Nanoscale particle therapies for wounds and ulcers

'Small is beautiful' – this should be the slogan of nanoscientists. Indeed, working with particles less than 100 nm in size, nanotechnology is on the verge of providing a host of new materials and approaches, revolutionizing applied medicine. The obvious potential of nanotechnology has attracted considerable investment from governments and industry hoping to drive its economic development. Several areas of medical care already benefit from the advantages that nanotechnology provides and its application in wound healing will be treated in this article.

KEYWORDS: bacterial infection ▪ biomaterials ▪ gene therapy ▪ growth factor ▪ hyaluronic acid ▪ inflammation process ▪ nanoparticle ▪ nanotechnology ▪ silver ▪ wound healing

Roberta Cortivo[1],
Vincenzo Vindigni[2],
Laura Iacobellis[1],
Giovanni Abatangelo[1],
Paolo Pinton[3]
& Barbara Zavan[†1]

[1]Department of Histology, Microbiology &Biomedical Technologies, University of Padova, Viale G. Colombo 3, 35131 Padova
[2]Plastic and Reconstructive Surgery Unit, University of Padova, Via Giustiniani 2, 35100 Padova, Italy
[3]Department of Experimental & Diagnostic Medicine, General Pathology Section, Interdisciplinary Center for the Study of Inflammation (ICSI) & Emilia Romagna Laboratory BioPharmaNet, University of Ferrara, Via Borsari 46, 44100 Ferrara, Italy
[†]Author for correspondence:
Tel.: +39 49 827 6096
Fax: +39 49 827 6079
barbara.zavan@unipd.it

Wound healing in normal hosts follows an orderly biological process involving several cellular and molecular events that are traditionally organized into three main phases. The purpose of this article is to describe current nanoparticle therapies for wound and ulcer healing, taking into account the nature of the material, the biological event in which they are involved and the biostrategies on which its application is based.

Nanobiotechnology has arisen from the convergence of engineering and molecular biology, leading to the development of structures, devices and systems in the atomic, molecular or macromolecular size range [1]. The potential of nanotechnology has been well known since 1959, when Nobel Laureate Richard Feynman predicted the emergence of a new science that deals with structures on a scale of 1–100 nm. While currently the nanoscale reaches the μm level, the true promise of nanotechnology lies in the ability to manipulate materials on the same unimaginably small scale used by nature [2]. Nanomedicine involves the cutting-edge combination of nanotechnology with medicine.

Several advantages are offered by small size, such as the ability to enter into the cytoplasmic space like Trojan horses, ferrying nanoparticles across cellular barriers and activating specific endocytic and transcytic transport mechanisms. [3,4]. The packaging of small-molecule drugs into nanoparticles could improve their bioavailability, biocompatibility and safety profiles [5] as the pharmacokinetics and pharmacodynamics of a drug-bearing particle is strongly related to particle size [6].

The field of wound healing emerges as one of the clinical applications that will most benefit from this fascinating technology. Successful repair of wounds and tissues remains a major healthcare and biomedical challenge in the 21st Century. In particular, chronic wounds often lead to loss of functional ability, increased pain and decreased quality of life, and can be a burden on healthcare and health system resources. Advanced healing therapies include biological dressings, skin substitutes, growth factor-based therapies and synthetic acellular matrices, all of which aim to correct irregular and dysfunctional cellular pathways present in chronic wounds [7].

This article aims to detail the current state of the art of nanotechnology-based therapies for the treatment of wounds and ulcers.

Wound healing process

By definition, wound healing is a complex predefined cascade of well-orchestrated histological events, aimed to repair the discontinuity of the epithelium due to trauma, compression, burns (external causes) or metabolic or vascular diseases (endogenous causes) [8]. These events reveal themselves through a series of molecular, biochemical and cellular phenomena, usually leading to the anatomical reconstitution of the biological barrier [9]. Indeed, the ultimate goal of wound healing research is rapid recovery with minimal scarring and maximal function [10]. In some types of wound, a pathological deviation from the physiological process of tissue repair can occur, resulting in an excessive or insufficient wound reparation [11]. The normal acute wound healing process takes place through three

future medicine · part of fsg

overlapping steps (FIGURE 1): inflammation, proliferation and remodeling. In severely damaged tissue, the choice of carrier is very important, as the wound healing process must not be disturbed by vehicle constituents [12]. The current nanostrategies, both carrier and drug related, that target the three phases of wound repair will be discussed, highlighting the cell signaling events involved.

Inflammation

Inflammation is the first event that spontaneously begins immediately after injury. It is characterized by clotting and chemotaxis of inflammatory cells that cleanse the wound. Inflammation arises 1–4 days after wounding and it involves the migration first of neutrophils into the wound site, followed shortly by macrophages and later by lymphocytes [8,13]. The fibrin clot matrix initially provides a 3D scaffold through which immune cells migrate to the wound and secrete a host of signaling molecules that act as chemoattractants and growth factors. Platelets produce factors such as PDGF, EGF, TGF-α, TGF-β and VEGF (FIGURE 2) [14].

