

Nanopharmaceuticals and Gene Therapy Applied to Wound Care

These new drugs hold great promise.

Objectives

- 1) Recognize the potential wound care applications of nano-pharmaceuticals and gene therapy.
- 2) Become familiar with nanotechnology and virus vectors for drug delivery.
- 3) Appreciate the clinical investigations regarding both nano-pharmaceuticals and gene therapy as they pertain to wound healing as found in the literature.



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Following this article, an answer sheet and full set of instructions are provided (p. 196).—**Editor**

By Robert G. Smith DPM, MSc, RPh

Introduction

Shelves are lined with volumes of aged, leather-bound journals with the title of "Nature" embossed on their spine dating back to 1886. The damp aroma of mildew fills the archive section of the medical

library. This fragrance has come to signify the passage of time as it reminds students and curious health-care professionals of the contributions made by investigators to life, medicine, and science. The history of topical wound care is rich with contributions dating back centuries. Over time, these contribu-

tions have changed the ideology and philosophy of topical wound care treatments. Collective works on aseptic practices, antibiotics, and treatment of wounds are represented by Lister, Fleming, and Winter.¹⁻⁵

George Winter is considered the

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father of moist wound healing.⁶ Winter questioned whether allowing a wound to dry out was indeed the best practice to achieve optimal healing.⁷ Winter's simple deduction that a wound's physical environment must be considered during evaluation has had a profound effect on modern wound care by stimulating and ushering in abundant research centered on the development of new wound care products and technology.

The realization and understanding of the process by which tissue injury produces a dynamic, interactive response that involves the complex, overlapping interaction of cell types, extracellular matrix molecules, and soluble mediators⁸ has enabled researchers to discover new information on the signals that control wound repair cell behavior. This knowledge has led to a number of novel therapeutic pharmacologic strategies to assist and enhance cutaneous wound repair in those

wounds identified as hard-to-heal.

The primary focus of this review is to present and elucidate pharmacological aspects of both nanomedicine and gene therapy as two therapeutic approaches to empower the podiatric physician when treating chronic lower extremity

*One nanometer
(1nm) is 10 times
the diameter
of a hydrogen atom
which is 0.1 nm.*

wounds. The rationale for both technological strategies to assist tissue repair will be first offered as a foundation. Secondly, specific creative techniques and methods employed with nano-medicine (technology) and gene therapy (vectors) to assist in wound healing will be

offered. Finally, as illustrated by clinical medical literature, applications for both these novel therapies as they apply to wound healing will be offered.

Nano-pharmaceuticals Rationale

Nanomedicine is defined as the use of molecular devices to address medical problems. The podiatric physician may remember from chemistry class that one nanometer (1nm) is 10 times the diameter of a hydrogen atom which is 0.1 nm.⁹ Further, nanomedicine is the science of creation and utilization of materials, devices, and systems applied in medicine using components and applications as tiny as 1 in 80,000 of the diameter of a human hair. On this scale of one nanometer, materials and devices can interact with cells and biological molecules in unique ways.¹⁰ These devices and particles are being applied as sensors to detect molecules such as proteins or DNA, as imaging enhancers, and

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TABLE 1
Nanotech in Medicine

Nanotechnology	Use	How It Works
Nanowires	Sensing	Conductive wire, 10-20 nanometers To detect proteins or DNA, probes made of complementary antibodies or DNA are attached to each wire When a protein meets its matching antibody, it binds to the probe and changes the conductive properties of the wire, allowing the event to be detected electronically
Quantum Dots	Imaging	Nanocrystals made of inorganic elements encased in latex or metal respond to light by emitting fluorescence at different wave lengths and intensities depending on their composition. Antibodies attached to the crystals can cause dots to bind to a select tissue, such as a tumor, which can then be more easily seen with conventional imaging devices
Nonoparticles	Tissue Targeting Delivery	Particles composed of a variety of materials can be constructed to contain therapeutic molecules in their core and to release them at a desired time and location.
Nanoshells	Tissue Targeting Imaging	Solid silica nanospheres, sometimes encased in a thin layer of gold, will travel through the blood stream without entering most healthy tissues, but tend to accumulate in tumor tissue. Depending on their composition, nanoshells can also absorb or scatter light, enhancing tumor images made with certain forms of spectroscopy

Adapted from Heath JR et al. Nanomedicine Targets Cancer Scientific American 2009; 300 (2) 48.

