Regenerative Medicine: Charting a New Course in Wound Healing

Geoffrey C. Gurtner^{1,*} and Mary Ann Chapman²

¹Stanford University School of Medicine, Stanford, California.

²TTC Group, Huntington, New York.

Geoffrey C. Gurtner, MD

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*Correspondence: Department of Surgery,
Stanford University School of Medicine, 257
Campus Drive, GK-201, Stanford, CA 94305-5148
(e-mail: ggurtner@stanford.edu).

Significance: Chronic wounds are a prevalent and costly problem in the United States. Improved treatments are needed to heal these wounds and prevent serious complications such as infection and amputation.

Recent Advances: In wound healing, as in other areas of medicine, technologies that have the potential to regenerate as opposed to repair tissue are gaining ground. These include customizable nanofiber matrices incorporating novel materials; a variety of autologous and allogeneic cell types at various stages of differentiation (*e.g.*, pluripotent, terminally differentiated); peptides; proteins; small molecules; RNA inhibitors; and gene therapies.

Critical Issues: Wound healing is a logical target for regenerative medicine due to the accessibility and structure of skin, the regenerative nature of healing, the lack of good limb salvage treatments, and the current use of cell therapies. However, more extensive knowledge of pathophysiologic targets is needed to inform regenerative strategies, and new technologies must demonstrate value in terms of outcomes and related health economic measures to achieve successful market access and penetration.

Future Directions: Due to similarities in cell pathways and developmental mechanisms, regenerative technologies developed in one therapeutic area may be applicable to others. Approaches that proceed from human genomic or other big data sources to models are becoming increasingly common and will likely suggest novel therapeutic avenues. To fully capitalize on the advances in regenerative medicine, studies must demonstrate the value of new therapies in identified patient populations, and sponsors must work with regulatory agencies to develop appropriate dossiers supporting timely approval.

SCOPE AND SIGNIFICANCE

THIS REVIEW DISCUSSES the current state of regenerative medicine and its applicability to wound healing. The definition and scope of regenerative medicine are considered, including the variety of approaches currently being explored across therapeutic areas and the potential of regenerative strategies to transform our approach to disease. Developments toward regenerative medicine in wound healing are explored, followed by challenges and opportunities

that the field faces in preparing for and incorporating these new advances.

TRANSLATIONAL RELEVANCE

Many regenerative medicine therapies being developed in nonwound areas will be relevant to wound healing due to similarities in signaling and cell development pathways. Moreover, big data such as genomics, proteomics, and electronic medical record analyses are informing basic research and population health

management. These developments are likely to enhance understanding of the pathophysiology of wounds and speed the dissemination of successful regenerative strategies throughout different areas of medicine.

CLINICAL RELEVANCE

Chronic wounds, such as venous, diabetic, and pressure ulcers, are difficult to heal and cause much human suffering. Complications include infection, amputation, and even death. Advanced therapies help a portion of patients, but significant percentages remain unhealed after treatment, and even more are at risk for recurrence and continued morbidity due to underlying pathophysiology. Regenerative medicine strategies have the potential to restore tissue, perhaps equaling or exceeding predamage levels, resulting in improved outcomes and quality of life.

BACKGROUND

Few dispute the high cost of chronic wounds, with an estimated annual burden of \$37 billion in the United States alone. Venous ulcers, for example, cost \$7,000 per patient per year, amounting to ~\$15 billion annually in the United States.¹ Among Medicare recipients, diabetic ulcers cost \$18,000 per patient per year²; given an estimated 29 million individuals in the United States with diabetes³ and a foot ulcer rate of 6% over 3 years,⁴ these costs amount to more than \$10 billion annually. The annual cost of pressure ulcers is ~\$11 billion.⁵ As the population ages and the absolute number of chronic wounds increases, these costs will likely become unsustainable.

Important contributors to chronic wound costs include outpatient/physician office visits, emergency department visits, hospitalizations, and, for diabetic foot ulcers, amputations.^{1,2} Costly complications such as infection only occur if wounds are open and thus treatments that heal a high proportion of wounds quickly, completely, and durably have the potential to substantially improve outcomes and reduce expenses—even if the initial cost of these therapeutics is high. Demonstrating this somewhat counterintuitive notion will require not only compelling efficacy and safety data but also comparative effectiveness, health economic, and population health analyses that illustrate the impact of technologies for both patients and healthcare systems. Aggregating these large data sets will require innovative partnerships between manufacturers, providers, and payers to properly evaluate the full range of real-world outcomes. Additionally, patient-centered measures

and patient participation in shared decision making will become increasingly important in assessing and realizing the true value of new treatment approaches.

These issues will become critical as new technologically advanced products are introduced in wound healing—many of which will likely emerge from the burgeoning field of regenerative medicine. Indeed, the regenerative medicine transformation has already begun in wound healing with the development of first-generation tissue-engineered and cell-based products that draw us closer to true skin regeneration.

OVERVIEW OF REGENERATIVE MEDICINE

Definitions of regeneration and regenerative medicine

Regeneration has been defined as the reactivation of development processes later in life to restore missing tissue.⁶ In wound healing, a distinction is typically made between repair, which culminates in a nonfunctional scar, and regeneration, which culminates in tissue that is comparable with the original in form and function.^{7,8} Various definitions of regenerative medicine have been proposed that differ based on whether they include (1) tissue repair and/or enhancement, (2) the development of tissue that was not previously established (e.g., in the case of genetic anomalies), and (3) techniques or technologies employed (e.g., stem cells, molecular medicines, genetic manipulations, tissue-engineered constructs, nanotechnology).^{9–11} The phrase “partial regeneration” has been used to denote healing that restores some, but not all, features of the missing tissue.