Fibrin clot
■ Thrombin

One of the first products of the hemostatic response is thrombin (also termed activated factor II or factor IIa) [15]. It is essential to the conversion of fibrinogen to fibrin and is responsible for aggregation of blood platelets in forming the 'platelet plug', as well for activation of other hemostatic factors [16]. Moreover, the activity of thrombin is critical in the later stages of the wound healing process, responsible for increasing vascular permeability and allowing cells and fluid to enter the wounded tissue [17]. In human plasma, the half-life of thrombin is shorter than 15 s due to close control by protease inhibitors and components of the vessel wall [18]. In order to provide drugs with long-term protection from their natural inhibitors, enzymatic degradation and other adverse elements, it has been suggested that therapeutic drugs be conjugated to nanoparticles. In the Ziv-Polat laboratory [19], thrombin was conjugated to maghemite (γ-Fe$_2$-O$_3$) nanoparticles (iron oxide nanoparticles) for the treatment of incisional wounds on rat skin. Results obtained by analyzing tensile strength and histological findings associated with the mechanical properties of the wound 28 days after treatment indicated that thrombin-conjugated (γ-Fe$_2$-O$_3$) nanoparticles accelerated the healing of incisional wounds significantly better than free thrombin and untreated wounds. The relatively greater skin tensile strength obtained with such strategies indicates that the novel conjugated thrombin may reduce complications of surgery such as wound dehiscence [19].

Bacterial infection & sepsis
■ Nanoparticle-bearing antibiotics

As reported previously, the fibrin clot aims to initially provide a 3D scaffold through which
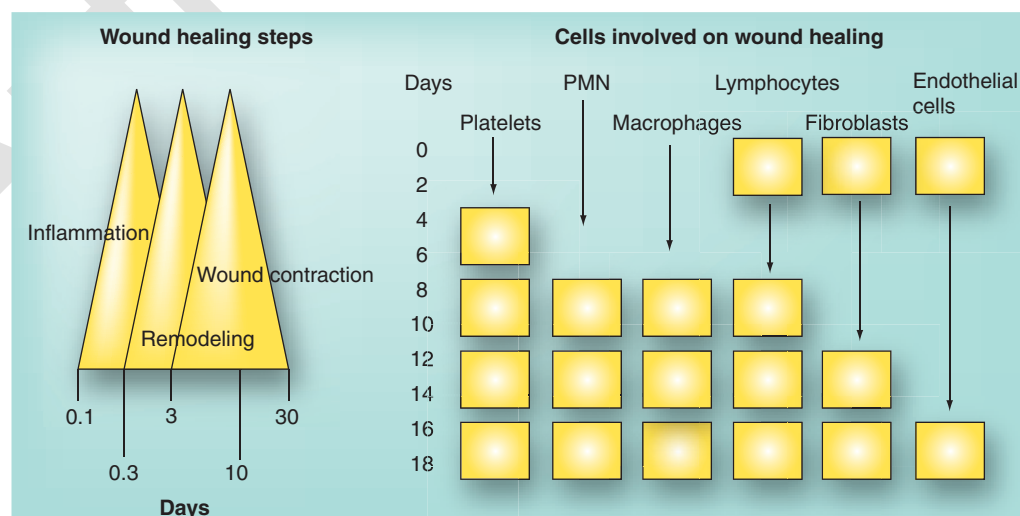

Figure 1. Main biological phases involved in wound healing. On the left, the overlapping events are well defined. None of the phases correspond to a precisely defined period of time, and all phases overlap to a certain degree. Indeed, no phase is initiated exactly at the completion of the previous one. On the right, cells involved in the healing process are reported in a chronological scheme.
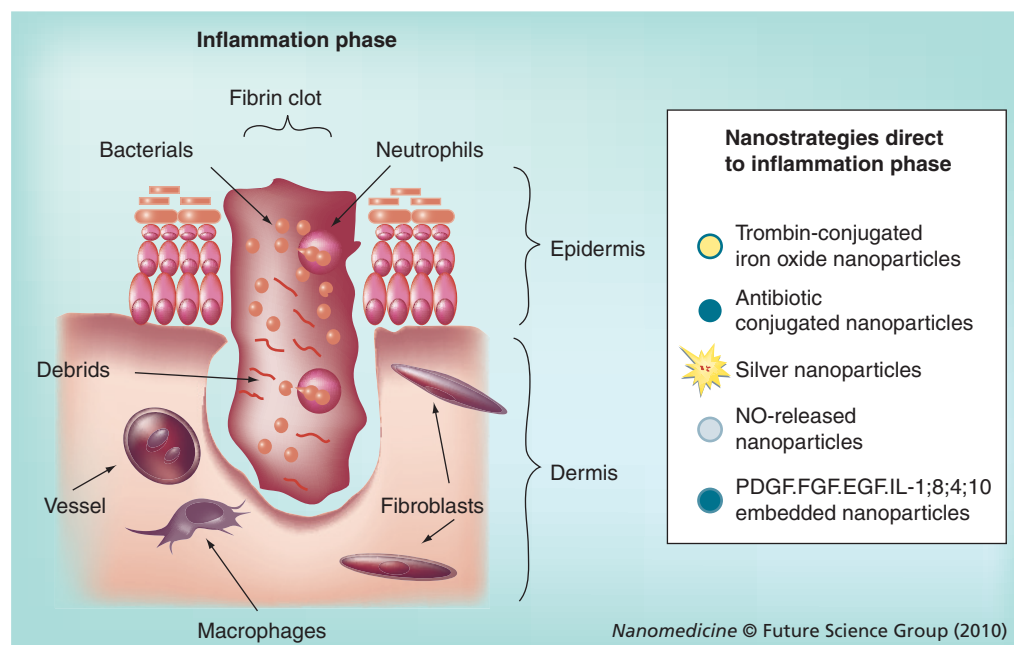PMN: Polymorphonuclear leukocytes.