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as a means to target specific tissues and deliver therapeutic agents.¹⁰ (Table 1)

Nano-pharmaceuticals are pharmaceuticals designed using "nanotechnology." Bottomley defines nanotechnology as it applies to the pharmaceutical industry as the generation of therapeutically relevant matter between 1 and 100 nanometers in size.¹¹

In addition, Bottomley proposes that nanotechnology can provide various technical solutions for effectively delivering anticancer agents and other medications.¹¹ More complex delivery vehicles with nanometer dimensions can be formulated from both organic and inorganic constituents. These creations possess complex functions to include controlled release, targeting and encapsulation.

Nanoparticles used in tissue targeting delivery are particles composed of a variety of materials constructed to contain therapeutic molecules in their core and to release them at a desired time and location.¹⁰ Nanotechnology can improve medication delivery to the site of therapeutic action either by associating drug nanoparticles with a carrier such as plasma albumin to improve organ targeting, or by encapsulating the active material in liposomes (Figure 1). Both these processes enhance the drug compound's half-life and improve medication targeting.¹¹ Finally, by limiting systemic exposure, the targeting of drugs using nanotechnology techniques can also reduce general drug-induced toxicities.¹¹

Gene Therapy Rationale for Tissue Repair Application

Gene therapy is a form of molec-

ular medicine. The major goal of gene therapy is to introduce a functional gene into a target cell and restore protein production that is absent or deficient due to a genetic disorder. The acceptance that wound

healing proceeds through an ordered cascade of events that is mediated by specific growth factors, growth factor receptors, and cytokines as well as acknowledging that deficiencies of these endogenous growth factors, elevated levels of matrix metalloproteinases

and other neutrophil proteins are the cause of stagnated chronic wounds has allowed science to explore gene therapy as a method to solve these molecular imbalances.¹²⁻¹⁵

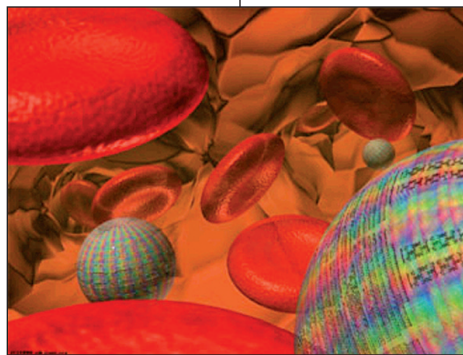


Figure 1: Nanotechnology can improve medication delivery to the site of therapeutic action either by associating drug nanoparticles with a carrier such as plasma albumin to improve organ targeting, or by encapsulating the active material in liposomes

Chandler and Sosnowski have stated that clinical success of growth factor proteins have revealed disappointing results, and only recombinant human platelet-derived growth factor BB has yielded modest improvements in healing.¹⁶ These authors postulate that these disappointing clinical experiences with growth factors have been attributed to the growth factors' short half-lives, degradation by wound proteases,

and the failure to maintain local protein levels above therapeutic thresholds.¹⁴⁻¹⁶

Eming, et al. builds on Chandler and Sosnowski's observations by acknowledging that current drug delivery strategies suffer from in-

herent loss of activity due to the combined effects of physical inhibition and biological degradation.¹⁷ Eming, et al. offer the assertion that a molecular genetic approach

in which genetically modified cells synthesize and deliver the desired growth factor proteins in a locally restricted manner to the wound site would be a powerful means to overcome the limitations associated with topical application of recombinant growth factor proteins.¹⁷ The

final idea that the function of genetically modified cells might be strengthened by implanting them on biomaterial scaffolds that promote cell adhesion, proliferation, migration, and differentiation, thus providing the basis for a regenerative rather than a reparative wound environment, is offered by Eming, et al.¹⁷