For the journal *Regenerative Medicine*, Mason and Dunnill sought a succinct definition, proposing that regenerative medicine replaces or regenerates human cells, tissue, or organs to restore or establish normal function.⁹ Like most definitions, this one focuses on function while omitting mention of tissue architecture or appearance—the latter being important for the skin because it also contributes to outward appearance and plays a role in social interactions. Given the substantial progress toward regeneration in wound healing, the inclusion of repair in a definition of regeneration is probably outdated and it is rapidly becoming untenable to refer to therapies that result in scars or omit important tissue structures as regenerative.

Scope of regenerative medicine

One aspect of regenerative medicine on which all experts agree is its multidisciplinary nature. Re-

generative medicine has grown out of diverse disciplines, such as surgery, organ transplantation, biomaterials science, engineering, developmental biology, and stem cell biology.^{9,12} Today, the scope of regenerative medicine includes technologies that induce the body to (re)develop missing tissue, regardless of their format, as well as engineered tissue or organs designed to fully replace the missing structures.

The merging of tissue engineering into regenerative medicine occurred with the progression of stem cell and therapeutic cloning research.¹³ In his discussion of the history of regenerative medicine, Kemp observed that tissue engineering, stem cell therapy, regenerative factors, specific acellular scaffolds, and therapeutic cloning united under a single umbrella term of regenerative medicine.¹³ The interactive relationships between these fields and regenerative medicine are depicted in Figure 1. This merging has been abetted by the recognition that various engineered constructs, some of which were originally designed to engraft and serve as replacement structures, stimulate endogenous processes that remodel the construct with the body's own tissue.

Nearly a decade ago, the U.S. Department of Health and Human Services issued a 2020 vision document that stated, "*What truly differentiates regenerative medicine from many current therapies is that regenerative medicine has the potential to provide a cure to failing or impaired tissues.*"¹⁴ Others have not explicitly used the word cure, but

rather the term restore, in describing the goal of regenerative medicine.^{15,16} Stocum has argued that regenerative medicine is not viable without an understanding of regenerative biology.¹⁷ In this conception, an important aspect of regenerative medicine is its link to regenerative biology or research on self-healing—the ability of the body to use its own systems in response to stimuli to recreate cells and rebuild tissues and organs.¹¹ Regenerative biology studies regeneration from the perspective of embryonic development, the use of adult and pluripotent stem cells, and adult tissue turnover and replacement primarily in animals with high regenerative capacities for complex organs such as fish or salamanders.¹⁸ However, important differences exist between regeneration and development, including the size of the structures formed, the dependence of some types of regeneration on nerves, and the requirement for positional identity in adult regeneration.¹⁹

Another characteristic of regenerative medicine today is its inherent recognition of relationships between biological systems. Many signaling pathways are common to different cell and tissue types, which may permit one regenerative treatment to have multiple uses as has occurred in cancer with the kinase inhibitors. Moreover, treatments such as mesenchymal stem cells are being developed for tissues that have a common developmental origin. Research in regenerative medicine increasingly incorporates relationships between tissues, organs, and systems, and even the body as a whole.

Figure 1. Multidisciplinary origins and scope of regenerative medicine. Regenerative medicine has its origins in many different areas of research and medicine and these fields continue to influence one another today.

This systems approach is leading to therapeutic strategies designed to have effects at multiple levels and is complementary to the molecular approach designed to identify and target single genes or proteins. Eventually, the merging of these strategies may result in the combination of several different molecular approaches into one course of treatment that can, for example, reduce inflammation, stimulate tissue development pathways, recruit endogenous stem cells, modulate immune function, and stimulate new blood vessel formation. Although multicomponent targeting is one of the ideas behind stem cell and biological extracellular matrix-based constructs, these approaches are currently administered with the hope that they will do everything nature does without the need for a complete understanding of which biological effects are responsible for the outcomes. A deeper understanding of regenerative biology, including relationships between systems, will inform not only multimolecule strategies but also cell and matrix-based strategies.^{20,21}

APPROACHES TO REGENERATIVE MEDICINE

Approaches to regenerative medicine are numerous and range from the application of a single molecule or peptide to the engineering of entire organs (Table 1). Some of these strategies may have broad applicability; for instance, a biochemical cocktail may be identified that stimulates the differentiation of different types of resident stem cells in adults. Additionally, many organs and tissues are developmentally related such that basic research may be broadly applied across a spectrum of diseases or health conditions. Conversely, tissue engineering strategies that work for one tissue may not be viable for another given the differences in three-dimensional (3D) shapes, extent of vascularization, and mechanical properties.

Biomaterials and matrices

Historically, biomaterials were designed primarily for mechanical functions and comprised inert materials intended to prevent immune rejection following implantation or application. As scientific understanding of complex biological processes increased, the field of biomaterials evolved to incorporate more biological or biologically inspired synthetic components designed to mimic one or more physical or chemical properties of tissue such as nanostructure and cytokines/growth factors.²² Today, investigators are developing programmable matrices with biomechanical, biochemical, and/or drug delivery properties tailored to specific tissues or clinical needs, as well as materials that can integrate endogenous inputs.

