowitz MC, et al. Human keratinocytes contain mRNA indistinguishable from monocyte interleukin 1 alpha and beta mRNA. Keratinocyte epidermal cell-derived thymocyte-activating factor is identical to interleukin 1. J Exp Med, 1986; 164(6): 2095-2100
22. Grossman RM, Krueger J, Yourish D, Granelli-Piperno A, Murphy DP, et al. Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proc Natl Acad Sci USA, 1989; 86(16): 6367-6371
23. Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K. Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumour necrosis factor. Immunology, 1989; 68(1): 31-36
24. Kupper TS, Lee F, Coleman D, Chodakewitz J, Flood P, Horowitz M. Keratinocyte derived T-cell growth factor (KTGF) is identical to granulocyte macrophage colony stimulating factor (GM-CSF). J Invest Dermatol, 1988; 91(2): 185-188
25. Eisinger M, Sadan S, Silver IA, Flick RB. Growth regulation of skin cells by epidermal cell-derived factors: implications for wound healing. Proc Natl Acad Sci U S A, 1988; 85(6): 1937-1941
26. Alsbjorn B. In search of an ideal skin substitute. Scand J Plast Reconstr Surg, 1984; 18(1): 127-133
27. Heimbach D, Luterman A, Burke J, Cram A, Herndon D, Hunt J, Jordan M, McManus W, Solem L, Warden G, et al. Artificial dermis for major burns. A multi-center randomized clinical trial. Ann Surg, 1988; 208(3): 313-320
28. Kohnlein HE. (Skin transplantation and skin substitutes). Langenbecks Arch Chir, 1970; 327: 1090-1106
29. Yanaga H, Udoh Y, Yamauchi T, Yamamoto M, Kiyokawa K, Inoue Y, et al. Cryopreserved cultured epidermal allografts achieved early closure of wounds and reduced scar formation in deep partial-thickness burn wounds (DDB) and split-thickness skin donor sites of pediatric patients. Burns, 2001; 27(7): 689-698
30. Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. Cell, 1975; 6(3): 331-343
31. Wood FM, Kolybaba ML, Allen P. The use of cultured epithelial autograft in the treatment of major burn wounds: eleven years of clinical experience. Burns, 2006; 32(5): 538-544
32. Atiyeh BS, Costagliola M. Cultured epithelial autograft (CEA) in burn treatment: three decades later. Burns, 2007; 33(4): 405-413
33. Munster AM. Whither (corrected) skin replacement? Burns, 1997; 23(1)
34. 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(11): 1588-1598
35. Voigt M, Schauer M, Schaefer DJ, Andree C, Horch R, Stark GB. Cultured epidermal keratinocytes

on a microspherical transport system are feasible to reconstitute the epidermis in full-thickness wounds. Tissue Eng, 1999; 5(6): 563-572
36. Woodley DT, Peterson HD, Herzog SR, Stricklin GP, Burgeson RE, Briggaman RA, et al. Burn wounds resurfaced by cultured epidermal autografts show abnormal reconstitution of anchoring fibrils. Jama, 1988; 259(17): 2566-2571
37. Herndon DN, Rutan RL. Comparison of cultured epidermal autograft and massive excision with serial autografting plus homograft overlay. J Burn Care Rehabil, 1992; 13(1): 154-157
38. Brown KW, Parkinson EK. Alteration of the extracellular matrix of cultured human keratinocytes by transformation and during differentiation. Int J Cancer, 1985 ;35(6): 799-807
39. Herzog SR, Meyer A, Woodley D, Peterson HD. Wound coverage with cultured autologous keratinocytes: use after burn wound excision, including biopsy followup. J Trauma, 1988; 28(2): 195-198
40. Rouabhia M. Permanent skin replacement using chimeric epithelial cultured sheets comprising xenogeneic and syngeneic keratinocytes. Transplantation, 1996; 61(9): 1290-1300
41. Horch RE, Kopp J, Kneser U, Beier J, Bach AD. Tissue engineering of cultured skin substitutes. J Cell Mol Med, 2005; 9(3): 592-608