Transduction

The process of gene delivery and expression is known as transduction. When genetic material is delivered directly to the target tissue, the process is called in-vivo gene transfer.¹⁶ The in-vivo gene transfer approach is a logical and preferred method for cutaneous wound healing applications because of the ready accessibility of the target tissue.¹⁶ There are three categories in which DNA can be delivered in-vivo: biologic, chemical, and physical. The biological method employs derivatives of viruses to deliver DNA. Viruses are natural vehicles for gene delivery, because they possess highly efficient mechanisms to enter cells, transport DNA to the nucleus, and use the cell's own machinery to activate gene expression, and in some cases, permanently integrate into the host cell's genome.^{16,17}

Viral vectors are derived from

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viruses with either RNA or DNA genomes and are represented as integrating and non-integrating gene products.¹⁸ Verma and Weitzman reveal that many viral infections lead to deleterious effects on the host characterized by destruction of infected host cells.¹⁸ Therefore, they explain, the basic principle of turning viral pathogens into a delivery system relies on the ability to separate the components needed for replication from those capable of causing disease.¹⁸

A number of authors have defined the latest developments in viral vectors for gene therapy.¹⁷⁻²⁰ These authors further explain how great hope was founded on rapid breakthroughs with gene therapy; however, the progress over the last decade has been slower than anticipated.¹⁷⁻²⁰ Finally, these investigators introduce and provide both advantages and disadvantages for the following viral vectors: adenovirus, adeno-associated virus, alphavirus, herpes simplex virus, lentivirus,

and retrovirus. A narrative summary as well as a graphic table (Table 2) of this information is presented to the podiatric physician so that an appreciation of this research may be realized.

Adenovirus

The adenovirus genome is packaged in a non-enveloped icosah-

The primary attachment site for adeno-associated virus serotype 2 is the ubiquitous heparin sulfate proteoglycan.

dral protein capsid.¹⁷⁻²⁰ The fiber protein projects from the virion, and the carboxy-terminal knob domain forms a high-affinity complex with the host cell surface receptor protein.¹⁸ Its genome consists of a

large double-stranded linear DNA molecule approximately 36 kb.^{16,18,20} The adenovirus replication cycle can be divided into two phases: early and late.²⁰ The human adenovirus consists of more than 50 serotypes.^{16,18} The majority of adenovirus serotypes attach to the host cells via the Cocksackie-adenovirus receptor.¹⁸

Adenoviral vectors possess several advantages over other viral vectors that include: efficient transduction of dividing and non-dividing cells, transduction efficiency of greater than 95%; and the facts that the adenovirus genome does not integrate into the host cell genome, transgene expression is transient, and there is no risk for insertional mutagenesis.¹⁶⁻²¹

On the other hand, one major disadvantage with adenovirus vectors is cytotoxicity of viral proteins and host cellular immune responses result in local inflammation and destruction of transduced cells. Also, there is a theoretical chance that pre-existing antibodies will limit the vector's effectiveness

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TABLE 2
Viral Vectors Used for Gene Therapy

Vector	Packaging Capacity	Host Range	Features
Adeno-associated virus	Low < 4 kb	Broad, infects non-dividing and dividing cells	Slow expression onset Inefficient large scale virus production
Adenovirus	Medium < 7.5 kb	Broad low transduction of neurons	Transient expression Strong immunogenicity
Alphaviruses	Medium < 7.5 kb	Broad, neurons glial cell specific strains	Transient, but extreme, Expression levels Low immunogenicity
Herpes simplex virus	High > 30 kb	Broad, neurons, stem cells muscle cells	Latent infection, Long term expression Low toxicity (mutants)
Lentivirus	Medium 8 kb	Broad, dividing and non-dividing cells	Genome integration Long term expression Safety concerns low titers Production inefficient
Retrovirus	Medium 8 kb	Restricted, dividing cells	Genome integration Long term expression