The extracellular matrix is a prime example of a tissue component that has inspired the development of biomaterials. The extracellular matrix is a structural and functional complex that surrounds and interacts with cells throughout their life cycle. During development, the extracellular matrix guides cell migration and differentiation through integrin binding, mechanical cues, and the sequestration and liberation of growth factors and other chemical signals.²³ Matrix composition varies by tissue, but collagen is the most abundant component overall, and collagen-based matrices are used in clinical conditions varying from wound healing to urinary incontinence.^{24–27} Other components of extracellular matrices such as proteoglycans and fibrin are also used in regenerative medicine either with or without collagen. Moreover, a variety of synthetic biomaterials are being developed to capitalize on the spatial and biomechanical cues of endogenous extracellular matrices during development.²⁸ The linking of bioactive molecules to synthetic or biological matrices is being investigated as a means to further stimulate cells, and still other matrices are being developed as carriers for cells.

Cells

The introduction of cells themselves as regenerative therapies is a highly active area of research. Depending on the type and source of cells, the goal may be for the applied cells to engraft and repopulate the tissue, modulate immune function, or to stimulate endogenous cell populations that then heal the tissue. Autologous cells intended to engraft may be fully differentiated adult cells or, increasingly, stem cells derived from the tissue to be treated or a tissue with related lineage. For instance, therapies are in development, in which stem cells are obtained from a readily accessible source such as thigh muscle, sent to the laboratory to differentiate into a specific cell type such as cardiomyocytes, then introduced back into the patient where they are thought to stimulate regeneration.²⁹ Differentiated autologous and allogeneic cells are already in clinical use for some wound healing applications, as discussed in the next section. The latter do not engraft, but rather survive for a limited time, presumably releasing growth factors, synthesizing matrix, and recruiting endogenous stem cells.^{30,31} Mesenchymal stem cells, found primarily not only in the bone marrow but also in adipose, vascular, muscle, and dermal tissue, are a highly active topic of research because of their ability to differentiate into multiple connective tissues, such as bone, cartilage, muscle, bone

marrow stroma, tendon/ligament, fat, and dermis.³² Furthermore, these cells secrete numerous immunoregulatory and regenerative molecules.

Another avenue of exploration is the induction of pluripotent stem cells from mature autologous cells that are stimulated to dedifferentiate through exposure to various biochemical stimuli. Although no therapies based on induced pluripotent stem cells are yet available, this is the most active area in regenerative medicine as gauged by the number of scientific publications.³³ Yet another area of research is the induction of readily available autologous cells into differentiated cells of another type without first returning to the pluripotent cell stage, termed transdifferentiation or lineage reprogramming. This has been reported for fibroblasts, which have been differentiated into nerve cells without an intermediate pluripotent stage using microRNAs and transcription factors; notably, these cells survived at least 6 months when injected into the brains of mice.³⁴ Embryonic stem cells are under study as the only type of cell that can differentiate into any cell in the body, although their investigation and use are constrained by ethical concerns that may be, at least partly, abrogated by another type of cell, the very small embryonic-like stem cells, which are purportedly rare pluripotent cells whose existence is somewhat controversial.³⁵

Gene therapies

Gene therapies are being evaluated to address defective or mutated genes needing correction or improved regulation through the insertion of properly functioning or genetically altered genes into a patient's cells.³⁶ Genes may be incorporated *ex vivo* into cells or vectors such as replication-deficient/nonreplicating viruses, and then introduced into patients. Gene therapy may be preferable to protein therapy due to its longevity in the body, which can be on the order of weeks to years compared with a protein half-life of only a few hours or days.³⁷ Moreover, gene therapy may lead to the synthesis of protein at biologically relevant levels, whereas direct introduction of the protein can be more difficult to regulate. Historically, gene therapy has been beset by serious safety issues, with the development of leukemia in some patients. However, these problems are being addressed with new approaches and many trials of gene therapies are currently underway for various diseases.

RNA interference

Several RNA interference strategies are under investigation in regenerative medicine, including the use of microRNAs to reprogram cells as described in the preceding section. MicroRNAs, short

single-stranded noncoding RNAs that inhibit gene expression, were identified only within the last few decades during which time they have been found to play a role in cell development, metabolism, proliferation, apoptosis, and regeneration.³⁸ Many studies are investigating the roles of microRNAs, with potential applicability of the findings to regeneration in many different disease states. For instance, microRNAs have been found to play a major role in the survival of cardiac progenitor cells³⁹ and thus may eventually be beneficial in cardiac regeneration.

Small interfering RNA (siRNA) is another strategy that inhibits gene expression. These exogenous double-stranded RNAs bind to mRNAs with sequences that are completely complementary. Investigators have immobilized siRNAs on biosynthetic matrices that promote their controlled delivery; such a system has been used to inhibit the transforming growth factor- β 1 pathway and improve scarring in an animal model.⁴⁰ Others have embedded siRNAs in hydrogels to prolong their release; this strategy has been used to enhance the osteogenic differentiation of stem cells.⁴¹

Peptides and proteins

Numerous peptides and proteins that play a role in cellular differentiation and development are routinely used to stimulate differentiation or dedifferentiation of cells in the laboratory and some are themselves potential therapies.⁴² In instances where a protein is missing, depleted, or dysfunctional due to a mutation, attempts have been made to replace it by introducing the protein directly into skin wounds due to their accessibility. For other disease states, novel delivery vehicles are under study to improve protein stability, pharmacokinetics, and targeted spatiotemporal release. This active area of research includes polyethylene glycol hydrogels,⁴³ copolymer microparticles,⁴⁴ heparin-conjugated nanospheres,⁴⁵ and protein engineering strategies.⁴⁶