42. Billingham RE, Reynolds J. Transplantation studies on sheets of pure epidermal epithelium and on epidermal cell suspensions. Br J Plast Surg, 1952; 5(1): 25-36
43. Fraulin FO, Bahoric A, Harrop AR, Hiruki T, Clarke HM. Autotransplantation of epithelial cells in the pig via an aerosol vehicle. J Burn Care Rehabil, 1998; 19(4): 337-345
44. Fredriksson C, Kratz G, Huss F. Transplantation of cultured human keratinocytes in single cell suspension: A comparative in vitro study of different application techniques. Burns, 2007
45. Gustafson CJ, Kratz G. Cultured autologous keratinocytes on a cell-free dermis in the treatment of full-thickness wounds. Burns, 1999; 25(4): 331-335
46. Navarro FA, Stoner ML, Park CS, Huertas JC, Lee HB, Wood FM, et al. Sprayed keratinocyte suspensions accelerate epidermal coverage in a porcine microwound model. J Burn Care Rehabil, 2000; 21(6): 513-518
47. Kaiser HW, Stark GB, Kopp J, Balcerkiewicz A, Spilker G, Kreysel HW. Cultured autologous keratinocytes in fibrin glue suspension, exclusively and combined with STS-allograft (preliminary clinical and histological report of a new technique). Burns, 1994; 20(1): 23-29
48. Freeman AE, Igel HJ, Herrman BJ, Kleinfeld KL. Growth and characterization of human skin epithelial cell cultures. In Vitro, 1976; 12(5): 352-362
49. Liu SC, Karasek M. Isolation and growth of adult human epidermal keratinocytes in cell culture. J Invest Dermatol, 1978; 71(2): 157-162
50. Horch RE, Debus M, Wagner G, Stark GB. Cultured human keratinocytes on type I collagen membranes to reconstitute the epidermis. Tissue Eng, 2000; 6(1): 53-67
51. Brysk MM, Raimer SS, Pupo R, Bell T, Rajaraman S. Grafting of leg ulcers with undifferentiated keratinocytes. J Am Acad Dermatol, 1991; 25(2 Pt 1): 238-244
52. Pellegrini G, Ranno R, Stracuzzi G, Bondanza S, Guerra L, Zambruno G, et al. The control of epidermal stem cells (holoclones) in the treatment of massive full-thickness burns with autologous keratinocytes cultured on fibrin. Transplantation, 1999; 68(6): 868-879
53. Ronfard V, Broly H, Mitchell V, Galizia JP, Hochart D, Chambon E, et al. Use of human keratinocytes cultured on fibrin glue in the treatment of burn wounds. Burns, 1991; 17(3): 181-184
54. Myers SR, Grady J, Soranzo C, Sanders R, Green C, Leigh IM, et al. A hyaluronic acid membrane delivery system for cultured keratinocytes: clinical “take” rates in the porcine kerato-dermal model. J Burn Care Rehabil, 1997; 18(3): 214-222
55. Matouskova E, Bucek S, Vogtova D, Vesely P, Chaloupkova A, Broz L, et al. Treatment of burns and donor sites with human allogeneic keratinocytes grown on acellular pig dermis. Br J Dermatol, 1997; 136(6): 901-907
56. Chester DL, Balderson DS, Papini RP. A review of keratinocyte delivery to the wound bed. J Burn Care Rehabil, 2004; 25(3): 266-275
57. Grant I, Ng RL, Woodward B, Bevan S, Green C, Martin R. Demonstration of epidermal transfer from a polymer membrane using genetically marked porcine keratinocytes. Burns, 2001; 27(1): 1-8
58. Kirsner RS, Falanga V, Eaglstein WH. The biology of skin grafts. Skin grafts as pharmacologic agents. Arch Dermatol, 1993; 129(4): 481-483
59. Dvorankova B, Smetana K, Jr., Konigova R, Singerova H, Vacik J, Jelinkova M, Kapounkova Z, Zahradnik M. Cultivation and grafting of human keratinocytes on a poly(hydroxyethyl methacrylate) support to the wound bed: a clinical study. Biomaterials, 1998; 19(1-3): 141-146
60. Haddow DB, France RM, Short RD, MacNeil S, Dawson RA, Leggett GJ, et al. Comparison of proliferation and growth of human keratinocytes on plasma copolymers of acrylic acid/1,7-octadiene and self-assembled monolayers. J Biomed Mater Res, 1999; 47(3): 379-387