Adapted from Lunndstrom K. Latest development in viral vectors for gene therapy. Trends in Biotechnology 2003; 21 (3) 118

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when administered repeatedly because previous exposure to adenovirus is common in humans.^{16,22}

Adeno-associated Virus

Adeno-associated viruses are nonpathogenic human parvoviruses that are small non-enveloped particles (20-25 nm) carrying a linear single-stranded DNA genome approximately 5kb in size.¹⁶⁻¹⁸ They require either adenovirus or herpes virus helper functions for replication; therefore, virus production is propagated only upon coinfection.^{17,20} The different serotypes of adeno-associated viruses utilize a variety of approaches for cell entry resulting in different host ranges.¹⁸ The adeno-associated virus serotype 2 is the best characterized and has been the most frequently employed recombinant vector.¹⁸ Thus, this establishes adeno-associated virus's position as one of the most popular delivery systems.¹⁹ The primary attachment site for adeno-associated virus serotype 2 is the ubiquitous heparin sulfate proteoglycan.²³

A significant advantage of recombinant adeno-associated virus vectors is they can infect both dividing and non-dividing cells and can express transgenes transiently from an episomal state.¹⁶ One identified major limitation for the use of adeno-associated viruses as a gene delivery vehicle is the relatively small packaging capacity; consequently, the packaging capacity relatively restricts large scale production because it has been proven to be inefficient.^{18,19} Finally, as with adenoviruses, there is the presence of circulating neutralizing antibodies against adeno-associated viruses in the majority of the population because of natural infection.^{16-20,24}

Alphaviruses

The recombinant particles from alphaviruses are generated by cotransfection of its replicon and helper vector RNA.¹⁹ Alphavirus

vectors have mainly been used for recombinant protein expression in cell lines, in expression studies in neurons, and for vaccine production.²⁵ There are limitations to the use of alphaviruses in the field of gene therapy due to their lack of targeting. Furthermore, the use of whole alphaviruses for gene therapy is of limited efficacy both because several internal alphaviral proteins are involved in the induction of apoptosis upon infection and also because the alphaviral capsid mediates only the transient introduction of mRNA into host cells. Neither of these limitations extend to alphaviral envelope pseudotypes of retroviruses or lentiviruses.¹⁶⁻²⁰

Herpes Simplex Virus

Human herpes viruses are a class of large DNA viruses with a large double-stranded genome (152 kb) capable of accommodating a large amount of foreign DNA.^{18,19} Despite its genome being cytotoxic to neurons, herpes simplex virus type-1 has been developed as a vector for gene delivery using multiple gene deletion.^{18,19,26} The herpes simplex virus type 1 virion consists of four components: envelope, tegument, capsid, and viral genome.¹⁸ The envelope is derived from the cellular membrane and contains approximately 12 viral glycoproteins essential for viral entry.²⁰ At present, two major classes of herpes simplex virus type-1 vectors have been developed: replication-defective viruses and replicational mutants.^{17,27,28}

A viral vector advantage is that it infects both dividing and non-dividing cells and does not integrate into the host cell genome. Secondly, the large packaging capacity of herpes simplex virus type-1 amplicons may be very useful for gene

therapy purposes to deliver complex genes and regulatory sequences or to deliver multiple copies of the transgene.²⁰ Conversely, the major problem of recombinant herpes simplex virus type-1 vectors is their cytopathic effect and the induction of an immune response by gene expression. This could be a reason that the compendium emphasizes that currently herpes simplex virus type-1 vectors are being used in only 3.3 percent of gene therapy trials.^{16,29}

Carbon nanotubes (CNTs) can provide strong antimicrobial activity against Escherichia coli.