Figure 2. Principal events during the inflammation phase. In left panel, the main biological events during the first phase of the healing is illustrated. The first events are directed to activating fibrin and platelets to produce a fibrin clot. Subsequently, cytokines, chemokines and growth factors are secreted to battle against infections. In right panel, nanostrategies currently in use to improve healing during this first phase are listed. These treatments take advantage of the characteristics of the biomaterial, the nature of the drug or molecules encapsulated (i.e., antibiotic, trombinin or growth factors), and/or their intrinsic antibacterial effects (i.e., silver or NO).
NO: Nitric oxide.

immune cells migrate to act against bacterial infections. Indeed, the presence of bacteria further exacerbates the tissue damaging processes [20]. We should not forget that wound infection was so ominous an event that at the beginning of 20th Century, infection of burn wounds was the major cause of morbidity and mortality (over 50%) in burn patients [21]. Burn injury disrupts the normal skin barrier as well as many systemic host defense mechanisms, making skin susceptible to microbial colonization and resulting in the development of burn wound sepsis [22].

Staphylococcus is one of the most common human pathogens, at the origin of a wide range of diseases either from direct staphylococcal bacteria invasion or through indirect production of toxins. The majority of infections caused by staphylococci are due to *Staphylococcus aureus*. Moreover, methicillin-resistant *S. aureus* (MRSA), an opportunistic microbe commonly found in skin abrasions and open wounds, has recently been identified as one of the major causes of hospital-acquired infections. Without treatment, these drug-resistant infections can lead rapidly to the onset of bacteremia, sepsis and toxic shock syndrome [23,24]. Treatment of staphylococcal infections is difficult because antibiotic resistant strains have become more

common, increasing the risk of serious complications. Delivery of antibiotics via nanoparticles is a promising drug delivery mechanism, particularly for controlled release or depot delivery of drugs, which would decrease the number of doses required to achieve a clinical effect.

More than 90% of *Staphylococcus* strains are resistant to penicillin [25], methicillin, aminoglycosides, macrolides and lincosamide [26,27]. Staphylococcal resistance to penicillin is mediated by penicillinase (a form of β-lactamase) production, an enzyme that breaks down the β-lactam ring of the penicillin molecule. In 1961, *S. aureus* developed resistance to methicillin, invalidating almost all antibiotics including the most potent β-lactams. Vancomycin is the latest generation antibiotic and can be assumed at the moment to be the most useful, although a fully vancomycin-resistant strain of *S. aureus* first appeared in the USA in 2002 [28]. The emergence of *S. aureus* strains with intermediate levels of resistance to vancomycin (vancomycin intermediate *S. aureus*) has also been identified [29]. A series of vancomycin-modified nanoparticles have been developed and employed in magnetic confinement assays to isolate a variety of Gram-positive and Gram-negative bacteria from aqueous solution by Kell *et al.* [30]. Hachicha *et al.*

prepared microparticles containing vancomycin for intraocular continuous release injection, maintaining drug concentrations above the minimal inhibitory limit for 24 h, a requirement for endophthalmitis prophylaxis after cataract surgery [31].

In a Chakraborty *et al.* investigation, a new method was reported for the preparation of nanoparticles based on carboxymethyl chitosan tagged with folic acid by covalent linkage through 2,2-(ethylenedioxy)bis-(ethylamine) [32]. Physicochemical characteristics of the prepared nanoparticles were examined by Fourier transform infrared spectroscopy, dynamic light scattering and transmission electron microscopy. Vancomycin was then loaded into the prepared nanoparticles through physical adsorption. These drug-loaded nanoparticles were found to be very effective against drug-resistant *S. aureus*.

Turos *et al.* recently identified *N*-methylthiolated β-lactams as a new family of antibacterial agents active against *Staphylococcus* bacteria, including MRSA [33]. Their results suggest that these lactams exert growth inhibitory effects on bacteria through a mode of action that is distinctively different from that of other β-lactam antibiotics, and possess structure-activity patterns unlike those already mapped for other β-lactam antibacterials such as the penicillins. One of the major limitations in the potential application of these *N*-thiolated β-lactam compounds, however, is their exceedingly low water solubility [34]. Thus, Turos and coauthors were interested in identifying an effective drug delivery platform that would enhance the water solubility of lactams, without sacrificing inherent bioactivity. The antibacterial drug was first converted to an acrylated derivative and then dissolved to homogeneity in a liquid acrylate monomer (or mixture of compatible liquid monomers) at 70°C. This mixture was then pre-emulsified in purified water containing 3% w/w sodium dodecyl sulfate with rapid stirring. The resulting homogenous solution of micelles was then treated with potassium persulfate (1% w/w), a water-soluble radical initiator, to induce free radical polymerization. The resulting emulsion was found to contain uniformly sized polyacrylate nanoparticles in which the drug monomer was covalently incorporated directly into the polymeric matrix of the nanoparticle [35]. A unique feature of this methodology is that only one step is required to build the nanoparticle emulsion containing the antibiotic agent from its monomer constituents, without the need for further chemical modification. Most importantly, these nanoparticles were shown to have potent antibacterial activity against MRSA. For these studies, four *N*-thiolated β-lactam derivatives were selected for use as antibiotic drug monomers in order to assess the effect of different lengths and locations of the acrylate linker on the resulting nanoparticle size and anti-MRSA bioactivity. *In vitro* screens have determined that these polyacrylate nanoparticles are nontoxic to human dermal fibroblasts, adding to their favorable characteristics and biocompatibility. Control experiments have indicated that nanoparticle emulsions were completely stable for at least 24 h at elevated temperatures (up to 60°C) and in blood serum, as determined by dynamic light scattering and antibacterial screening (minimum inhibitory concentration analysis).