61. Hecht J, Hoefter EA, Hecht J, Haraida S, Nerlich A, Hartinger A, et al. (Cultivated keratinocytes on micro-carriers: in vitro studies of a new carrier system). Handchir Mikrochir Plast Chir, 1997; 29(2): 101-106
62. Cuono CB, Langdon R, Birchall N, Barttelbort S, McGuire J. Composite autologous-allogeneic skin replacement: development and clinical application. Plast Reconstr Surg, 1987; 80(4): 626-637
63. McKay I, Woodward B, Wood K, Navsaria HA, Hoekstra H, Green C. Reconstruction of human skin from glycerol-preserved allodermis and cultured keratinocyte sheets. Burns, 1994; 20 Suppl 1: S19-22
64. Kangesu T, Navsaria HA, Manek S, Fryer PR, Leigh IM, Green CJ. Kerato-dermal grafts: the importance of dermis for the in vivo growth of cultured keratinocytes. Br J Plast Surg, 1993; 46(5): 401-409
65. Leary T, Jones PL, Appleby M, Blight A, Parkinson K, Stanley M. Epidermal keratinocyte self-renewal is dependent upon dermal integrity. J Invest Dermatol,

1992; 99(4): 422-430
66. Coulomb B, Friteau L, Baruch J, Guilbaud J, Chretien-Marquet B, Glicenstein J, et al. Advantage of the presence of living dermal fibroblasts within in vitro reconstructed skin for grafting in humans. Plast Reconstr Surg, 1998; 101(7): 1891-1903
67. Krejci NC, Cuono CB, Langdon RC, McGuire J. In vitro reconstitution of skin: fibroblasts facilitate keratinocyte growth and differentiation on acellular reticular dermis. J Invest Dermatol, 1991; 97(5): 843-848
68. Coulomb B, Lebreton C, Dubertret L. Influence of human dermal fibroblasts on epidermalization. J Invest Dermatol, 1989; 92(1): 122-125
69. Metcalfe AD, Ferguson MW. Harnessing wound healing and regeneration for tissue engineering. Biochem Soc Trans, 2005; 33(Pt 2): 413-417
70. Cha J, Falanga V. Stem cells in cutaneous wound healing. Clin Dermatol, 2007; 25(1): 73-78
71. Weissman IL. Stem cells: units of development, units of regeneration, and units in evolution. Cell, 2000; 100(1): 157-168
72. Baharvand H, Kazemi Ashtiani S. Embryonic stem cells: Concepts and Potentials. Yakhteh, Autumn 2005; 7(3): 178-193
73. Potten CS, Hendry JH. Letter: Clonogenic cells and stem cells in epidermis. Int J Radiat Biol Relat Stud Phys Chem Med, 1973; 24(5): 537-540
74. Lavker RM, Sun TT. Heterogeneity in epidermal basal keratinocytes: morphological and functional correlations. Science, 1982; 215(4537): 1239-1241
75. Morris RJ, Potten CS. Slowly cycling (label-retaining) epidermal cells behave like clonogenic stem cells in vitro. Cell Prolif, 1994; 27(5): 279-289
76. Morris RJ, Potten CS. Highly persistent label-retaining cells in the hair follicles of mice and their fate following induction of anagen. J Invest Dermatol, 1999; 112(4): 470-475
77. Lavker RM, Sun TT. Epidermal stem cells: properties, markers, and location. Proc Natl Acad Sci U S A, 2000; 97(25): 13473-13475
78. Bickenbach JR, Mackenzie IC. Identification and localization of label-retaining cells in hamster epithelia. J Invest Dermatol, 1984; 82(6): 618-622
79. Cotsarelis G, Sun TT, Lavker RM. Label-retaining cells reside in the bulge area of pilosebaceous unit: implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell, 1990; 61(7): 1329-1337
80. Michel M, Torok N, Godbout MJ, Lussier M, Gaudreau P, Royal A, et al. 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 (Pt 5): 1017-1028
81. Bickenbach JR. Identification and behavior of label-retaining cells in oral mucosa and skin. J Dent Res, 1981; 60 Spec No C: 1611-1620
82. Lajtha LG. Stem cell concepts. Differentiation, 1979; 14(1-2): 23-34