The use of peptides in regenerative medicine is concentrated in several areas. One of these is the incorporation of adhesion sequences onto biomaterials. Various amino acid sequences have been identified as the bioactive regions of large proteins such as fibronectin that are responsible for binding the extracellular matrix to cellular integrins, the best studied of which is the RGD sequence. This sequence and other short synthetic adhesion peptides are being integrated into biomaterials to enable cell binding and to guide the behavior of cells.⁴⁷ Another strategy is that of self-assembled peptide nanofibers designed to mimic aspects of the

extracellular matrix, with the goal of altering cell adhesion, proliferation, differentiation, or other matrix-mediated behaviors. These peptides can assemble into a variety of forms, such as spheres, cylinders, or tubes, and can be administered as implantable gels, injected as supramolecular nanostructures, or injected as liquids that gel *in vivo*.⁴⁸ Yet another strategy is the use of peptides to alter the activation of key proteins. An example of this is a fibronectin peptide known as P12, which binds platelet-derived growth factor BB (PDGF-BB) and enhances its effects on burn healing and fibroblast survival under stress.⁴⁹ Evidence suggests that the mechanism by which P12 acts is to slow internalization of the PDGF-receptor complex by redirecting it from the clathrin-dependent endocytic pathway to a macropinocytosis-like pathway.⁴⁹

Small molecules

Small-molecule pharmaceuticals are also being developed for their potential to trigger regenerative processes. Compared with proteins, small molecules have several advantages, including their ease of dose titration, reversibility, (usual) cell permeability, amenability to thorough physical and chemical characterization, and lack of immunogenicity.⁵⁰ Small molecules are also impervious to attack by proteases, which could be an advantage in conditions such as chronic wounds that are characterized by excessive proteolytic activity.⁵¹ Receptors and signaling pathways that control the activation, differentiation, proliferation, and maintenance of stem cells *in vivo* are important targets for small-molecule pharmaceuticals that are being actively pursued. These efforts may target a variety of pathways that control either adult stem cells or their niches or may seek to influence direct reprogramming of differentiated cells *in vivo*. Small molecules are also being investigated for the mobilization, homing, and integration of stem cells.⁵⁰ Advanced high-throughput techniques are used today for screening millions of compounds simultaneously to identify targets and potential therapies.⁵²

Overarching approaches

Chemical and biomechanical engineering aspects. In addition to the aforementioned approaches are features of tissue that are not under the purview of any single strategy. For instance, biochemical and physical gradients are known to provide signals for cell migration. Thus, biomaterials are being engineered to incorporate signal gradients in both planar and 3D environments.⁵³ Gradients may further be used to define and target stem cell niches; for instance, the hematopoietic

stem cell niche is low in oxygen tension, with these cells localizing to the most hypoxic region of the oxygen gradient within bone marrow.⁴²

The importance of biomechanical forces such as tension to embryogenesis and tissue regeneration has led to strategies designed to mimic these features in engineered biomaterials. Local variations in the extracellular matrix of developing tissue influence cell shape, movement, and cytoskeletal mechanics through integrins, stretch-activated ion channels, and cytoskeletal filaments.⁵⁴ *In vivo*, tissue exists in a state of isometric tension that enables simultaneous and coordinated responses at multiple levels.⁵⁵ A greater understanding of how tension controls developmental processes such as alignment and orientation will inform the development of regenerative biomaterials. The influences of other mechanical forces, such as shear, compression, and hydrostatic pressure, are also under investigation for their role in cell orientation and migration; this is an area in which research directed at understanding cancer cells may yield insight and advances in regenerative medicine.⁵⁶

Targeting development pathways. Given the link between development and regeneration, genes and pathways that regulate development are being targeted with a variety of strategies. An important example is the Wnt/beta-catenin signaling pathway that regulates the expression of genes critical for embryonic development and adult stem cell biology and mutations, which are associated with cancer.^{57,58} Investigators are attempting to selectively influence this pathway using many of the approaches described here: Wnt proteins delivered using novel strategies,⁵⁹ gene-based strategies such as microRNAs and small inhibitory RNA transgenes, and small-molecule inhibitors.⁶⁰ Figure 2 illustrates the many points along the Wnt pathway that can be targeted with regenerative technologies. Cell and matrix-based strategies may exert more overarching effects on this pathway through multiple mechanisms.

In silico models and big data. *In silico* or computer-based models are increasingly used to synthesize experimental findings in tissue development, permitting alterations of the model's inputs to predict and guide subsequent *in vivo* study. These integrative models enable the pursuit of questions such as how cells coordinate interactions over time and how molecular interactions eventually lead to the formation of structures; such questions are difficult to examine from experimentation on isolated tissues.⁶¹

Figure 2. Points along the Wnt pathway that may be targeted with regenerative approaches. As a primary regulator of development, the Wnt pathway is a target for regenerative strategies. Protein and small-molecule therapeutics are in development, as are inhibitory RNA strategies and gene therapies. Cells and matrix-based therapies may influence the Wnt pathway in more programmatic ways such as cell–cell or cell–matrix adhesion and stimulation or inhibition of gene expression. Illustration reproduced courtesy of Cell Signaling Technology, Inc.