Silver-based nanoparticles

Although antibiotics are of great importance, their overuse and the failure of healthcare facilities to apply basic infection control policies and procedures have contributed to the high mortality and morbidity of burn wound patients due to infections caused by multidrug-resistant nosocomial pathogens (e.g., *Pseudomonas aeruginosa*, methicillin-resistant staphylococci, vancomycin-resistant enterococci) [36–39]. Thus, antimicrobial therapy that controls colonization and proliferation of microbial pathogens, including multidrug-resistant organisms, is the most important aspect of skin wound care [40]. The recent introduction of antimicrobial agents containing silver has revolutionized burn wound care [41]. Interestingly, for thousands of years, silver and silver ions have been used for their bactericidal properties [42–44], which include:

- Multilevel antibacterial effects that considerably reduce the chances of developing resistance, since this effect of silver is thought to be due to blockage of respiratory enzyme pathways and alteration of microbial DNA and the cell wall [45];

- Effectiveness against multidrug-resistant organisms [46,47];

- Low systemic toxicity [48,49].

Nanotechnology has provided the means of producing pure silver nanoparticles (SNPs), markedly increasing the rate of silver ion release. Jain *et al.* [49,50] synthesized silver (Ag^+) nanoparticles by a proprietary process that involves photoassisted reduction of Ag^+ to metallic nanoparticles and their biostabilization. The

gel formulation containing SNPs in the size range of 7–20 nm has been tested to identify the minimum inhibitory concentration and minimum bactericidal concentration against standard reference cultures, as well as multidrug-resistant organisms. SNP have been shown to destroy Gram-negative bacteria more effectively than Gram-positive bacteria. They also exhibit good antifungal activity, synergism when associated with commonly used antibiotics such as ceftazidime, additive effects when associated with streptomycin, kanamicin, ampiclox, polymyxyn B, as well as antagonistic effects with chloramphenicol.

Surprisingly, SNP also exhibited good anti-inflammatory properties. These results were confirmed by Tian *et al.* in an *in vivo* model that demonstrated promotion of wound healing by silver through reduction of cytokine-modulated inflammation [51]. Silver-induced neutrophil apoptosis and decreased metalloproteinase (MMP) activity may also have contributed to overall dampening of the inflammatory response and as a consequence, an accelerated rate of wound healing. Moreover, the authors showed that in wounds treated with SNPs, the high levels of TGF-β normally present in keloids and hypertrophic scars were lower and associated with higher IFN-γ levels, compared with nontreated wounds in the period before wound closure. As IFN-γ has been demonstrated to be a potent antagonist of fibrinogenesis through its ability to inhibit fibroblast proliferation and matrix production, its control on TGF-β production may play a role [51].

Since cytokines play an important role in wound healing, the authors investigated the expression patterns of IL-6, TGF-β1, IL-10, VEGF and IFN-γ with quantitative real-time PCR. Levels of IL-6 mRNA in the wound areas treated with SNPs were maintained at statistically significantly lower levels throughout the healing process, while mRNA levels of TGF-β1 were higher in the initial period of healing in the site treated with SNPs. The same trend was observed for IL-10, VEGF and IFN-γ mRNA. Moreover, in this study, better cosmetic results were observed in animals treated with SNPs. In terms of wound healing, enhanced expression of TGF-β1 mRNA was found in both keloids and hypertrophic scars.

Cumulative evidence has suggested that TGF-β1 plays an important role in tissue fibrosis and postinjury scarring. The authors demonstrated that lower levels of TGF-β coincided temporally with increased levels of IFN-γ before wound closure in the SNP-treated group. Since IFN-γ has been demonstrated to be a potent antagonist of fibrogenesis through its ability to inhibit fibroblast proliferation and matrix production, its control of TGF-β production may play a role in the positive effects of silver on wound healing. Regarding angiogenesis, it is well known that VEGF promotes healing [52]. Tian *et al.* detected much higher levels of VEGF mRNA in keratinocytes present at the wound edge and in those that migrated to cover the wound surface. Besides a scarce expression in mononuclear cells, VEGF was not expressed in other cell types in the wound, indicating that keratinocytes are a major source of VEGF in the wound. As this factor is highly specific for endothelial cells, it is likely to have a paracrine function in the sprouting of capillaries on the wound edge and in granulation tissue. It appears from these findings that silver treatment not only acts as an antibacterial, but also directly acts on dampening the process of inflammation, thus promoting scarless wound healing [51].

Nitric oxide-delivering nanoparticles

Despite its undoubted success, Ag+ treatment of wounds does have some undesirable effects, such as a cosmetic blue gray coloration of the skin (argyria) with prolonged use and the recent insurgence of silver-resistant mutant bacteria [53–55]. Recent studies have investigated the antimicrobial properties of nitric oxide (NO), a reactive free radical produced by inflammatory cells (e.g., neutrophils and macrophages) during bacterial infection [56–59]. Using small molecules as NO donors, DeRosa *et al.* demonstrated that NO possesses a broad spectrum of antibacterial properties against both Gram-positive and Gram-negative bacteria [60]. Miller *et al.* reported the efficacy of NO in destroying MRSA biofilms, which are complex communities that form when a group of microorganisms self-secrete a polysaccharide matrix that retains nutrients for the constituent cells and protects them from both the immune response and antimicrobial agents [61]. Barraud *et al.* reported that NO-releasing small molecules promoted cell dispersal in *P. aeruginosa* biofilms [62]. As an alternative strategy for delivering NO to pathogenic bacteria, Hetrik *et al.* reported on the antibacterial properties of NO-releasing silica nanoparticles [63] that exhibited enhanced bactericidal efficacy against planktonic *P. aeruginosa* cells compared with small molecule NO donors. The rapid diffusion characteristic of NO may result in enhanced penetration into the biofilm matrix

and thus improved efficacy against biofilm-embedded bacteria [64]. Moreover, the authors demonstrated that with respect to wounds, NO exerted beneficial secondary effects on the healing process by modulating inflammation, angiogenesis and tissue remodeling. Since wounds are known to be deficient in NO [65], application of NO-releasing silica nanoparticles may accelerate healing by killing bacteria and overcoming the general NO deficiency.