Lentivirus

Lentiviruses belong to the retrovirus category.¹⁹ Lentiviruses have the unique ability among retroviruses of being able to infect non-cycling cells. Vectors derived from lentiviruses have provided a huge advancement in technology and seemingly offer the means to achieve significant levels of gene transfer in-vivo. Several of the lentivirus vectors used in gene therapy are based on the human immunodeficiency virus.^{19,30}

The limitations of using this virus vector in clinical trials include: the lack of sufficient methods for production of high-titer virus stocks and safety concerns related to their origin from HIV, despite the engineering of packaging cell lines and deletions of genes required for viral replication.¹⁹ To improve the biosafety of lentiviral vectors, all nonessential viral sequences are removed from packaging constructs.²⁰

Retroviruses

Retroviruses are a large family of enveloped RNA viruses found in all vertebrates.²⁰ The enveloped virus particle contains two copies of the RNA genome, surrounded by a cone-shaped core.^{18,20} The viral RNA contains three essential genes: gag, pol, and env and is flanked by long terminal repeats.^{18,20} Retroviruses infect cells via cell surface receptors.¹⁶ After binding to its receptor, the viral capsid containing the RNA

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One advantage of using a retrovirus vector is its ability to integrate into the host genome.

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genome enters the cell through membrane fusion.¹⁸

The RNA genome is reverse transcribed into double-stranded DNA, which then integrates into the host cell genome.^{16,18,31}

One advantage of using a retrovirus vector is its ability to integrate into the host genome and therefore sustain heterologous gene expression for an extended period.¹⁹ This may be one reason for recombinant retroviruses being one of the most successful methods for gene transfer and clinical applications.¹⁷ On the other hand, its ability to integrate into the genome of target cells raises the theoretical risk of insertional mutagenesis.¹⁶ Another risk is generating a replication competent retrovirus during manufacture.^{16,31,32}

Nanomedicine Wound Healing Applications

Given that a major obstacle to wound closure is the presence of infection, some investigators have created antibacterial nanomedicine bio-inspired and synthetic devices. Yacoby and Benhar recently reviewed clinical studies which present nine nanomedicine-oriented applications of antiseptics, disinfectants, and antibacterial therapeutics as found in the literature.³³ The studies were divided into

two groups: antibacterial nanomedicines that are based solely on synthetic materials and those antibacterial nanomedicines based on natural biological substances.³³ The first six studies described synthetic nanomaterials with antibacterial activity.³³ These studies

investigated the following compounds: carbon nanotubes (cnts), fullerene c60, bioactive glasses of the $\text{SiO}_2\text{-Na}_2\text{O-CaO-P}_2\text{O}_5$ system, nano-mgo nanoparticles, encapsulated silver and electrospun

tecophilic nanofibers.

Carbon Nanotubes (CNTs) are single-walled nanotubes with a mean diameter of 0.9 nm that can provide strong antimicrobial activity against *Escherichia coli* by causing severe membrane damage and subsequent cell inactivity. Fullerene C60 may function by puncturing

bacterial membranes or by producing radical-oxygen species that are toxic in both gram-positive and gram-negative bacteria. Bioactive glasses of the $\text{SiO}_2\text{-Na}_2\text{O-CaO-P}_2\text{O}_5$ system have

some antimicrobial activity when suspended in aqueous solutions; they release their ionic compounds over time. Nano-MgO nanoparticles are 45 to 75 nm in size. The bactericidal efficacy of Nano-MgO increases slowly with decreasing particle size. Their mechanism of action and toxicity involves chemical changes that occur in the proteins of the bacteria cell wall.