So-called big data such as those obtained from genomics and other omics, sciences, and electronic medical records are likewise a burgeoning field, fueling a reverse research approach that begins with human data and works backward toward models and treatments. Big data are also being generated from high-throughput technology and have already resulted in international databases of nucleotide and protein sequences, protein crystal structures, and gene expression measurements.⁶²

Offshoots: microfabrication, 3D bioprinting, whole organ engineering

Microfabrication, the production of structures and devices on the micrometer scale or smaller, is enabling tissue engineering that mimics the microscale nature of native tissue. Microfabrication techniques include photolithography, electrospinning, emulsification, fluid dynamics, and rapid prototyping methods.⁶³ These methods are directed at overcoming current challenges in tissue engineering, such as vascularization, control of cell–cell and cell–matrix interactions, and the guidance of stem cell fate.

Three-dimensional bioprinting has garnered extensive press coverage due to its similarities with

ink-jet printing processes and the promise of bio-printed organs. A critical component of 3D bioprinting is the layered positioning of biomaterials such as cells into a design that mimics the architectural elements of a tissue or organ. Tissues are built up in layers, with channels or spaces in distinct locations designed to resemble biological tissue. Different strategies have as their goal the printing of entire organs or minitissues—smaller functional components that can be assembled into larger ones.⁶⁴ A major advance in 3D bioprinting came in 2014 with the successful bioprinting of vascular networks within hydrogel-based engineered tissue constructs—the inability to print blood vessel networks was formerly a major hindrance to progress in bioprinting.⁶⁵

Given the immediate need for more organs than can be procured through organ donations, several other whole organ engineering strategies besides bioprinting are being pursued. One method that is being examined is the decellularization of donor organs and their reseeded with various cell populations.⁶⁶ This strategy may help minimize organ rejection, thereby increasing the potential donors for individual patients. Other investigators are

Table 1. Examples of regenerative medicine strategies* in development for nonwound applications

Type of Technology	Examples of Specific Application(s) Under Study
Induced pluripotent stem cells	Endothelial cells, neurons, and hepatocytes for preclinical applications
Transdifferentiation of cells	Preclinical stage for transdifferentiation of fibroblasts into brain cells
Embryonic stem cells	Severe heart disease and macular degeneration
Autologous stem cells	Retinal conditions, reconstructive surgery, heart disease, bone damage, and populating matrices for organ transplantation
Allogeneic stem cells	Diabetes, cirrhosis, and other liver diseases, neurologic diseases, stroke, and critical limb ischemia
Gene therapies encoding growth factors	Chronic limb ischemia, ischemic heart failure, and diabetic neuropathy
Small-molecule therapeutics	Preclinical stage inhibition of nuclear export
Human tissue or organ engineering	Small intestine, bone, trachea, and pancreas

*The extent of regeneration versus repair or partial regeneration induced by these strategies has not been established.

attempting to develop whole organs from cells—a feat that has recently been reported for thymus by reprogramming fibroblasts and implanting them into laboratory animals.⁶⁷

TOWARD REGENERATIVE MEDICINE IN CUTANEOUS WOUND HEALING

Cutaneous wound healing has long been a magnet for tissue engineering and regenerative medicine due, in part, to the accessibility of skin, its flat structure and relatively avascular composition, and the fundamentally regenerative nature of healing (Table 2). Cell therapies are already used for wounds, and good limb salvage options are lacking, rendering wound healing an attractive option for regenerative strategies. The field has witnessed pioneering efforts in tissue-engineered, cell-based, gene-based, and growth factor therapies. As per earlier definitions, to date, none of the commercially available products can truly be considered regenerative as none leads to the regeneration of adnexal structures of the skin such as hair follicles and sweat glands. Nevertheless, some of the tissue-engineered/regenerative medicine technologies have made valuable contributions to the field as demonstrated by significantly improved healing rates over standard of care in randomized

trials. Thus, the field is evolving toward regenerative medicine even as most of the current tissue-engineered/regenerative medicine technologies are more in line with Yannas' characterization of partial regeneration.⁸

Toward regeneration with matrix-based products and technologies

Several of the earliest successes in tissue engineering were products designed to mimic important aspects of skin and targeted for use as skin replacements for burn patients who lacked adequate autologous tissue for grafting.^{68,69} This is still an important use for some of these products and a major area of development, although other early products found their primary use in the treatment of chronic wounds; that history has been reviewed elsewhere.¹³

Today, more than 40 matrix-based products for wound healing are commercially available, comprising a variety of materials derived from animals and plants, as well as synthetic polymer components. The majority of matrix-based products are made of allogeneic or xenogeneic tissue that has been decellularized. Many of the allogeneic matrices are derived from cadaver skin, comprising both dermis and epidermis or dermis only. Over the past few years, numerous matrices based on human

Table 2. Examples of regenerative medicine strategies* in development for wound applications

Type of Technology	Comments	Examples of Specific Application(s) Under Study
Autologous skin cells harvested and prepared using novel systems	Differentiated cells	Burns and scars
Autologous adipose-derived cells harvested and prepared using novel systems	Cells also known as adipose tissue-derived stromal vascular fraction cells	Burns and scleroderma
Autologous genetically modified dermal fibroblasts	Fibroblasts engineered to express collagen VII	Epidermolysis bullosa
Allogeneic placenta-derived cells	May have immunomodulatory, anti-inflammatory, angiogenic, and reparative properties	Peripheral arterial disease and diabetic foot ulcers
Allogeneic peripheral blood cell suspension	White blood cells may help fight infection	Diabetic foot ulcers and venous leg ulcers
Antisense oligonucleotide therapy for connexin 43	Designed to inhibit gap junction protein connexin 43	Diabetic foot ulcers and venous leg ulcers
Protein therapeutic	Recombinant human collagen VII	Epidermolysis bullosa
Synthetic extracellular matrix protein	Contains vitronectin protein adherent and attachment sequences	Venous ulcers
Three-dimensional nanofiber scaffolds	May be used as cell or drug delivery vehicle	Multiple wound types

