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INVITED MEDICAL REVIEW

Wound healing and regenerative strategies

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Wound healing is a complex biological process that affects multiple tissue types. Wounds in the oral cavity are particularly challenging given the variety of tissue types that exist in close proximity to one another. The goal of regenerative medicine is to facilitate the rapid replacement of lost or damaged tissue with tissue that is functional, and physiologically similar to what previously existed. This review provides a general overview of wound healing and regenerative medicine, focusing specifically on how recent advances in the fields of stem cell biology, tissue engineering, and oral disease could translate into improved clinical outcomes.

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Introduction

The oral cavity is a complex environment comprised of multiple tissue types, including soft tissue, muscle, bone, skin, mucosa, and teeth. The orchestration of wound healing and tissue regeneration in this milieu is challenging, as these processes require coordinated growth of structures that are spatially proximate but physiologically and structurally disparate. This review represents a discussion of the stages of wound healing and a general overview of regenerative medicine, with special emphasis on recent advances in stem cell biology, tissue engineering and oral disease research.

Obstacles in wound healing

Although skin is a convenient model to study tissue regeneration, wound healing, and scar formation affects multiple tissues in the body, including maxillofacial region following traumatic injury or surgery, the heart after acute myocardial infarction and the healing cirrhotic liver. Therefore, wound healing and the development of scar reduction therapies are of interest to all clinicians.

Wound healing is a complex process involving the integration of multiple biological pathways. Although this process is highly evolved, the replacement of lost or damaged tissue can be negatively influenced by multiple factors, including concurrent disease, such as diabetes, vascular disease, and renal failure, malnutrition, smoking, radiation exposure, infection, and immunocompromise. In the presence of these factors, wounds can fail to adequately heal, resulting in chronic ulcer formation (Guo and Dipietro, 2010).

A classic example of compromised wound healing is seen in the diabetic foot, in which decreased sensory innervation and peripheral vascular disease can result in chronic ulcer formation, gangrene, and resultant limb amputation. Diabetes is a global health concern, as the number of diabetic patients worldwide is projected to increase from 197 million reported in 2003 to 366 million by the year 2030 (Hosoya *et al*, 2008). In the United States, the estimated cost of diabetes in 2007 was 174 billion dollars, 58 billion of which was spent on diabetes-related complications (ADA, 2008).

Of interest to the readers of this review, diabetes, and poor blood glucose control affect wound healing in all tissues of the body. Therefore, diabetic patients are also more susceptible to periodontal diseases and oral infection. In particular, periodontitis, one of the most common inflammatory diseases, has been linked to chronic diseases such as diabetes and often results in significant loss of tissue, including alveolar bone, periodontal ligament, root cementum, gingiva and – in advanced stages – teeth (Pihlstrom *et al*, 2005).

Even in the most ideal wound-healing conditions, postnatal tissue replacement results in scar formation. Scars maintain only 70% of normal tensile tissue strength and are characterized histologically by disorganized collagen formation (Rhett *et al*, 2008). In burn patients, when tissue injury occurs across joints, wound contraction, and scar formation can lead to substantial functional impairment. Moreover, scar formation in facial tissues, particularly in the pediatric population, can lead to poor cosmetic outcomes, hindrance of growth, and

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negative psychological consequences, such as anxiety, depression, and social avoidance. Finally, when injury occurs around the mouth or eyes, scar formation can result in ocular and oral dysfunction. For these reasons, even a normal or non-pathologic scar can be problematic.

Finally, the wound healing process can occasionally go into overdrive, resulting in pathologic or excessive healing and the formation of fibroproliferative scar. Keloids and hypertrophic scars can be disfiguring and cause itching, burning, and pain at the site of injury (Kose and Waseem, 2008).

The wound healing cascade

Wound repair occurs through three distinct and overlapping phases – inflammation, cell proliferation, and remodeling (Figure 1). When a breach to the skin's integrity occurs, platelets are the first responders, initiating hemostasis through fibrin clot formation. Platelets also release various wound healing mediators that signal macrophages and fibroblasts to migrate to the site of tissue injury (Singer and Clark, 1999).