Gastrointestinal ulcers

Bacterial infections are also the cause of non-healing wounds in the mucosal environment, giving rise to gastrointestinal ulcers. *Helicobacter pylori* is the major cause of gastrointestinal infection in adults and children worldwide [66,67]. Treatment of *H. pylori* infection [68] is obstructed by two main factors:

- The bacteria live underneath the gastric mucous lining adherent to the gastric epithelium, and hence access of antimicrobial drugs to the site of infection is restricted [69,70];

- Antibiotics are not delivered to the site of infection in effective concentrations and in fully active forms [71].

The formulation of nanoparticles embedded with specific antibiotics has been shown to be successful in clinical applications for gastrointestinal infection. Indeed, the greatest advantage of smaller particles is their ability to be more adhesive and to better act against bacteria present in the gastrointestinal mucosa [72]. Two principal mechanisms have been identified that facilitate particle adhesion in ulcerated tissue:

- Abundant mucus production in the gastrointestinal tract, especially in the stomach, favors small particle adhesion to the mucus layers due to their relatively small mass. In addition, an inflammatory state in the gastrointestinal tract increases mucus production and results in a higher quantity of particle attachment [73];

- Small particles are taken up more easily by immune cells, such as macrophages, in the area of active inflammation; particles in the µm range are taken up by macrophages less effectively [74] and thus constitute the upper limit in size.

With these concepts in mind, the design for anti-*H. pylori* nanoparticle-based therapies is defined by two main aspects: the choice of antibiotic and the quantitative and qualitative adhesive behavior of the nanocarrier in the ulcerated tissue of the stomach.

Nanoparticle-bearing antibiotics

Regarding antibiotics, the most effective treatments for *H. pylori* infection are combinations of two antibiotics (e.g., clarithromycin, tetracycline or metronidazole) and a proton pump inhibitor. At present, therapeutic approaches are available in standard tablet formulations [75]; thus, the drug is released into the stomach and provides a localized effect. In fact, therapy choices are preferentially selective and locally active with limited availability to other tissue [76].

Ramteke *et al.* have prepared clarithromycin- and omeprazole-containing gliadin nanoparticles with desolvation methods using pluronic F-68 as a stabilizing agent [77]. Gliadin defines a group of polymorphic proteins extracted from gluten that are soluble in an ethanolic solution and show a remarkably low solubility in water except at extreme pH. Due to these physico-chemical properties, gliadin nanoparticles can be prepared by desolvation methods for macromolecules using environmentally acceptable solvents such as ethanol and water. These macromolecules showed a high capacity for drug loading and were soluble without further chemical or physical crosslinking.

With *in vitro* and *in vivo* studies, the authors tested the mucoadhesive properties and antibacterial activity of these nanoparticles. Findings indicated greater eradication with the dual therapy nanoformulation compared with traditional formulations [78].

Polystyrene fluorescent nanoparticles were studied by Hasani *et al.* [79] in a detailed analysis of adherence to ulcerated gastric tissue. After induction of gastric ulcers in mice, nanoparticle therapy was administered and inflammation monitored by myeloperoxidase activity, a reliable index that quantifies infiltration of activated neutrophils into the inflamed tissue [80]. At the end of the treatment, confocal laser scanning microscopy analyses qualitatively localized the particles and fluorescent spectroscopy quantitatively determined particle deposition. Results confirmed that nanoparticle deposition was significantly higher in ulcerated compared with healthy tissues [81] and that embedded drug nanoparticles acted in selected areas [82,83].

■ Heparin

A complete study of pH-responsive chitosan/heparin nanoparticles for stomach-specific anti-*H. pylori* therapy was performed by Lin *et al.* [84]. In this study, pH-responsive nanoparticles were produced instantaneously upon the addition of a heparin solution to a chitosan solution

with magnetic stirring at room temperature. The nanoparticles appeared to have a particle size of 130–300 nm, with a positive surface charge, and were stable at pH 1.2–2.5, allowing them to protect the incorporated amoxycilline from destructive gastric acids. Through *in vivo* studies, they demonstrated that the prepared nanoparticles adhered to and infiltrated cell–cell junctions and interacted locally with *H. pylori* infection sites in intercellular spaces.

■ Proliferation

Factors secreted during inflammation trigger and drive the proliferation stage, which normally takes place between 4 and 21 days after wounding. During this stage, fibroblasts are stimulated by FGF and PDGF to invade the wound site and produce extracellular matrix (ECM) components, such as collagen, elastin and glycosaminoglycans, to generate granulation tissue. Fibroblasts also secrete FGF, which can, along with VEGF secreted by platelets and neutrophils, act as an angiogenic factor to stimulate endothelial cell proliferation and migration, thus promoting vascularization at the healing site. While fibroblasts and endothelial cells mostly invade the wound site from its bed, keratinocytes at the margin of the wound undergo a transient burst of proliferation that will sustain epithelialization over the wound (FIGURE 3) [85].

■ Growth factors

The end of the inflammation phase is characterized by the presence of peptides needed during the subsequent phase, proliferation. These factors are mainly growth factors such as PDGF, EGF, CTGF and FGF-β. The aim of growth factors is to attract cells into the wound and to promote cell migration into the wound area, to stimulate the growth of epithelial cells and fibroblasts, to initiate the formation of new blood vessels, and to modulate matrix formation and remodeling of scars [86].