*The extent of regeneration versus repair or partial regeneration induced by these strategies has not been established.

amnion, chorion, and/or umbilical cord tissue have become commercially available. Xenogeneic matrices are largely collagen based, although some contain other biochemicals such as glycosaminoglycans. These products are derived from various porcine, bovine, equine, and even piscine tissues and are available in multiple formats such as sheets, particles, and gels. This category also includes a hydrogel matrix replacement, a hyaluronic acid-based matrix sheet, a monosaccharide derived from microalgae, and a biosynthetic material designed without the inclusion of cells (*i.e.*, acellular). Matrix-based products tend to be relatively easy to use, and most are available off-the-shelf, with no freezing or thawing required. These products have been extensively reviewed elsewhere.^{70–72}

Although a few of the matrix-based products are supported by randomized controlled trials, most lack adequate evidence of efficacy, with many companies providing small uncontrolled case series as the sole supportive evidence. However, given the differences in composition, source, and manufacturing/processing methods, it cannot be assumed that data from any one of these products apply to the entire group. For instance, different processing strategies may lead some products to retain more or different growth factors and adhesive proteins that are important to the mechanism of action; moreover, structural features such as cross-linked versus native collagen versus collagen particles may contribute to bioactivity.⁷³ For the few products whose mechanism of action has been studied, results suggest the involvement of cytokines/growth factors and possibly other unspecified noncollagen components.^{74–76}

New developments in matrix-based technologies include customizable synthetic or biosynthetic nanofiber matrices.^{77,78} These structures mimic the nanometer scale of proteins in natural extracellular matrices. Electrospun nanomaterials combined with collagen are being designed for wound healing, and nanoparticles are being developed as delivery vehicles for cells, molecules, proteins, etc.⁷⁷ Nanofiber hydrogels that exist as liquids at room temperature, but form solid structures at body temperature, are also under investigation in wound healing, either for use alone or as carriers for the controlled release of small molecules or proteins.

Toward regeneration with living autologous cell-based products and technologies

Autologous cell therapies in wound healing began with an autologous keratinocyte product that is currently available in the United States as a

humanitarian use device for deep dermal burns. In addition to refinements in harvesting, processing, and delivering autologous keratinocytes, researchers are pursuing the use of autologous adipose-derived regenerative cells for the treatment of skin conditions such as scleroderma and chronic ulcers.^{79,80} Adipose-derived stem cells have also been induced to differentiate into keratinocyte-like cells that form a stratified epidermis.⁸¹ Stem cells derived from hair follicles are another key area of interest due to their ability to differentiate into multiple skin cell types, including melanocytes, and the ease with which they can be harvested from patients.⁸²

Yet another line of promising research is the transdifferentiation of terminally differentiated adult cells into other cell types using only drug-based induction, thereby avoiding the need for stem cells altogether. Most of the research in this area focuses on transdifferentiating readily accessible fibroblasts into other cell types such as endothelial cells⁸³ and insulin-expressing clusters, which may have potential for the treatment of type 1 diabetes.⁸⁴

Toward regeneration with living allogeneic cell-based products and technologies

Allogeneic cell-based therapy in wound healing began with a bilayer product containing keratinocytes and fibroblasts in self-generated matrix. This product is still marketed today and is accompanied by several additional single-layer and bilayer living cell matrix-based products. Although most of these incorporate fibroblasts and/or keratinocytes derived from neonatal foreskin, at least one product contains mesenchymal stem cells in a chorionic membrane matrix.

The mechanism of action of these products is not well understood as the allogeneic cells do not engraft, but are instead degraded by the body after ~4 weeks.^{30,31} Before their degradation, the allogeneic cells presumably release growth factors, matrix components, and other biochemicals that may promote healing, but only one study has examined the role of growth factors in the activity of these products. This study found that an antibody against hepatocyte growth factor/scatter factor blocked the angiogenesis and enhanced motility of human vascular endothelial cells induced by the single-layered product containing human fibroblasts.⁸⁵ Studies have not even definitively ruled out the contribution of cells versus the matrix components of these products, although one study suggests that metabolically active cells are important for biological response and, further, that ex-

tremely high activity can actually interfere with a response.^{86,87}

An understanding of the mechanism of action may be important to future regenerative allogeneic cell therapies in wound healing. For instance, if the allogeneic cells act by recruiting the patient's own cells, they may be more effective if the endogenous cells are healthy and responsive—features not always observed in chronic wound patients. If a certain growth factor or series of growth factors is responsible for the benefits of allogeneic cell therapy, different cell types could eventually be selected or engineered for the secretion of these biochemicals. Alternatively, if the presence of allogeneic cells and/or the process of their degradation contribute(s) to healing, strategies designed to capitalize on these effects could be developed. Moreover, the extent to which matrix components contribute to healing when combined with cells is unknown and thus it is difficult to determine whether future strategies should focus on cells and matrix in isolation or in combination.