The inflammatory process begins with the recruitment of neutrophils, macrophages, and lymphocytes. The proliferative phase largely overlaps with the inflammatory phase and results in formation of the epithelium through the migration of cells across a provisional matrix, the formation of blood vessels, the deposition of collagen, and the creation of an extracellular matrix. Following the proliferative phase, collagen remodeling begins, along with vascular maturity and regression; this process typically lasts 6–24 months from the time of injury (Singer and Clark, 1999).

Some eukaryotes are capable of replacing lost or damaged tissue with tissue that is nearly identical in its phenotype and function to the tissue damaged. This phenomenon occurs in human skin during pre-natal development, but regenerative ability is lost in adult life (Colwell *et al*, 2003). Instead, in response to tissue injury, human skin deposits a disorganized extracellular matrix made up of collagen. The final result is a scar, which retains approximately 70% of the tensile strength of the original tissue (Singer and Clark, 1999).

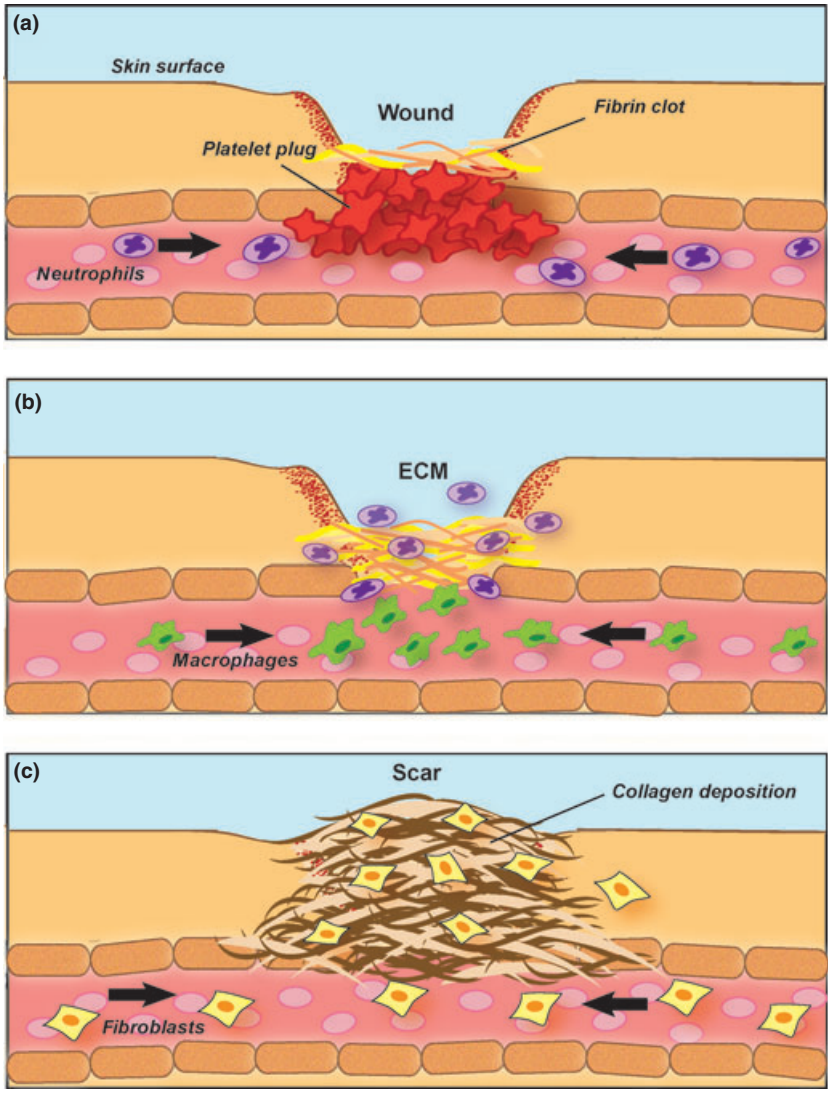

Figure 1 Distinct and overlapping phases of wound healing. (**a**) Coagulation/early inflammatory phase. In response to tissue injury, platelets migrate from blood vessels with increased permeability. Fibrinogen is converted to fibrin, which is deposited along with platelets to create a fibrin plug. Platelets secrete factors such as transforming growth factor-β and platelet-derived growth factor, which attract neutrophils to the wound. (**b**) Late inflammatory phase. Macrophages migrate to the wound and join neutrophils in scavenging debris, releasing growth factors, and reorganizing the extracellular matrix (ECM). (**c**) Proliferative phase. Fibroblasts migrate to the wound. Collagen is deposited in a disorganized manner, creating a scar. The remodeling phase (data not shown) follows. During this phase, collagen crosslinking and reorganization occurs. This process can last years

