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Wound Healing

Research is a dynamic enterprise that generates a wealth of knowledge. It provides a forum for debating ideas and working them into evidence-based theories. The clinical trial setting puts these theories to the test and may lead to evidence-based medicines that can alleviate symptoms or cure disease. But the process of taking research from bench to bedside is a lengthy one, and demands not only vision but also years of hard work and dedication on the part of scientists, physicians and patients. This document presents basic information about wound healing and frames the context for the discussion that follows about how lessons learned from stem cell research may help fight this disease. Readers may also wish to peruse additional web resources or speak with their physicians for more information about this condition.

About Wound Healing

Skin wounds can happen for a myriad of reasons during the course of one's life. Injuries, cuts, burns, poor circulation, ulcers from pressure sores, and illnesses such as diabetes can all cause wounds that temporarily compromise the normal function and structure of the skin. If the body is unable to heal these wounds, they become chronic and fester over time. Surprisingly, this happens more often than one would expect, and at any given time one per cent of the population is living with a chronic skin wound. Half of these wounds never heal. The burden to the individual and society is tremendous in that quality of life decreases and healthcare costs skyrocket: in the USA alone, the cost of chronic wound care amounts to greater than 20 billion dollars.

Anatomy of the skin

As our largest organ, the skin forms a natural barrier to the outside world and protects the underlying tissues from the wear, tear, and infection. The three layers of the skin – epidermis, middle dermis and hypodermis – each contribute something special to maintain the normal structure and function of the skin.

The top layer is the epidermis and it is composed of four different types of cells: keratinocytes, Merkel cells, Langerhans cells, and melanocytes. Keratinocytes contain the protein keratin that gives skin its water resistant quality, and Merkel cells detect sensation. Melanocytes give the skin its pigment, and Langerhans cells rid the body of debris and help prevent infection. Together these cell types are arranged in multiple layers that cover the outer surface of the body – most thickly on the hands and feet.

The epidermal layer is a powerhouse of regeneration. Keratinocytes make up the majority of the cells, and the ones found on the surface are mostly all are dead. As they are sloughed off, new keratinocytes from underneath move up to replace them. This bottom-up, conveyor-style process revolves around the clock so that the entire surface of the skin gets renewed every 15 to 30 days. The epidermis is one of the few tissues that lacks blood vessels and this feature combined with the rapid turnover of the outer layers helps prevent infectious agents from taking a foothold in the body.

A basement membrane separates the epidermis from the underlying dermis. The dermal layer is made of fibroblasts, blood vessels, immune cells, skin appendages (hair follicles, and sweat glands), and extracellular matrix proteins that glue everything together and support the cells. As with the epidermis, the components of the dermis determine its function. Blood vessels nourish the skin, immune cells protect the skin, and fibroblasts make collagen proteins for strength and elastin proteins for stretchiness. The skin would be dry and brittle without the sweat glands because they produce an oily secretion called sebum that bathes the surface of the skin. Sebum also has anti-infection properties.

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The deepest part of the skin is the hypodermis, a fatty layer under the dermis that cushions the underlying muscle and bones and acts as an additional barrier to protect against infection. It is thought that the adipocytes within the hypodermis play an important role in regulating skin homeostasis and growth of cells within skin appendages such as hair follicles.

Wound healing

Wounds develop when the epidermal layer of skin is breached by injury or infection and the severity increases when the underlying layers of skin become involved. Three steps need to occur for wounds to heal normally: inflammation, proliferation and remodeling. Inflammation lasts approximately four days. In that time the key events that happen are the generation of a fibrin matrix and the formation of a blood clot to cover and protect the wound. Immune cells, such as macrophages, also migrate into the area to clean up any cellular debris and to help prevent infections from taking hold. During the proliferation phase, inflammatory signals recruit many different cell types to the wound, including fibroblasts and vascular endothelial cells. Fibroblasts make collagen and can also turn into myofibroblasts that close the wound, and vascular endothelial cells form new blood vessels around the wound. All this cellular activity makes what is called a 'granulation tissue' that is located just below the blood clot. Keratinocytes then migrate to the wound from the wound edges and also from the base of hair follicles. The dividing keratinocytes move over the granulation tissue to knit the epidermis together. This process continues during the remodeling phase and once the epidermis is restored, keratinocytes and fibroblasts lay down the matrix proteins for the new basement membrane that regenerates the interface between the epidermis and the underlying dermis.