Encapsulated Silver

Encapsulated silver (Nano-SiO₂) and zinc-silver-loading nano-SiO₂ specimen show superior antibacterial effects; however, the zinc-silver-loading nano-SiO₂ specimen demonstrated greater activity because it involves the presence of both zinc and silver positive ions. Electrospun tecophilic nanofibers encapsulate the toxic material silver (I)-imidazole cyclophane. The encapsulation of the silver heterocyclic carbene complexes with the tecophilic nanofibers increases the bioavailability of active silver

species while reducing the amount of silver used. These complexes were demonstrated to be promising materials for sustained and effective delivery of silver ions with maximum bactericidal activity over a longer pe-

riod of time when compared to aqueous silver.

The last three studies involved bio-inspired, antibacterial, nanomedicine applications based on biological substances: Quaternized chitosan electrospun nanofiber mats, Nisin-loaded poly-L-lactide nanoparticles, and targeted

drug-carrying bacteriophages.³³ Electrospun quaternized chitosan (QCh)/poly (vinyl alcohol) (PVA) fibers have diameters of 60-200 nm with a narrow diameter of distribution. Photo-cross-linked electrospun

QCh/PVA nanofiberous mats had good bactericidal activity against the gram-negative bacteria *Escherichia coli* and the gram-positive bacteria *Staphylococcus aureus*.

Nisin-loaded poly-L-lactide nanoparticles formulation provides for slow protein release and protein stabilization, which yields an efficient antimicrobial system useful in food and pharmaceutical preservation. Targeted drug-carrying bacteriophages are prepared by genetic engineering of filamentous phages to display a targeting moiety on their coat and chemical modification that loads the phage particles with a large payload of drug. The drug is linked to the phage coat through a link that is subject to controlled release at the target cells. This was the only study that was carried out with an intention to develop a therapeutic approach.^{33,34}

Hromadka, et al. present a detailed summary of the beneficial outcomes found in burn wound care with the use of nanofibers created from a host of natural and synthetic compounds which mimic collagen fibrils in the extracellular matrix.³⁵ These authors summarize that nanofiber technology has the capability of dramatically accelerating the development of innovative dressing materials for wound healing.³⁵ Collagen nanofiber mats have demonstrated increased wound healing properties.³⁵ Finally, nanofibers have significant potential in the targeted delivery of drugs

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The process of gene delivery and expression is known as transduction.

Alphavirus vectors mainly have been used for recombinant protein expression in cell lines, in expression studies in neurons and vaccine productions.

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to include: antibiotics, analgesics, and growth factors that will promote burn healing, decrease wound infection, and improve scarring.³⁵

Greenhalgh and Turos recently report their results based on in-vivo studies of polyacrylate nanoparticle emulsions for topical and systemic applications.³⁶ These authors report the first in-vivo study of penicillin-conjugated nanoparticle emulsion in determining toxicity response initiated upon systemic and topical application in a murine model.³⁶ They suggest and conclude that polyacrylate nanoparticle-containing emulsions may afford promising opportunities for treating both skin and systemic infections.³⁶

Gene Therapy Wound Healing Applications

Successful gene therapy strategy relies not only on efficient delivery of the transgene into the target cell, but maximum control over localization and duration of transgene expression.¹⁷ The skin is an attractive tissue site for the development of new genetic therapeutic approaches because of its accessibility.¹⁷ Also, inherited skin disorders, metabolic disorders, tumor disease and wound repair are among the diseases that may benefit from genetic therapeutic approaches.^{17,37}

Eming, et al. have summarized successful in-vitro investigations that centered on dermal fibroblasts that have been genetically modified to express a variety of genes relevant to angiogenesis, fibroblast cell function to include VEGF, PDGF-A and PDGF-B.¹⁷ Further, Eming, et al.'s review described several successful in-vivo approaches to include direct injection of DNA into the skin, DNA particle bombardment, DNA delivery from polymer coatings, microspheres, and synthetic matrices.¹⁷

Specifically, directed to lower extremity medicine, Rogers and Lear present two excellent reviews centered on gene therapy as it applies to diabetic patients and the potential for podiatric physicians to be at the forefront to use this science, to assist in wound closure.^{38,39} After providing summaries

of the available research of gene therapy specific to the lower extremity, these authors conclude with a recommendation that the podiatric physician become familiar with the process of gene therapy and the disease targets currently under investigation.^{38,39}