Targeting the early inflammatory phase

The early inflammatory phase that sets in within minutes of tissue injury seems to determine areas in which scar will form, as this phase is responsible for clearing the injured area of debris. Growth factors and cytokines stimulate neutrophils and macrophages to migrate to the wound. Upon arrival, neutrophils release enzymes such as metalloproteinases and collagenases which, along with infiltrating macrophages, break down large amounts of tissue with free radicals. The resulting area, left devoid of matrix, is ultimately filled with scar tissue through the migration and proliferation of fibroblasts, the production and deposition of collagen and angiogenesis (Singer and Clark, 1999).

For this reason, investigators have focused on the inflammatory response as a potential target to reduce scar formation, as many wound healing experts debate the necessity of the inflammatory phase to effective tissue repair. One study showed that the PU.1 null mouse, which is devoid of both macrophages and neutrophils, healed both incisional and excisional wounds at statistically similar rates to wild type littermates, but without scar formation. The cytokine and growth factor profiles at the wound site in the PU.1 null mouse differed from those of the wild type wound. As a result, cell death was reduced, and scar formation did not occur (Martin *et al*, 2003). Other studies have focused on platelets and mast cells as targets and have shown that neither of these mediators are essential to effective wound repair, further suggesting that a dampened or modified inflammatory response could reduce scar formation (Egozi *et al*, 2003; Szpaderska *et al*, 2003).

Regenerative healing and scar reduction theory

The skin has provided a convenient and applicable model for studying tissue regeneration. The observation that fetal cutaneous wounds in the first 6 months of gestation heal without scar (Lorenz *et al*, 1992) has prompted researchers to investigate the differences between fetal and adult skin, as well as environmental conditions that promote scarless fetal wound repair (Longaker *et al*, 1994; Cass *et al*, 1997; West *et al*, 1997; Soo *et al*, 2000; Hsu *et al*, 2001). Through studying this model of scarless wound healing, researchers hope to develop therapies that promote the rapid deposition of collagen in a fine reticular pattern akin to uninjured skin, rather than the dense, disorganized pattern with increased cross-linking that is characteristic of scars (Whitby and Ferguson, 1991).

To date, several differences between the structure and molecular makeup of fetal and adult skin have been identified. Hyaluronic acid, present in higher amounts in fetal skin, increases the fluidity of the tissue, which is permissive to the influx of fibroblasts essential for wound repair. In addition, hyaluronic acid creates an environment with space between cells so that proliferating cells can evade the inhibitory signals of their neighbors (West *et al*, 1997). Finally, the ratio of collagen type III to type I in fetal skin is higher than adult skin and decreases with age (Whitby and Ferguson, 1991).

During the repair process, signaling molecules are active at different concentrations between healing fetal and adult cutaneous wounds. For example, the ratio of transforming growth factor beta-3 (TGF-β3) to TGF-β1 and TGF-β2 in fetal wounds is higher, and fetal fibroblasts do not produce collagen in the TGF-β1-induced pathway characteristic of adult fibroblasts (Hsu *et al*, 2001). Furthermore, the ratio of matrix metalloproteinases to tissue inhibitors of metalloproteinases is higher in the fetal wound environment, tipping the balance in favor of remodelling over collagen deposition (Soo *et al*, 2000). Homeobox genes, transcription factors that stimulate organogenesis, have been shown to be more active in the fetus and are thought to initiate fetal skin wound repair by a mechanism currently under investigation (Yeh *et al*, 2009).

Detailing the multiple differences between fetal and adult wound healing is outside the scope of this review. However, it is important to emphasize that evolution has essentially favored accelerated healing with the cost of scar formation, possibly due to the survival benefit of sealing off the wound, minimizing blood loss, avoiding infection, and preventing deformation. The main goals of regenerative medicine are to replace damaged tissue with equally functional tissue and to accelerate healing. Scar formation hallmarks the replacement of absent or damaged tissue with a weaker variant of compromised functionality. Therefore, regenerative therapies would ideally both shorten wound-healing time and reduce the formation of scar.