Toward regeneration with other approaches in wound healing

Although no gene therapies are currently approved in the United States, several other countries have approved at least one such treatment and more are under study. A 2006 report from Italian investigators described a combination gene and stem cell approach in a single patient with epidermolysis bullosa—a severe genetic and often fatal disease, in which the skin blisters due to mutations in proteins at the dermo-epidermal junction.⁸⁸ Epidermal stem cells were obtained from the patient's palm and transfected with a retroviral vector expressing the normal form of the gene encoding laminin 5, which was mutated in the patient. These cells were then used to produce genetically corrected cultured epidermal grafts, which were transplanted onto the patient's legs. The procedure resulted in functional laminin 5, an adherent epidermis, and a resolution of blistering for up to 1 year. The patient has been followed for 6.5 years and continues to show no signs of the disease.⁸⁹ Interestingly, the transplanted stem cells still retained molecular features of the skin of the palm and have not adopted features of leg skin cells.

In 2009, a group of researchers in the United States reported results of a phase 1 study of an adenoviral construct expressing PDGF for venous ulcers.⁹⁰ This study demonstrated the safety and feasibility of the gene therapy, with documented transfection of cells in the wound, migration of endothelial precursor cells to the wound, formation

of granulation tissue, and a decrease in wound size at 28 days in nearly all patients.

Several other gene therapies are in development for conditions such as critical limb ischemia that could have an impact on chronic wounds, including a DNA plasmid encoding stromal cell-derived factor-1.⁹¹ Still other gene therapies are being investigated in preclinical models, such as those for diabetes. In one study, insulin gene constructs corrected hyperglycemia and metabolic abnormalities when injected into diabetic rats.⁹²

Numerous proteins and peptides have been investigated for use in wound healing, although their longevity and/or bioactivity in chronic wounds can be variable given the highly proteolytic environment.⁵¹ The most popular proteins developed for wounds have been growth factors. A topical preparation of PDGF is commercially available for diabetic foot ulcers, but, overall, the efficacy of topical growth factors has been relatively disappointing in humans compared with their robust effects in other animals, and a considerable number of negative studies have gone unpublished.⁹⁰ Single matrix proteins such collagen, laminin, and fibrin have also been examined in wound healing; however, these have not yet gained traction as therapies, possibly due to their susceptibility to peptidase attack and consequent limited half-lives. Of course, collagen, hyaluronic acid, and several other matrix proteins are often injected as dermal fillers in cosmetic procedures. However, in wound healing, investigators are using peptide sequences from some of these proteins in novel ways, such as attaching them to various biomaterials engineered to mimic mechanical features and, possibly, functions of the extracellular matrix. Fibronectin sequences are one example of this; in one study, three fibronectin domains attached to a hyaluronan matrix rapidly recruited fibroblasts in an animal wound model.⁹³ Another example is the collagen peptide E1, which is liberated when collagen is degraded. This peptide increased wound contraction and reepithelialization in an animal model.⁹⁴

Additional nonmatrix proteins involved in wound healing are also being investigated that may eventually be useful in some format as treatments. For example, protein kinase D1 has been found to play a critical role in the reversal of keratinocyte differentiation in culture, and deletion of this enzyme in mice led to delayed wound reepithelialization that correlated with decreased proliferation and migration of keratinocytes at the wound edge.⁹⁵ Gap junction proteins such as connexin-43 are also being targeted with synthetic peptides⁹⁶ and antisense oligonucleotides.⁹⁷

Overall, it is possible that the targeting of individual proteins, genes, or even pathways (irrespective of the approach used) will be insufficient to effect regeneration. Instead, multiple components may need to be targeted at once or in sequence, particularly in chronic wounds where some processes may need to be inhibited and others stimulated. Such a multicomponent strategy is perhaps what we hope to gain from stem cells; however, even here it is unclear whether an adjunct or complementary strategy will be beneficial or needed.

CHALLENGES AND OPPORTUNITIES

Given the advances toward regenerative medicine and their likely application to cutaneous wounds, an important question becomes the extent to which the field of wound healing is prepared for their integration. The field faces a number of critical challenges and opportunities that will shape the adoption and outcomes of these therapies for the foreseeable future.

One major challenge is a need for characterization of the population. Population-wide epidemiologic studies of chronic wounds are decidedly lacking, and prevalence numbers are repeatedly cited that may not have a traceable primary data-based source (*e.g.*, 2.5 million people in the United States with venous ulcers).^{24,98–100} The prevalence, incidence, and cost of diabetic ulcers in the Medicare population, as well as the prevalence and mortality associated with amputation, have been well documented by Margolis and colleagues,^{2,101–103} but there is still a need for epidemiologic data on diabetic ulcers for the overall U.S. population. Pressure ulcer data are also needed, particularly given the increased emphasis on prevention implemented over the last few years. Ideally, prevalence studies would also include comorbidities and other health-related data (*e.g.*, quality of life, pain) to provide a more comprehensive characterization of the populations of interest.

Similarly, more extensive research is needed into the pathophysiology of chronic wounds. It is difficult to develop superior treatments in the absence of knowing the appropriate targets or the underlying microenvironmental factors that must be addressed for such treatments to deliver improved outcomes. This challenge includes our lack of understanding of how matrix- and cell-based therapies may work and incorporates attendant opportunities as to what we can do to improve them. Key questions include the precise mechanisms involved as well as the role of chronic in-

flammation¹⁰⁴; similarities and differences among the chronic wound types; and the reasons for prolonged nonhealing in a portion of patients versus those that heal with several weeks to months of standard of care.

Another overarching challenge and opportunity in wound healing—as with every area of medicine in today’s environment—is the demonstration of value. Value begins with evidence-based medicine, and wound care is plagued by the paucity of well-designed, comparative clinical trials in well-defined patient cohorts. Despite a few prominent exceptions, most of the products for skin wounds lack adequate data on clinical outcomes,¹⁰⁵ which may be partly due to their approval along the device 510K regulatory pathway that does not require randomized controlled trials comparing them with standard of care, as well as the historical ability of products to achieve market access without such data. Moreover, even randomized studies in wound healing tend to be relatively small and lack an attempt at blinding, such as raters who are unaware of group assignment.