The clinical use of growth factors in wound healing has been of great interest recently. One problem with their use is their rapid breakdown by numerous proteolytic enzymes that enrich the wound site [87]. A means of enhancing the *in vivo* efficacy of growth factors is to facilitate the sustained release of these bioactive molecules over an extended time period by incorporation into polymer nanocarriers. Implantation of a drug delivery device directly into the tissue in need of treatment facilitates localized drug delivery. Delivery systems have been designed in a variety of configurations and have been fabricated from different types of natural and synthetic polymers (degradable and nondegradable) [88,89]. These devices have a common ability to control the release of bioactive proteins for extended periods of time by different mechanisms [90].
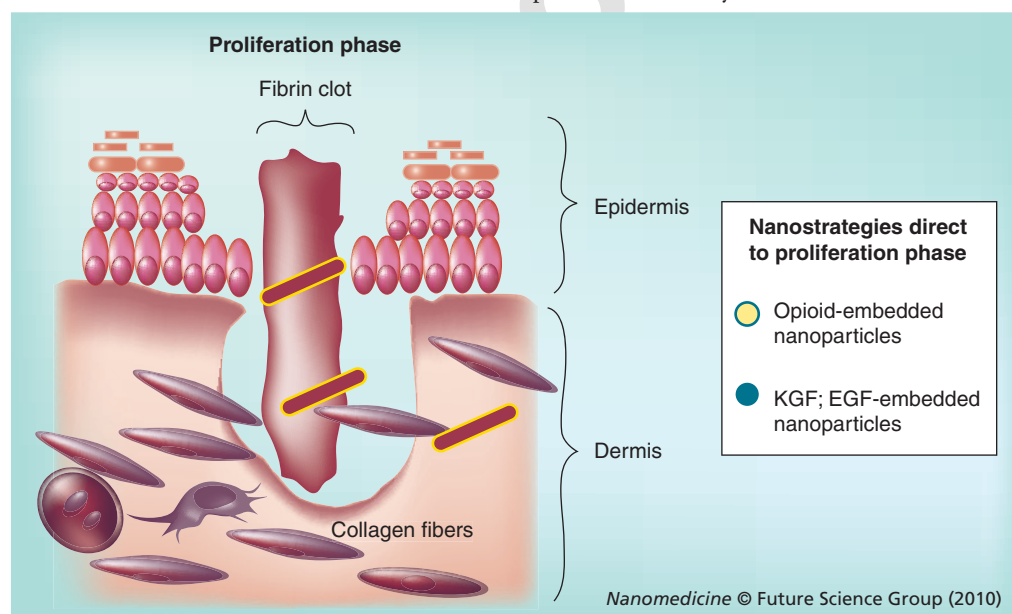

Proliferation phase
Fibrin clot
Epidermis
Dermis
Collagen fibers

Nanostrategies direct to proliferation phase

○ Opioid-embedded nanoparticles

● KGF; EGF-embedded nanoparticles

Nanomedicine © Future Science Group (2010)

Figure 3. Principal events during the proliferation phase. On the left, the main biological events involved in the second phase of healing, proliferation, is illustrated. During this phase, capillaries, fibroblasts and macrophages invade the dermis, giving rise to granulation tissue. Fibroblasts produce a new extracellular matrix over which the re-epithelialization process occurs. On the right, nanostrategies currently in use as therapy in promoting healing during this second phase are listed. Nanosphere-based strategies usually act as drug delivery systems when used during this proliferative phase of healing.

Through incorporation into polymeric devices, protein structure and thus biological activity can be stabilized, prolonging the length of time over which growth factors are released at the delivery site. The period of drug release from a polymer matrix can be regulated by the drug loading, type of polymer used and the processing conditions. Adverse processing conditions that cause protein aggregation or denaturation have to be avoided. In biodegradable carriers, growth factor release is controlled by the polymer matrix's rate of degradation, which causes changes in the morphological characteristics of the materials, such as porosity or permeability [91,92]. The use of porous materials offers advantages that are particularly important for drug release systems: higher specific surface for the adsorption/release of active components and enhancement of the drug release rate for erodible particulate systems. These two aspects are particularly relevant for particles that have nanostructured porosity [93].

These particles are usually produced by double emulsion-solvent evaporation or spray drying techniques. The limits associated with these processes are excessive use of organic solvent, which leads to pollution of the product and waste disposal problems, toxicity for incomplete solvent removal, and thermal and chemical degradation of substances [94]. Other techniques, such as spray drying, operate at temperatures that can thermally denature thermosensible compounds, such as proteins. One of the advantages of using supercritical fluids in polymer processing is the possibility of producing different solid shapes and structures at low temperature with a minimum amount of residual organic solvent [95,96]. Traditional techniques used so far include mechanical treatment, recrystallization of solute from a solution using liquid antisolvent, freeze drying and spray drying. The limits associated with these processes are excessive use of organic solvent, which again leads to pollution of the product and waste disposal problems, and thermal and chemical degradation of bioactive substances. Conversely, processes based on supercritical fluids, and especially on CO_2, have the advantages of being environmentally safe, preserving the properties of thermally labile compounds, and being inexpensive. Drug-loaded polymeric microparticles can be produced by a semicontinuous gas antisolvent precipitation process, in which solutes are precipitated from organic solutions upon contact with compressed CO_2 antisolvent fluxed through a high-pressure precipitation unit [97].