Of note, wounds in the oral cavity proceed through the same three overlapping phases. However, breaches to the integrity of the oral mucosa tend to heal at a faster rate and with decreased scar formation (Whitby and Ferguson, 1991). Predictably, fibroproliferative scars, such as hypertrophic scars and keloids, also rarely develop in this location (Wong *et al*, 2009). The only identified exception to this rule is the healing rate of excisional wounds placed in the hard palate of the mouse, which is much slower than injuries to other areas of the oral mucosa (Graves *et al*, 2001).

One reason for the difference in healing of oral mucosal wounds in relation to skin wounds may be the dampened inflammatory response seen in response to injury in the oral cavity. For example, during wound repair, the ratio of TGF-β1 to TGF-β3 is lower in the oral mucosa than in the skin (Schrementi *et al*, 2008). On histology, lower levels of inflammatory cells infiltrate mucosal wounds at early time points, and fewer inflammatory cytokines and chemokines are active in the wound. In addition, research has shown that angiogenesis in murine oral mucosal skin wounds occurs to a lesser degree than in skin wounds, which is similar to what is seen in fetal scarless repair (Mak *et al*, 2009).

Despite the improvement in oral mucosal wound repair over skin wound repair, scar formation, and delayed healing are major obstacles in oral disease, as the oral cavity is made up of many tissue types that exhibit significant restriction in their regenerative capacity.

Scar reducing therapies

To date, no satisfactory FDA-approved therapy is available for the prevention and treatment of scar, which is a testament both to the complexity of scar formation and the redundancy of the pathways involved. In general, surgical techniques that avoid incisions across joints and support low inflammatory response and tension-free closure are encouraged. Occlusive therapy has shown some benefit. In addition, whenever possible, minimally invasive techniques should be employed, as mucosal incisions heal with less scar formation than skin incisions (Mak *et al*, 2009).

Topical hyaluronic acid and saponins, which upregulate hyaluronic acid production, may have anti-scarring effects (Mast *et al*, 1991). Other solutions under investigation include TGF-β3 formulations and neutralizing antibodies to TGF-β1 and TGF-β2, as well as solutions that decrease the activity of connexin 43, a mediator of TGF-β signaling (Rhett *et al*, 2008). Decorin is a small chondroitin/dermatan sulfate proteoglycan that limits the duration of TGF-β influence on inflammation and tissue repair, promoting regenerative repair and limiting tissue fibrosis (Border and Ruoslahti, 1992; Border *et al*, 1992; Jarvelainen *et al*, 2006). Recently, a fusion protein was developed that exploits the regenerative properties of decorin and enhances its regenerative capacity by linking decorin to a peptide that recognizes angiogenic blood vessels and migrates to the site of injury. When this biotherapeutic was administered systemically to mice with excisional wounds, skin healing, and regeneration were enhanced compared to control groups with decorin administered alone (Jarvinen and Ruoslahti, 2010).

Other cytokines and growth factors identified as targets for scar prevention and therapy include tumor necrosis factor alpha (TNF-α), platelet derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (ILGF), and epidermal growth factor (EGF) (Lawrence, 1998). These targets are under continual investigation as researchers attempt to manipulate existing biological pathways, upregulating targets that support scarless regeneration and downregulating those that promote fibrosis and scarring.

Stem cell therapy and tissue regeneration

Stem cells are self-renewing cells that are capable of differentiating into multiple cell types. As such, these cells are ideal candidates for maintaining homeostasis and promoting tissue repair. In theory, the application of stem cells to wounds is advantageous over using a single agent, as stem cells have the ability to differentiate and replace lost or damaged tissue, as well as influence multiple biological pathways at once via paracrine signaling. In fact, paracrine interactions may be the primary means by which stem cells influence tissue repair, as conditioned media from stem cell culture have shown similar efficacy in wound healing (Chen *et al*, 2008; Gnecchi *et al*, 2008).