In cases where the dermal layer is injured, as happens with very bad burns or cuts, the hair follicles and sweat glands may also be destroyed and that will lessen the number of keratinocytes available to repair the epidermis. As a result, deep wounds take longer to heal and skin grafts may be necessary. Age also slows the healing process, possibly due to the weakening of the skin or compromised blood flow that may be the result of inactivity or lifestyle choices, such as smoking.

Treatment

Split thickness skin grafts are the standard of care for serious skin wounds. They can either be sourced as autografts (from the patient) or allografts (from a donor). In either case, a thin slice of skin is shaved from a donor site, usually from the buttocks or inside the thigh, and applied to the patient's wound. Split thickness skin grafts always contain the epidermis and may also contain a small portion of the dermis. Thicker skin grafts will help a patient's wound to heal faster, but one must be careful not to take too thick a slice or the donor site may not heal properly. Full thickness skin grafts include the epidermis and all the dermis but they are used sparingly as the donor site must be closed with stitches and typically scars over. Multiple grafts can be taken from the same donor site, once it has healed, but again one must be careful not to do this too often because the dermis typically exhibits poor regeneration (and typically scars), and it will get thinner and thinner over time resulting in poor healing of the donor site.

The ideal graft is an autograft because it comes from the patient and will not be rejected. If an autograft is not immediately available, an allograft will be used as a temporary skin cover but because it is usually rejected within a week or so an autograft will inevitably be necessary. Skin grafts that come from animals other than humans, for example pigs, are called xenografts (xenos is Greek for foreign) and may also serve as temporary wound coverings until autografts are available at a later date.

The limited supply of autografts (particularly in patients with large surface area burns or disease) led the scientific community to explore a tissue engineering approach to wound healing. Skin products were the first successful tissue engineered products and today they can be made from a biodegradable matrix or cell-based product. The goal of using a biodegradable matrix is to create an organic scaffold that will integrate into a wound and allow enough time for blood vessels to reform before the skin contracts and an autograft can be applied. Donor allografts can also be engineered as matrices by stripping them of all living cells. This technique leaves only a protein scaffold and reduces the chance of graft rejection or disease transmission. Allograft matrices will also inevitably need an autograft applied to fully heal a wound.

The age of cell-based products for treating wounds was born when laboratory techniques advanced to the point

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where keratinocytes could be readily grown and expanded in culture. In 1988, the first cultured skin product (Epicel) was made by using an autologous skin biopsy to grow an epidermal graft. This technique is extremely expensive and the resulting epidermis is very fragile and tricky to use so it. More recently, a technique for mincing full thickness autografts has been devised to minimize the amount of donor tissue. In this case, the minced autograft, containing both epidermal and dermal tissue, is combined with a gel and distributed over the wound. Similarly, 'meshing' of split-thickness grafts is frequently used to increase surface area coverage and reduce the amount of autograft required for transplant. These approaches are far cheaper than growing skin biopsies and requires less donor tissue.

Allografts (not from the patient) can also be used to create cell-based skin products. One example (Dermagraft) is composed of neonatal dermal fibroblasts that grow on a biodegradable synthetic mesh. The fibroblasts produce an extracellular matrix that integrates into the wound and helps the dermis to heal. Another example (Apligraf) is made from neonatal foreskin containing fibroblasts and keratinocytes. The fibroblasts are mixed with collagen to form a scaffold and the keratinocytes are seeded onto it. The multiple layers that result resemble the dermis and epidermis. All of these types of allografts are useful but they are all ultimately rejected by the patient and autografts must be applied to heal the wound. Moreover, despite their minimal utility and effectiveness, their enormous cost is also prohibitive for large wounds.

Can Stem Cells Help?

Although wound management has greatly advanced over the years, the current treatments fall short of healing some 50 per cent of chronic wounds. Conventional treatments such as split thickness skin grafts lack the dermal layer which is crucial for regenerating hair follicles, sweat and oil glands. If these components cannot be regenerated in the wounded area, the resulting skin is dry, brittle, itchy and generally looks aesthetically unappealing.