Moreover, health-related quality of life and, more generally, patient-reported outcomes are increasingly recognized as critical components of value, as exemplified by the 2009 Food and Drug Administration (FDA) guidance document that provides recommendations for manufacturers on the use of patient-reported outcomes in clinical trials.¹⁰⁶ These outcomes may include patient satisfaction or preference as well as more elaborate and validated health-related quality of life questionnaires. Overall, the inclusion of these measures reflects the growing importance of patient participation in clinical decision making and disease management.

In addition to clinical and patient-reported outcomes, health economic analyses will be needed to support both appropriate market access and pricing for new regenerative medicine technologies in wound healing. As the US healthcare system shifts toward an emphasis on outcomes and affordability, value—as opposed to fee per service rendered—will be the predominant paradigm.¹⁰⁷ Thus, regenerative medicine products not accompanied by demonstrated value dossiers are unlikely to be integrated into the healthcare system even if they hold significant therapeutic advantages.

Regulatory evolution and challenges are also concerns with the development of regenerative medicine technologies as many fall outside the current classification and characterization schemes developed for drug therapies (*e.g.*, dose–response pharmacokinetics). These challenges have been

observed in wound healing with the approval of cell therapies and development of the PDGF gene therapy. New technologies such as gene therapy, diagnostic devices and services, and nanotechnology may require novel clinical trial designs and value concepts. Pluripotent stem cells, which are on the horizon for a variety of different disease states, will require some completely new regulatory considerations in the form of maintenance and induction of pluripotency,¹⁰⁸ and other stem cell types are also likely to require unique regulations and dossier requirements. Exceptions already exist for conditions such as genetic skin diseases that lack any adequate therapies; in these cases, gene or other therapies that show promise of curing the genetic abnormalities in phase 1 studies may not undergo randomized trials, not only because a standard of care is lacking but also for ethical and practical reasons such as resource use.

It has been proposed that early engagement, collaboration, and flexibility are key ingredients to the successful regulation of regenerative medicine products¹⁰⁸ and, thus far, regulators in key markets across the globe appear to be following this strategy. Notably, some analysts predict that FDA reforms are likely to occur soon and these may include modernization of clinical trials, use of real-world evidence in product development, streamlined review of combination products, expedited approval pathways, the use of biomarkers and statistical models, and even the potential for harmonized approvals with coverage determinations.¹⁰⁹

Overall, the field of wound healing remains a primary target for regenerative medicine therapies. To adequately capitalize on advances that may substantially help patients with cutaneous wounds, stakeholders in the wound care community must collaborate to meet the challenges and opportunities outlined here to prepare the way for the adoption of regenerative medicine. Ultimately, we need to meet these challenges so that the entire field can move forward technologically, scientifically, medically, and socially—by making available treatments that are worth their price and truly improve patients' lives.

SUMMARY

The high cost of chronic wounds in the United States will be unsustainable as the population

TAKE-HOME MESSAGES

- Chronic wounds are prevalent, costly, and stressful for patients; new, highly effective treatments are urgently needed.
- Regenerative medicine strategies are in clinical trials in all areas of medicine that have the potential to dramatically reshape our approach to disease.
- Wound healing is a logical target for early development of regenerative strategies due to the regenerative nature of wound healing, the accessibility and structure of skin, the lack of good limb salvage treatments, and the field's early adoption of cell therapies.
- Regenerative medicine strategies developed in other areas of medicine may also be useful for wound healing given the commonalities in signaling and cell development pathways.
- For the impact of regenerative medicine to be fully and rapidly integrated into wound healing, regenerative technologies must be accompanied by demonstrated value to payers and improved outcomes for patients.

ages. More effective treatments are needed to heal these wounds, prevent serious complications such as infection and amputation, and improve patients' lives. Advances in regenerative medicine have the potential to dramatically enhance outcomes for patients with chronic wounds, but they will require documentation of the health economic value to be adopted for widespread clinical use.

Regenerative technologies are in clinical trials across different areas of medicine and are, due to commonalities in cell signaling and developmental pathways, likely to have applications for multiple tissue types and disease states. Wound healing is a logical target for regenerative medicine given the regenerative nature of healing as well as the physical features of skin (*i.e.*, relatively avascular, flat, accessible). Moreover, few good limb salvage treatments are available and the field has a history of using cell therapies. For wound healing to capitalize on advances in regenerative medicine, the field would ideally have a strong understanding of wound pathophysiology and therapeutic targets. Also needed are established study designs capable of demonstrating the value of these new therapies, methods to identify patients most likely to benefit, and regulatory strategies that would ensure timely approval.

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ABOUT THE AUTHORS

Geoffrey Gurtner, MD, is the Johnson and Johnson Professor of Surgery and Materials Science (by courtesy) at Stanford University and Executive Faculty in the Stanford Biodesign Program. Dr. Gurtner is the author of more than 180 peer-reviewed publications and is the Director of the Stanford Advanced Wound Care Center. **Mary Ann Chapman, PhD**, is a scientific communications writer based in Mead, WA.

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Abbreviations and Acronyms

3D = three-dimensional
 FDA = Food and Drug Administration
 PDGF = platelet-derived growth factor
 siRNA = small interfering RNA
 Wnt = wingless integrated (gene name)