With this technology, Zavan et al. used hyaluronan-based porous nanoparticles, obtained with a high-pressure CO_2 antisolvent technique, as a growth factor delivery system for the in vivo treatment of skin ulcers [98]. The particles had a nanostructured porosity that was particularly suitable for absorbing bioactive molecules. HYAFF11®, the benzyl ester of hyaluronic acid (Fidia Advanced Biopolymer, Italy), is a well-known biopolymer in tissue engineering applications, such as in vitro reconstruction of skin, cartilage and bone, and has recently been used for the in vivo regeneration of small arteries and veins [99,100]. In their study, PDGF was embedded in HYAFF nanoparticles as a delivery system designed to improve full-thickness wound repair. HYAFF particles have the ability to absorb different growth factors, cytokines and bioactive peptide fragments, and to release them in a temporally and spatially specific event-driven manner. This timed and localized release of cytokines, enzymes and pharmacological agents should promote optimal tissue repair and regeneration of full-thickness wounds. PDGF was chosen because it is a potent activator for cells of mesenchymal origin, and a stimulator of chemotaxis, proliferation and new gene expression in monocytes, macrophages and fibroblasts, accelerating ECM deposition [101]. Moreover, PDGF and its relative proteins were the first approved proteins for promoting diabetic foot healing and other chronic nonhealing ulcers. Knighton et al. reported their successful treatment of chronic ulcers since 1986 with an autologous platelet-derived wound healing formula [102]. In their study, the authors optimized timing and localization of release of PDGF to promote optimal tissue repair and regeneration of full-thickness wounds.

Another growth factor that has found great success in clinical application is FGF. The FGF family regulates proliferation, differentiation, migration, ECM deposition and angiogenesis through various pathways [103]. Ikada et al. [104] used bFGF-impregnated gelatin nanospheres on diabetic mice skin wounds, concluding that this is a clinically useful treatment in patients with deficient wound repair such as in decubit ulcers [105,106]. Poly(D,L-lactic-co-glycolic) acid (PLGA) microspheres embedded with EGF, another potent mitogen growth factor, has also been successfully used in the treatment of chronic gastric ulcers [107].

◾ Opioids

While keratinocyte migration indicates a positive effect on the healing process, delayed migration indicates delayed healing. Of the several growth

factors involved in this process (e.g., EGF and KGF), recently Wolf *et al.* [108] indicated that opioids enhance wound healing by promoting keratinocyte migration. This novel finding is of great interest because one of the side effects of a severe skin wound is intense pain. For efficient pain reduction, topical application of opioids appears advantageous. Opioid receptors (μ) are present on nociceptive endings [109] and in the human skin [110]. The endogenous ligand β-endorphin induces keratinocyte migration [111], and opioid receptor agonists, such as dalargin and morphine, accelerate wound healing in rats [112], possibly due to stimulated migration of keratinocytes.

Clinical success using these molecules appears limited thusfar [113], possibly due to too low concentrations of opioid reaching the target site over time. Since nanoparticulate carrier systems can increase skin penetration several folds and slowly release the loaded drug, Wolf *et al.* [108] used both solid lipid nanoparticles and dentritic core-multishell nanotransporters embedded with several opioids to test the influence on keratinocyte migration and local tolerability. HaCaT cells were selected to study keratinocyte physiology and migration in this study. Initial experiments with modified Boyden chambers proved both keratinocyte types to respond to opioids and the positive control, TGF-β1. Inducing the expression of fibronectin receptors, TGF-β1 stimulated keratinocyte migration towards fibronectin covering the membrane, consistently duplicating the number of migrated cells counted in the membrane compared with the negative control. In the chemotaxis model, opioids also enhanced HaCaT cell migration in a concentration-dependent manner. Maximum opioid effects corresponded to TGF-β1 efficacy and were completely inhibited in the presence of naloxone, whereas this TGF-β1-modulated effect was not influenced by opioid receptor antagonism. Thus, opioid-induced migration enhancement was due to specific receptor interaction. To investigate whether the effect of the opioids was chemotactic or chemokinetic in a second set of experiments, drugs were added to the upper, as well as to the lower, chambers to abolish the gradient. Findings showed that TGF-β1 enhanced HaCaT cell migration both chemotactically and chemokinetically; however, the chemokinetic approach eliminated migration induced by morphine, fentanyl and hydromorphone. This indicated that opioids had a chemotactic rather than chemokinetic effect on HaCaT cells. By contrast, buprenorphine still enhanced keratinocyte migration to

a limited extent even after abolishing the gradient, indicating that buprenorphine had at least a partial chemokinetic effect on HaCaT cells. As an acidic pH has to be considered in inflamed wound areas, the efficacy of the opioids in this condition was also evaluated. Adjusting the cell culture medium to pH 6.5, the opioids still evoked a significant chemotactic stimulation of HaCaT cell migration, although slightly less than that observed at physiological pH level. In these different migration assays, the tested opioids evoked almost identical effects, thus the authors focused on morphine as a representative opioid for subsequent investigations.

In the end, they aimed to verify the opioid effect using an independent approach. The so-called scratch test allows for evaluation of the ability of a substance to accelerate closure of a wound that has been created artificially in a cell monolayer. After a repair period of 48 h at 37°C, the HaCaT cells had more efficiently proliferated and migrated into the damaged area in the presence of TGF-β1 in the medium. The addition of morphine also increased the ability of the HaCaT cells to close the wound [113].

To summarize, using the human keratinocyte-derived cell line HaCaT, the authors found that opioids stimulated cell migration and closure of experimental wounds. Enhancement of migration was concentration dependent and could be blocked by the opioid receptor antagonist naloxone, indicating a specific opioid–receptor interaction. These findings indicate that opioids loaded to solid lipid nanoparticles or CMS may be suitable for topical pain reduction and improved wound healing.

Remodeling

The last step of normal acute wound healing is remodeling, during which ECM components are modified by the balanced mechanisms of proteolysis and new matrix secretion, and during which the wound becomes gradually less vascularized. The initial granulation tissue is weak but gains strength over time due to the gradual replacement of immature type III collagen with mature type I collagen (**FIGURE 4**) [86].