Stem cells have been actively explored as therapies for wound therapy for many years because they have a tremendous potential to differentiate into different types of cells, especially various components of the skin. In fact, the ability to control the differentiation of epidermal stem cells into keratinocytes in the laboratory has fuelled the field of tissue engineered skin grafts for the past 30 years. These grafts have been somewhat successful in treating skin wounds caused by diabetes, ulcers, genetic skin disorders such as epidermolysis bullosa (blistering) and burns, but they too are unable to regenerate the dermis – the key component that restores the function and appearance of the skin long term. Engineered products are also very expensive which tends to limit their widespread use.

In an effort to address these issues, researchers are looking at other stem cells and the cells they make (progeny) to see if they retain the potential to restore all the components of the dermis. High on the list of stem cells being studied are adult stem cells (skin stem cells, mesenchymal stem cells, and adipocyte stem cells) and pluripotent stem cells (induced pluripotent stem cells (iPSCs).

Research and clinical directions

Understanding the role of stem cells in wound healing

Stem cells are known to play an important role during the normal course of events that distinguish skin regeneration and wound healing. The skin is home to many different types of stem cells, including epidermal stem cells, melanocyte stem cells (which make pigment-producing cells), and epithelial and mesenchymal stem cells that reside in hair follicles. Together, these stem cells are responsible for making the multitude of different skin cells that are important for perpetually renewing normal, healthy skin. In response to general injuries the number of circulating stem cells in the body increase. Hair follicle stem cells and epithelial stem cells are thought to interact with bone marrow stem cells, which are recruited to a wound during the inflammatory phase of wound healing, and together these stem cells bring about speedy wound closure and tissue repair. As yet, it is not clear whether stem cells from the bone marrow might also contribute to the healing process by actually making new skin cells.

Epidermal stem cells

Canadian scientists Jeff Biernaskie and Vincent Gabriel in Calgary are searching for ways to improve split thickness

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skin grafting (STSG), the surgical procedure most commonly used to repair deep burns and other wounds. In isolating a dermal stem cell that lives in mammalian hair follicles they found that it is involved in hair follicle formation and regeneration as well as repair of the dermis. These dermal stem cells are also able to make a number of different types of cells of the dermis. The group has since isolated a similar precursor that resides in human hair follicles and is setting about to test whether the precursors can populate a split thickness skin graft with new dermal cells in a model of human STSG. They plan to use non-invasive imaging techniques to measure the extent of wound healing. As part of the study they will interview patients who have had STSG to better understand the experiences and the issues around living with STSGs. Their hope is to tailor the cellular features of the graft to the functional qualities that are most important to STSG recipients.

Freda Miller at the Hospital for Sick Children in Toronto is well known for having discovered skin-derived precursors (SKPs). These are adult dermal stem cells that not only repair wounded skin but also prevent skin from aging and promote hair growth. She and her team are actively searching out drugs, small molecules and genes that can ramp up the activity of SKPs. The idea being that if they can increase the number of SKPs, they might be able to enhance skin repair and maintenance, especially as abnormal wound healing and premature aging have been linked with low levels of SKPs. Using an approach called high throughput screening technology, which enables researchers to quickly test millions of compounds, her team has identified a number of drugs that increase self-renewal in both human and mouse SKPs. When applied on the skin of mice one drug was also able to increase the speed of wound healing, and others were able to increase dermal thickness. Taken together their results suggest that small molecules can stimulate SKPs and play a role in wound healing and maintenance. Building on this theory, they have also identified drugs that turn on a stem cell gene called Sox2 which plays an important role in wound healing, hair growth and stem cell maintenance.

Clinical studies using skin stem cells are still at a very early stage but they are helping to provide proof-of-concept for the possibility of using skin stem cells to treat wounds. For example, there are several phase I clinical trials around the world exploring whether epidermal stem cells can be safely used to treat epidermolysis bullosa, a group of genetic skin disorders inherited by children. For these individuals, minor skin injuries can develop into blisters and open wounds because the top layer of skin (epidermis) does not anchor properly to the middle layer of skin (dermis) and flakes off leaving painful sores that are akin to 3rd degree burns. In ongoing phase I trials, epidermal stem cells harvested from patients are being genetically corrected and then expanded into sheets of skin that can grafted back onto a patient's wounds. One of the big advantages with this approach is that the grafted skin sheets are made from the patient's own epidermal stem cells which means that they are not rejected.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are perhaps one of the most promising types of adult stem cell therapies currently being applied to wound healing. This is due in part to their differentiation potential and also because they are so accessible within the body. MSCs reside in many different tissues (bone marrow, fat tissue, umbilical cord, dermal layers of the skin) and are able to grow into a variety of different cell types, including skin cells. For the purposes of transplant experiments, MSCs are the easiest to harvest from the bone marrow body and grow in culture. A huge benefit of these cells is that they are immune response modulators, helping transplanted cells to fly 'under the radar' without provoking the same type of vigorous immune response that so often leads to graft rejection.