Tissue regeneration can be approached through the following three routes: cell-based therapy, the use of biomaterials alone or a combination of the two (biomaterials seeded with cells). Biocompatible scaffolds are used either to promote tissue regeneration or to act as vehicles of cell delivery in appropriate *in vivo* conditions. When cells are used to promote regenerative healing, they can either be harvested from the same type of tissue to be replaced or, when conditions are unfavorable, from other tissue sources (e.g. stem cell reservoirs). Stem cells can be harvested at various stages of differentiation and can be either implanted directly or grown out in culture with directed differentiation prior to *in vivo* implantation. Each cell type requires its own customized culture conditions to promote growth and directional development. Likewise, the *in vivo* environment must be appropriately accommodating to the introduction cells and their differentiation from precursors to functional tissue (Atala and Yoo, 2009).

Autologous stem cell reservoirs are preferred over allogenic reservoirs because these cells avoid rejection by the host's immune system. However, expanding an appropriate number of cells *in vitro* can prove to be difficult, and allogenic sources are often the only option (Atala and Yoo, 2009). Embryonic stem cells (ESCs), induced pluripotent cells (iPS cells) and adult stem cells are potential sources for cell-based therapy and regenerative medicine.

Embryonic stem cells

The inner cell mass of the blastocyst forms only a few days after fertilization and eventually forms the primitive ectoderm that then differentiates into the three primary germ layers: endoderm, mesoderm and ectoderm. Embryonic stem cells are derived from the inner cell mass. As such, these cells are pluripotent, capable of forming all three primary germ layers (Figure 2). To demonstrate this ability, human ESC-derived

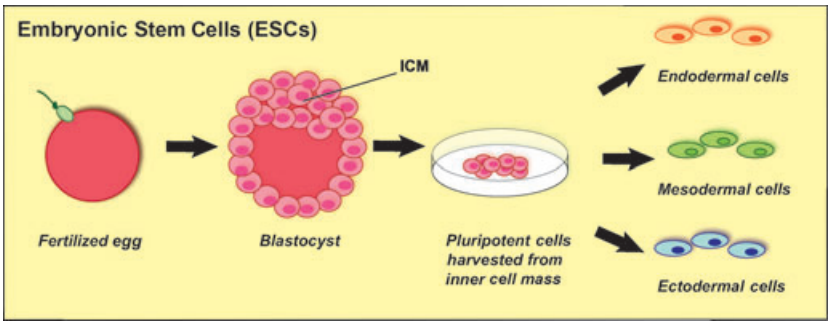

Figure 2 Pluripotent stem cells: embryonic stem cells (ESCs). ESCs are derived from the inner cell mass (ICM) of an early stage embryo. These cells are capable of generating all three primary germ layers, even after they are maintained in culture over many passages

progenitors were injected under the kidney capsule of nude mice. Results showed teratoma formation containing all three germ layers (Yao *et al*, 2006). Researchers have demonstrated that not only are ESCs pluripotent, but they are also capable of retaining pluripotency after being maintained in culture over multiple passages (Beddington and Robertson, 1989).

The ESCs express the following markers common to pluripotent and undifferentiated cells: Oct-4, Nanog, alkaline phosphatase, CD9, CD24, LIN28, Rex-1, SOX-2, Thy-1, and endometrial bleeding-associated factor. These cells also have high levels of telomerase, which is largely responsible for their unlimited self-renewal capacity (Bongso *et al*, 1994).

Changes in culture conditions allow ESCs to emerge from an undifferentiated, pluripotent state along a path of differentiation into various lineages. In regard to wound healing, three research groups in particular have been successful in demonstrating that mouse ESCs are capable of differentiating into cells of epidermal lineage after exposure to *in vivo*-like conditions, such as exposure to bone morphogenetic protein-4 signaling and extracellular matrix (Bagutti *et al*, 1996; Aberdam, 2004; Troy and Turksen, 2005).

Researchers have also attempted to create artificial skin from ESCs directed along the epidermal lineage pathway *in vitro*. This is a complicated task, as the skin is a stratified, complex organ. One group used an organotypic culture model to grow stimulated ESCs on a cell-free inert filter substrata at the air-liquid interface. The tissue that arose under these conditions formed both epidermal and dermal components with appropriately stratified differentiation markers (Coraux *et al*, 2003). These epidermal cells behaved like epidermal cells would *in vivo*, expressing markers in a temporally appropriate manner. Furthermore, a sub-population of these cells retained the ability to generate epidermal cells in response to environmental cues (Coraux *et al*, 2003).