Clinical Investigations

In order to investigate the current status of nanomedicine and gene therapy clinical investigations as they relate to wound treatments presently, an investigation of the available published clinical reports was researched using an electronic data base. The available published clinical reports on www.ncbi.nlm.nih.gov/PubMed used the following MESH terms: "Gene" and "Therapy" and "Cutaneous" and "Wound" with the

The bactericidal efficacy of Nano-MgO increases slowly with decreasing particle size.

limits of two years, including only human investigations, clinical trials, and meta-analysis. No citations were found. It was not until removing the limitations of clinical trials and meta-analysis and including animal investigations that 13 citations were discovered. Of these citations, one focused on human studies as a review, seven used animal models, and five investigations performed experiments on cell culture models and compared existing technology. The one human investigation reviewed current therapy to prevent and manage hypertrophic scars and keloids in burns in children.⁴⁰

A similar investigation of the available published clinical reports on www.ncbi.nlm.nih.gov/PubMed using the following MESH terms: "Nanomedicine" and "Cutaneous" and "Wound" and "Healing" with the limits of two years and first including only human investigations, clinical trials, and meta-analysis re-

sulted in no citations being found. Again, it was not until the removal of clinical trials and meta-analysis and including animal investigations that eight citations presented themselves. Four citations actually centered on either cutaneous wound healing or wound repair. Half of these citations were cell models, specifically the "porcine" type. Nadworny, et al. investigated the anti-inflammatory activity of nanocrystalline silver using a porcine model.⁴¹ They offer data that demonstrates nanocrystalline silver may be responsible for anti-inflammatory activity and improvement in healing. The last citation centered on photomedicine in a controlled laboratory environment.

The results of these two cross-sectional observations give slight validity and insight to Eming, et al., Verma and Weitzman, and Lundstrom's sad declaration that, despite the initial flurry of viral vector experimentation conducted involving gene therapy, current efforts especially with regard to wound healing therapies are disappointing.¹⁷⁻¹⁹ It is acknowledged that these two isolated observations have a number of limitations. The greatest one is investigator's bias because only one electronic data base was searched and the investigator was not blinded. The investigator selected the MESH terms used and they may have been too limiting for the search criteria.

Conclusions

Cutaneous tissue repair is a dynamic, interactive response that involves the complex, overlapping interaction of many cell types. An understanding of this process has led to a number of novel therapeutic pharmacologic strategies to assist and enhance wound repair. This review introduced the pharmacological aspects of both nanomedicine and gene therapy as they apply to wound care. The rationale for the application of both strategies in tissue repair was offered as a foundation. Specific creative and exciting new techniques and methods employing nanomedicine technology and gene therapy vectors in the context

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of wound healing as found in medical literature was offered. It is hoped that podiatric physicians may appreciate these new developments and strive to become more familiar with them so they may assist their patients to achieve maximum positive outcomes by employing these technologies. ■

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Dr. Smith completed his post-graduate training with the College of Medicine at the University of Wales, Cardiff, Wales, UK in wound care and tissue repair. He



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See answer sheet on page 197.

1) According to this review one nanometer (1 nm) is 10 times the diameter of a

- A) Sodium atom
- B) Helium atom
- C) Hydrogen atom
- D) Potassium atom

2) Which investigator is considered the father of moist wound healing?

- A) John Lister
- B) George Winter
- C) Alexander Fleming
- D) George Washington

3) Nanotechnology can improve medication delivery to the site of therapeutic action with a carrier such as _____ or encapsulating the active material in _____

- A) Plasma platelets, liposomes
- B) Plasma RBCs, liposomes
- C) Plasma WBCs, liposomes
- D) plasma albumin, liposomes

4) Chandler and Sosnowski have stated that clinical success of growth factors reveals disappointing results and only _____ has yielded only modest improvements in healing.