Preclinical studies using rodent models of skin wounds have clearly shown that mesenchymal stem cells from donor animals are able to accelerate wound closure, recruit immune cells and endothelial progenitors, increase the formation of blood vessels in recipient animals. Researchers have also shown that a population of mesenchymal stem cells called human umbilical cord perivascular cells (HUCPVC) are able to promote wound healing in mouse models. Moving to larger animals with more relevance to humans, they are now testing the pig analogue of HUCPVCs in swine models of wound healing to gauge whether the results warrant moving from preclinical studies in animals to clinical studies in humans. The preliminary research tested the cells on very small wounds, and although promising, larger wounds will provide a better idea of how well the pig mesenchymal stem cells are working to close the wounds.

A handful of small clinical trials using bone marrow derived mesenchymal stem cells are underway. In a very small trial with three participants having chronic wounds, bone marrow cells were injected into the edge of the wound and then autologous bone marrow derived MSCs grown in the laboratory were applied to the surface of the wound. This com-

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bination approach was able to completely close the three chronic wounds, and opened the door to larger trials. One randomized trial involved 24 people with non-healing ulcers due to diabetes or vasculitis. In this case bone marrow-derived autologous MSCs were grown in culture and then injected intramuscularly into the edges of the wound. After 12 weeks, the wound decreased by 73 per cent in the treated group as compared with only 23 per cent in the untreated group. The treated group also reported much less pain when walking.

These preliminary results are very encouraging but there remain a number of hurdles yet to be worked out. For example, results from different researcher labs still vary greatly, possibly because they used different populations of MSCs or or different laboratory techniques used to sort the cells. One strategy to level the field would be to identify cell surface markers that could distinguish particular subsets of bone marrow-derived MSCs. Another approach might be to use common growing and sorting methodology for isolating the subsets. These measures could help to standardize the starting population of MSCs derived from bone marrow and make the comparison of experimental results from lab to lab more meaningful.

Although autologous MSCs are easily harvested from the bone marrow, their numbers naturally decrease as we age. Given the increasing elderly population, this natural stem cell depletion will make it difficult to treat wounds in older people. Researchers are investigating whether non-bone marrow MSCs or other types of stem cells might work better in this group.

Adipose-derived stem cells (ASCs)

It comes as no surprise that adipose-derived stem cells (ASCs) can make fat cells, but it is rather amazing to find that they can also make bone, cartilage, muscle cells, and even promote the formation of new blood vessels (angiogenesis). This range of capabilities makes them a very intriguing new candidate for wound healing.

ASCs can be isolated through minimal liposuction techniques from fat tissue and then be put through a series of isolation and purification steps in the laboratory. ASCs have already been used with some success in preclinical studies in animal models where wound closures have occurred much faster and without any abnormalities. Providing ASCs with a 3D scaffold to grab onto is an important step for ensuring their ability to heal wounds, and to that end researchers have been comparing the properties of different biomaterials to see which one has the most advantages. A human dermal matrix lacking cells (called an acellular dermal matrix; ADM) has advantages over a synthetic matrix in that ADMs are easier to handle and more resistant to infection. In animal models, ADMs impregnated with adipocyte stem cells promoted enhanced wound healing and vascular networks and the grafted cells were still viable after two weeks of engraftment.

As yet, there are very few clinical trials using ASCs for wound healing. A small pilot study with 20 participants looked at using ASCs for chronic wounds caused by radiation treatment for cancer. The dramatic results - ASC therapy transformed the damaged tissue into normal tissue – are predicted to be a result of the ability of ASCs to promote blood vessel formation. This early success is very promising and paves the way for additional trials testing the safety and efficacy of ASCs for wound healing.