The advent of embryonic stem cell biology and the development of human embryonic stem cell lines have allowed researchers to study various disorders from discarded *in vitro* fertilized embryos (Evans and Kaufman, 1981). Embryonic stem cell biology has also allowed for the creation of knockout mice, a discovery that has allowed biologists to study the role of specific pathways in disease (Doetschman *et al*, 1987; Thomas and Capecchi, 1987).

Embryonic stem cells are one of the most attractive sources for cell-based therapy due to their theoretically unlimited supply and pluripotent potential. However, embryonic stem cell biology is not without its problems. One major limitation is that differentiation of embryonic stem cells is difficult to control, and the ability of these cells to form all tissue types poses concerns regarding tumorogenicity and teratoma formation *in vivo*. Additionally, chromosomal stability is difficult to maintain when ESCs are maintained in culture over multiple passages (Baker *et al*, 2007). Translating what has been accomplished *in vitro* to *in vivo* poses challenges, as it is unclear what predifferentiated cells could do to potentially disrupt the developmental milieu if introducted *in vivo*. Furthermore, if a small percentage of cells were not fully differentiated, the potential for neoplasia is high (Cha and Falanga, 2007).

Ethical controversy also poses barriers to embryonic stem cell research. Isolating embryonic stem cells involves destroying the embryo as the cells are extracted, a process that has prompted objection from political, religious, and social groups. To address the ethical reservations associated with ESCs, researchers have explored the process of parthenogenesis, or conversion of an egg to an embryo in the absence of sperm (Koh *et al*, 2009). Despite some success, parthenogenesis has not proven to be the solution to the controversy surrounding embryonic stem cell research. As a result, the use of ESCs in clinical practice remains largely theoretical and ESC research is mainly directed toward studying disease states and biological processes.

One exception to this generalization is the recent approval of the first clinical trial involving a human ESC-derived product, oligodendrocyte progenitor cells (OPC1). Geron, a biotechnology company based in Menlo Park, California received clearance from the FDA to treat spinal cord injury patients with progenitor cells that have shown nerve growth-stimulating and remyelinating properties (Alper, 2009). Despite this breakthrough for ESC research, the Geron trial is primarily focused on safety rather than efficacy and clinical benefit, as the conditions of approval only allow for a small number of cells to be administered to only the sickest of patients (Lebkowski, 2009).

Induced pluripotent stem cells

In response to the ethical considerations associated with the use of ESCs, Kazutoshi Takahashi and Shinya Yamanaka developed a novel technology that allows the conversion of differentiated somatic cells to pluripotency using just four defined transcription factors: c-Myc, Klf4, Oct4, and Sox2. The cells that result from this process are called induced pluripotent stem cells (iPS cells) (Takahashi and Yamanaka, 2006) (Figure 3). iPS cells resemble ESCs, in that these cells exhibit immortal growth characteristics *in vitro,* express genes characteristic of ESCs, and produce teratomas *in vivo* (Takahashi and Yamanaka, 2006). Since Takahashi and Yamanaka published their results in 2006, other researchers have reported the ability to reprogram somatic cells to iPS cells using fewer transcription factors, further refining iPS technology (Kim *et al*, 2009b).

The first iPS cells were derived through reprogramming of mouse dermal fibroblasts. Since then, other cell sources have successfully been isolated from both mice and humans and reprogrammed to iPS cells (Aasen *et al*, 2008; Hanna *et al*, 2008; Park *et al*, 2008; Kim *et al*, 2009b). Researchers in the field of oral disease recently demonstrated that human dental stem cells originally derived from ectomesenchyme can be reprogrammed into iPS cells (Yan *et al*, 2010). Another group in the field demonstrated that human fibroblasts obtained from the oral mucosa can be reprogrammed to iPS cells (Miyoshi *et al*, 2010). This discovery is particularly exciting because of the ease of isolation of