- A) Recombinant human platelet-derived growth factor BC
- B) Recombinant human platelet-derived growth factor BE
- C) Recombinant human platelet-derived growth factor BB
- D) Recombinant human platelet-derived growth factor AB

5) Viral vectors are derived from viruses with either _____ and are represented as integrating and non-

integrating gene products.

- A) RNA or DNA genomes
- B) proteins or fats
- C) viral particles or antibiotics
- D) platelets or WBC

6) Viruses are natural vehicles for gene delivery because they _____

- A) possess highly efficient mechanisms to enter cells
- B) transport DNA into the nucleus
- C) use the cell's own machinery to activate gene expression
- D) all answers are correct

7) Two major disadvantages with adenovirus vectors are _____ and _____, resulting in local inflammation and destruction of transduced cells.

- A) induced HIV infections, and HVB infections
- B) fatal response to host cells, and transient transgene expression
- C) cytotoxicity of viral proteins, and host immune responses
- D) efficient delivery of genes, and resulting host destruction

8) The primary attachment site for adeno-associated virus serotype 2 is the _____

- A) ubiquitous insulin receptor site
- B) ubiquitous glucagon proteoglycan
- C) ubiquitous heparin sulfate proteoglycan
- D) ubiquitous sodium/potassium gateway

9) Lentiviruses belong to the _____ category.

- A) alphaviruses
- B) retroviruses
- C) herpes simplex viruses
- D) adeno-associated viruses

10) A viral vector advantage of herpes simplex virus type 1 is that _____.

- A) it infects only dividing cells
- B) it infects only non-dividing cells
- C) it infects only diploid cells
- D) it infects both dividing and non-dividing cells

11) One advantage of using a retrovirus vector is its ability to _____.

- A) integrate into the host genome
- B) infect all non-vertebrates
- C) replicate pol, gag, and short terminal repeats
- D) not integrate into the host genome

12) Carbon nanotubes (CNTs) can provide strong antimicrobial activity against _____.

- A) Staphylococcus aureus
- B) Pneumococcus
- C) Escherichia coli
- D) Clostridium difficile

13) According to this review, Hromadka, et al. presented a detailed observation that concluded that nanofibers mimic _____.

- A) procollagen fibrils in the intracellular matrix
- B) collagen fibrils in the extracellular matrix
- C) collagen gels in the intracellular matrix
- D) procollagen precursors in the extracellular matrix

14) Greenhalgh and Turos recently reported their results

Continued on page 196

(cont'd)

using _____ for topical and systemic applications.

- A) Electrospun tecophilic nanofibers
- B) Carbon nanotubes
- C) Polyacrylate nanoparticle emulsions
- D) Fullerene C60

15) The following are bio-inspired antibacterial nanomedicine applications _____.

- A) Electrospun quaternized chitosan nanofiber mats
- B) Nisin-loaded poly-L-lactide nanoparticles
- C) Targeted drug-carrying bacteriophages
- D) All the above

16) Alphavirus vectors mainly have been used for recombinant protein expression in cell lines, in expression studies in neurons and _____.

- A) recombinant vectors
- B) vaccine productions
- C) DNA and RNA
- D) proteins

17) The process of gene delivery and expression is known as _____.

- A) transmission
- B) transadhesion
- C) transduction
- D) transmigration

18) The bactericidal efficacy of Nano-MgO increases slowly with _____.

- A) decreasing particle size
- B) increasing particle size
- C) electrically stimulating particle size
- D) ionic charging particle size

19) Nadworny, et al. examined a porcine model to examine _____ activity of nanocrystalline silver to promote improved healing.

- A) anti-platelet
- B) anti-adhesion
- C) anti-inflammatory
- D) anti-bacterial

20) The following are categories in which DNA can be delivered in-vivo by which methods?

- A) biologic
- B) chemical
- C) physical
- D) All the above are delivery methods

See answer sheet on page 197.

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