Pluripotent stem cells

Embryonic stem cells (ESCs) are the powerhouses of development and can make all the different cell types in the embryo. Scientists have been able to grow skin in a dish from mouse embryonic stem cells, but the issue of graft rejection is a barrier to translating this success to the clinical setting. In contrast, induced pluripotent cells (iPSCs), which were first derived from human skin in 2007, offer a way around this limitation. Because they are generated from patient skin cells reprogrammed to a stem cell-like state, skin cells made from iPSCs are perfectly compatible as skin grafts. The barrier to widespread use of iPSCs is safety, given that the viral components currently used to reprogram skin cells may be tumorigenic. Scientists are finding safer methods to push back the clock on reprogramming mature cells, and are working towards achieving this goal without having to genetically alter cells at all. Another safety concern is that pluripotent stem cells, such as ESCs and iPSCs, as well as abnormal multipotent stem cells are able to form tumours when transplanted so future therapies will depend heavily on being able to sort out the safe cells from the dangerous ones.

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Future Challenges

A common issue with using any stem cells for treating wounds is the challenge of reliably growing and expanding them for topical applications. One can imagine achieving the scale-up numbers for small wounds, but it is not as simple to ramp up the numbers so that there are enough cells to cover a larger area of skin. Understanding the skin microenvironment, signaling mechanisms and culture conditions that promote stem cell expansion will be very important if the use of stem cells is to reach beyond the treatment of small chronic wounds.

Stem cell delivery is another conundrum. For the vast majority of studies, stem cells are introduced into wounds simply by injecting them into the dermis or around the edge of the wound. Alternative methods that are easier and might also lead to better outcomes are being explored. For example, researchers are testing whether stem cells applied topically in a fibrin protein spray or impregnated in a collagen-containing scaffold or hydrogel may work better than the traditional route of injection.

Researchers are also faced with the issue of how to treat wounds in the aging population because collecting stem cells from the elderly may not be a viable reality given that the stem cell pool to draw from decreases as we age.

Despite these and other inherent challenges, stem cells do offer a realistic alternative to conventional therapies which lack the ability to create hair follicles and secretory glands. Stem cells have the potential to overcome this limitation and also to address the issue of limited donor graft availability and graft rejection. In time, they may someday rival split thickness skin grafting, the current standard of care for chronic wound treatment.

Web Resources

Readers may wish to peruse the recommended sites or review the selected reading list below for more information about the application of stem cells to treat chronic wounds.

- Canadian Association of Wound Care: http://cawc.net/
- Wound Care Canada Journal: http://cawc.net/en/index.php/resources/wound-care-canada-journal/

Selected Reading List

- Arwert E et al. Epithelial stem cells, wound healing and cancer. Nature Reviews 2012;12:1701-180; Chen M et al. Stem Cells for Skin Tissue Engineering and Wound Healing. Crit Rev Biomed Eng. 2009;37(4-5):399-421.
- Badiavas EV and Falanga V. Treatment of chronic wounds with bone marrow-derived cells. Arch. Dermatol. 2003:139:510-516.
- Boggio P et al. Is there an easier way to autograft skin in chronic leg ulcers? 'Minced micrografts', a new technique. JEADV 2008;22:1168–1172.
- Chen JS, Wong VW, Gurtner GC. Therapeutic potential on bone marrow-derive mesenchymal stem cells for cutaneous wound healing. Frontiers in Immunology. 2012;3(102):1-9.
- Chen M et al. Stem Cells for Skin Tissue Engineering and Wound Healing. Crit Rev Biomed Eng. 2009;37(4-5):399-421.
- Cherubino M et al. Adipose-Derived Stem Cells for Wound Healing Applications. Annals of Plastic Surgery. 2011;66(2):210-215.
- Dash NR et al. Targeting non-healing ulcers of lower extremity in human through autologous bone marrow-derived mesenchymal stem cells. Rejuventation Res. 2009:12:359-366.
- Mak TW and Saunders ME. Primer to The Immune Response. Update Edition, 2011. Academic Press, Elsevier
- Martini F and Timmons M. Human Anatomy, 3rd edition, 2000. Prentice-Hall.
- Wang P, Na J. Mechanisms and Methods to Induce Pluripotency. Protein Cell. 2011;2(10):792-799.
- Solovey P et al. Xenografts—liophilized pig skin as a burn wound cover. Burns. 2007;33(1Supplement 1):S85-S6.t