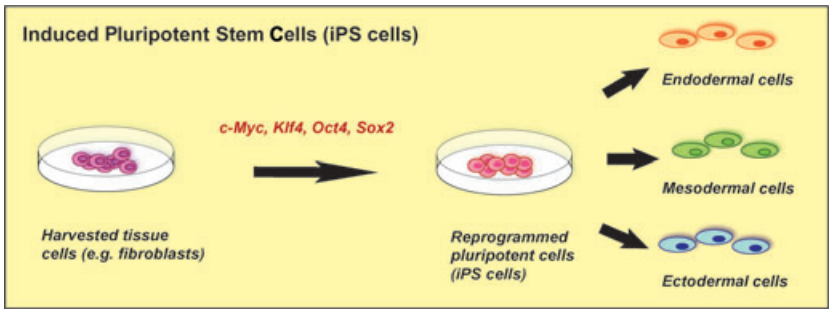

Figure 3 Pluripotent stem cells: induced pluripotent stem cells (iPS cells). iPS cells are pluripotent cells reprogrammed from tissue-specific cells. The process of reprogramming has traditionally involved the integration of four transcription factors via viral vector transduction. Since initially described by Takahashi and Yamanaka, the number of transcription factors needed for reprogramming has been reduced, and non-viral transduction methods have been used with some success. The resultant cells are capable of generating all three primary germ layers

oral fibroblasts and the rapidity with which oral mucosal wounds incurred for cell harvest can heal (Whitby and Ferguson, 1991).

In general, iPS cells are attractive because of their potential clinical applications; somatic cells could be isolated from a patient, reprogrammed, and then used in the same patient, theoretically evading the immune system. iPS technology can also be used to screen new drug therapies and study the differentiation of both normal and diseased cells. An additional advantage of iPS technology is that the use of somatic cells avoids the ethical controversy associated with ESC research.

Despite these advantages, iPS technology is not without its limitations. First, the reprogramming efficiency of iPS technology is exceedingly low, estimated between 0.01% and 10% (Takahashi *et al*, 2007; Yamanaka, 2009). In addition, the reprogramming process has traditionally involved the use of modified viruses, which creates speculation regarding the safety of these cells for use in therapeutics. In response to safety concerns, researchers have investigated other methods of gene transfer (Stadtfeld *et al*, 2008; Kim *et al*, 2009a; Zhou *et al*, 2009) and small molecule chemical methods of inducing pluripotency (Shi *et al*, 2008a,b). Jia *et al* were able to produce human iPS cells from human adipose derived stromal cells using a non-viral, single minicircle vector. This minicircle vector is comprised of four reprogramming vectors, Sox2, Lin28, Nanog, and Pou5F1 (Jia *et al*, 2010). Other groups have attempted to create iPS cells with the removal of vectors following the reprogramming reaction (Kaji *et al*, 2009; Soldner *et al*, 2009; Woltjen *et al*, 2009). However, this research is still in its infancy, and any reaction that uses viral vectors at any stage will attract speculation regarding safety. Moreover, one of the advantages of iPS cells – their ability to differentiate into all three germ layers – is also a limitation, as concerns regarding tumorogenicity and formation of teratoma are common between ESCs and iPS cells.

Adult mesenchymal stem cells

In response to the salient issues associated with ESCs and iPS cell research, attention has shifted to adult mesenchymal stem cells (MSCs) as a potential cell population to develop cell-based therapies. Like iPS cells, the use of adult stem cells in cell-based therapy could avoid many of the barriers associated with ESCs, as the cells are harvested from adult tissues, and autotransplantation of cells should theoretically avoid immune rejection.

Progenitor cell populations are found in many tissue types. The first adult stem cells isolated were bone marrow derived mesenchymal stem cells (BM-MSCs), defined by their self-renewal ability and by their capability to differentiate into different cell types. MSCs are more restricted than ESCs and iPS cells and tend to be more lineage-specific. These multipotent adult progenitor cells are a heterogenous population that have the ability to differentiate into mesodermal, endodermal, and neuroectodermal cells (Chamberlain *et al*, 2007).

Of all the sources of adult stem cells, bone marrow cells have attracted the most attention. However, adult stem cells with similar characteristics have been isolated from other tissue types, such as skeletal muscle, brain, fat, and skin (da Silva Meirelles *et al*, 2006). Adipose-derived stromal cells (ASCs) are particularly attractive for regenerative therapies due to their relative ease of isolation and large tissue reservoir (Zuk *et al*, 2002).

The oral mucosa is thought to contain multiple stem cell niches. Adult stem cells have been isolated from the dental pulp (Gronthos *et al*, 2000), exfoliated deciduous teeth (Miura *et al*, 2003), the periodontal ligament (Seo *et al*, 2004), and – most recently the lamina propria of the oral mucosa (Marynka-Kalmani *et al*, 2010). Dental pulp stem cells in particular have been shown to possess self-renewal capacity and multi-lineage potential common to other adult MSC populations (Gronthos *et al*, 2002; Laino *et al*, 2006).