83. Alonso L, Fuchs E. Stem cells of the skin epithelium. Proc Natl Acad Sci USA, 2003; 100 Suppl 1: 11830-11835
84. Trainer AH, Alexander MY. Gene delivery to the epidermis. Hum Mol Genet, 1997; 6(10): 1761-1767
85. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med, 1989; 321(17): 1174-1178
86. Gluckman E, Rocha V, Arcese W, Michel G, Sanz G, Chan KW, et al. Factors associated with outcomes of unrelated cord blood transplant: guidelines for donor choice. Exp Hematol, 2004; 32(4): 397-407
87. Gluckman E, Rocha V, Boyer-Chammard A, Locatelli F, Arcese W, Pasquini R, et al. Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. N Engl J Med, 1997; 337(6): 373-381
88. Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med, 2000; 6(11): 1229-1234
89. Orlic D, Kajstura J, Chimenti S, Jakoniuk I, Anderson SM, Li B, et al. Bone marrow cells regenerate infarcted myocardium. Nature, 2001; 410(6829): 701-705
90. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science, 1999; 284(5411): 143-147
91. Azizi SA, Stokes D, Augelli BJ, DiGirolamo C, Prockop DJ. Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats--similarities to astrocyte grafts. Proc Natl Acad Sci USA, 1998; 95(7): 3908-3913
92. Bordignon C, Notarangelo LD, Nobili N, Ferrari G, Casorati G, Panina P, et al. Gene therapy in peripheral blood lymphocytes and bone marrow for ADA-immunodeficient patients. Science, 1995; 270(5235): 470-475
93. Jackson KA, Majka SM, Wang H, Pocius J, Hartley CJ, Majesky MW, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J Clin Invest, 2001; 107(11): 1395-1402
94. Kocher AA, Schuster MD, Szabolcs MJ, Takuma S, Burkhoff D, Wang J, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. Nat Med, 2001; 7(4): 430-436
95. Mangi AA, Noiseux N, Kong D, He H, Rezvani M, Ingwall JS, et al. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. Nat Med, 2003; 9(9): 1195-1201
96. Fathke C, Wilson L, Hutter J, Kapoor V, Smith A, Hocking A, et al. Contribution of bone marrow-derived cells to skin: collagen deposition and wound repair. Stem Cells, 2004; 22(5): 812-822
97. Deng W, Han Q, Liao L, Li C, Ge W, Zhao Z, et al. Allogeneic bone marrow-derived flk-1+Sca-1- mesenchymal stem cells leads to stable mixed chimerism and donor-specific tolerance. Exp Hematol, 2004; 32(9): 861-867
98. Brittan M, Braun KM, Reynolds LE, Conti FJ, Reynolds AR, Poulsom R, et al. Bone marrow cells engraft within the epidermis and proliferate in vivo with no evidence of cell fusion. J Pathol, 2005; 205(1): 1-13

99. Badiavas EV, Abedi M, Butmarc J, Falanga V, Quesenberry P. Participation of bone marrow derived cells in cutaneous wound healing. J Cell Physiol, 2003; 196(2): 245-250
100. Borue X, Lee S, Grove J, Herzog EL, Harris R, Diflo T, et al. Bone marrow-derived cells contribute to epithelial engraftment during wound healing. Am J Pathol, 2004; 165(5): 1767-1772
101. Mansilla E, Drago H, Marin GH, Sturla F, Ibar R, Soratti C. Mesenchymal stem cells, could they be the link between tolerance and regeneration? Burns, 2007; 33(2): 137-138
102. Fried DA, Munster AM. Does immunosuppression by thermal injury depend on the continued presence of the burn wound? J Trauma, 1975; 15(6): 483-485
103. Lyle S, Christofidou-Solomidou M, Liu Y, Elder DE, Albelda S, Cotsarelis G. The C8/144B monoclonal antibody recognizes cytokeratin 15 and defines the location of human hair follicle stem cells. J Cell Sci, 1998; 111(Pt 21): 3179-3188
104. Han B, Fu XB, Han B, Lei YH, Chen W, Sun TZ. Chemotactic effects of burn rat serum on mesenchymal stem cells derived from different sources. Zhonghua Shao Shang Za Zhi, 2007; 23(1): 25-28