The wound microenvironment attracts MSCs, and local inflammation and oxidative stress influences the behavior of these cells by creating a low-oxygen environment. Hypoxia induces both BM-MSCs and ASCs to proliferate at a faster rate and promote wound healing through increased epithelialization, accelerated wound closure, and angiogenesis (Ren *et al*, 2006; Grayson *et al*, 2007; Lee *et al*, 2009). In addition, some reports claim that both autologous and allogeneic MSCs transplanted locally or systemically evade the host's immune system (Mansilla *et al*, 2005; Liu *et al*, 2006).

In the wound, MSCs have been shown to modestly differentiate into endothelial cells, epidermal keratinocytes, pericytes, and sebocytes *in vivo* (Li *et al*, 2006; Wu *et al*, 2007; Sasaki *et al*, 2008). Of note, MSCs have been shown to promote tissue repair in most organ systems, demonstrating the broad applicability and efficacy of MSC-associated treatments.

Although MSCs are thought to have self-renewal capabilities, issues of poor engraftment *in vivo* (Wu *et al*, 2007) suggest that paracrine interactions may be the primary means by which stem cells influence tissue repair. This theory is supported by research showing that conditioned culture media from both MSCs and ASCs also improves tissue repair (Kim *et al*, 2007; Lee *et al*, 2009).

The fact that MSCs show only a modest amount of differentiation *in vivo* has limited the cells' ability to reduce scar formation. Although the application of conditioned media accelerates repair, amplifying the ability of MSCs to differentiate into functional and organized tissue could in theory enhance their regenerative capacity. Genetic modification may be a solution to the obstacles of poor engraftment and low rates of differentiation *in vivo*.

Genetic modification of stem cells can be performed to reprogram cells to direct differentiation (Li *et al*, 2007) or to deliver specific growth factors to an affected area (Conrad *et al*, 2007). This process could increase adult stem cells' supportive role in the wound through increased gene expression. In addition, genetic manipulation could improve the cells' substitutive role through increased differentiation *in vivo* (Barzilay *et al*, 2009).

To date, the most effective means of gene delivery to stem cells has been through the use of modified viral vectors, specifically lentivirus (McMahon *et al*, 2006). Although gene delivery could enhance survival and differentiation *in vivo*, the use of viral transfection methods limit the potential of genetically modified stem cells for us in translational medical applications (Nair, 2008). As discussed previously in regard to iPS technology, other non-viral transfection methods are currently under investigation to address this issue.

Conclusion

Continued enthusiasm in the field of stem cell biology has led to research related to increasingly sophisticated delivery systems for cells, matrices, and growth factors. Microfluidic channels, for example, are a new technology that could allow for precise delivery of cells, growth factors, and cytokines in response to changes sensed in the wound's microenvironment. Biodegradable matrices and biogels could provide advanced vehicles of cell delivery that would integrate into the healing wound and reduce the risk of infection associated with non-biodegradable structures. Smart biomaterials could be applied to wounds to recruit the organism's endogenous stem cells from their reservoirs to the site of injury, avoiding the complications associated with the introduction of exogenous cells.

In complex wound environments, the ability to coordinate the regeneration of multiple tissue types at once from single or multiple stem cell sources could allow for regeneration of more functional tissue. To this end, in the field of oral surgery, researchers have shown some success using scaffolds seeded with autologous stem cells from tooth and bone to bioengineer teeth that are both functional and contain all necessary tissue components, including periodontium, supporting roots, and alveolar bone (Zhang *et al*, 2009). This research is particularly exciting given the complexity of these constructs and the clinical implications of being able to create functional teeth using a single surgical approach.

Stem cell biology and regenerative medicine are rapidly expanding fields. The introduction of new technology and the emergence of novel stem cell populations have allowed researchers to take a more in-depth approach to the study of specific diseases and biological processes. Although many therapies are still in the discovery phase, the field of stem cell biology holds great promise for clinical medicine in general and tissue regeneration in particular.

Author contributions

Drs. Nauta, Gurtner, and Longaker all contributed to the research, drafting, and editing of this manuscript.

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