The clinical manifestations of wound maturation include contraction, decreased redness, decreased thickness, decreased induration and increased strength. Although these changes continue to develop over a period of weeks, months and even years, their initiation overlaps with the production of granulation tissue during the proliferative phase of wound healing. As such, fibroblasts and their products, collagen and MMPs,

along with blood vessels, constitute the main participants in wound maturation. Excessive contraction in large wounds can lead to contractures, while excess formation of scar tissue leads to hypertrophic scars or even, if the scar tissue extends into the healthy tissue around the wound, a keloid scar [114].

The inverse relationship between increasing wound strength and decreasing wound thickness over time is attributed primarily to remodeling of the collagen-based ECM. This process of remodeling constitutes a balance between collagen production, breakdown and remodeling. Collagen breakdown is attributed primarily to MMP and tissue inhibitor of MMP (TIMP) production and activity. Increased mRNA levels of MMP-2, TIMP-2, and MMP-7 have been demonstrated during the early phases of ECM remodeling [115]. Conversely, expression of MMP-1 and MMP-9 and TIMP-1 mRNA levels decreases prior to ECM remodeling. Thus, it appears that the tightly coordinated expression of specific combinations of MMPs and TIMPs may be necessary for proper wound maturation [116,117].

The absence of appendages is another characteristic associated with mature scars. The most clinically obvious appendage loss involves missing hair follicles. Unlike keratinocyte stem cells, which repopulate the neoepidermis and contribute to the indefinite production of the epithelial layer, the stem cells responsible for epithelial appendages do not appear to repopulate a scar.

Several authors have speculated that this phenomenon may be due in part to the requirement that stem cells live in a special 'microenvironment' or 'niche' [118]. Furthermore, the inability of scar tissue to reproduce a suitable appendage-specific niche may contribute to the absence of these structures in mature scars [119].

In light of these considerations, research approaches to improve the results of the remodeling phase are directed to:

- Manage enzymatic activity of MMP and TIMP to control tissue scar formation;

- Manage stem cell biology to improve appendage formation in mature tissue scars.

Nanotechnology applied to gene therapy as a therapeutic treatment is attracting much interest in the research community, leading to noteworthy developments over the past two decades. Nonviral vectors have recently received increasing attention in order to overcome the safety problems of their viral counterparts. Nanoparticles, with their special characteristics such as small particle size, large surface area and the ability to change surface properties, have numerous advantages over other gene delivery systems [120,121].

Gene therapy

Nonviral polymeric gene delivery systems offer increased protection from nuclease degradation, enhanced plasmid DNA (pDNA) uptake
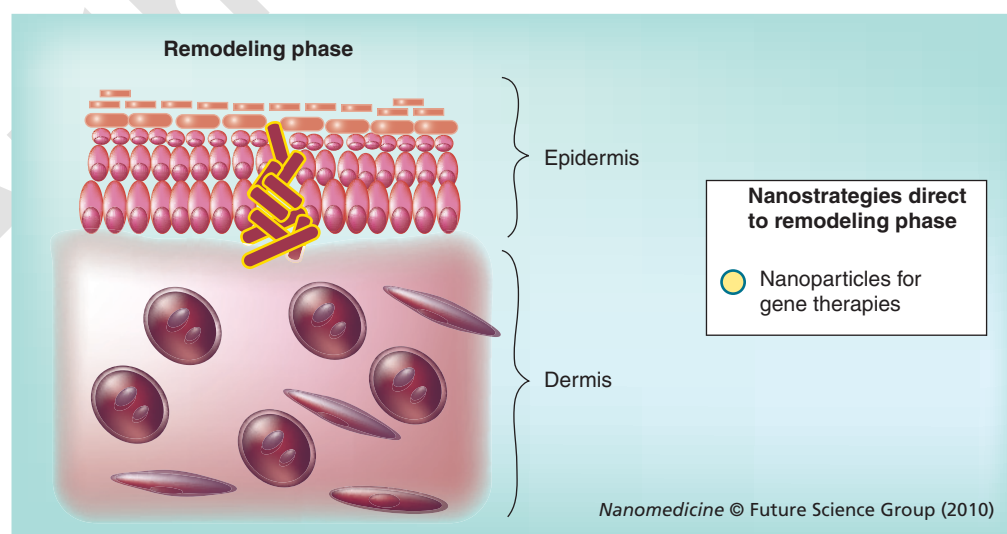

Figure 4. Principal events during the remodeling phase. On the left, the biological events involved during the last phase of the healing, remodeling, is illustrated. The clinical manifestations of wound maturation include contraction, decreased redness, decreased thickness, decreased induration and increased strength. As such, fibroblasts and their products, collagen and metalloproteinases, along with blood vessels, constitute the main participants in wound maturation. In the right panel, nanostrategies currently in use for promoting healing during this second phase mainly act as gene delivery systems.

and controlled dosing to sustain the duration of pDNA administration. Such gene delivery systems can be formulated from biocompatible and biodegradable polymers such as PLGA [122]. Experimental loading of hydrophilic macromolecules, such as pDNA, is low in polymeric particles. The study of Mayo et al. [123] aimed to develop a supercritical fluid extraction of emulsions (SFEE) process based on CO_2 for preparing pEGFP–PLGA nanoparticles with high plasmid loading efficiency. Another objective was to determine the efficacy of pFlt23k, an anti-angiogenic pDNA capable of inhibiting VEGF secretion, following nanoparticle formation using the SFEE process. Results indicated that the SFEE process allowed high loading of pDNA (19.7%, w/w), high loading efficiency (>98%) and low residual solvents (<50 ppm), due to rapid particle formation from efficient solvent removal provided by the SFEE process. pFlt23K–PLGA nanoparticles were capable of in vitro transfection, significantly reducing secreted VEGF from human lung alveolar epithelial cells (A549) under normoxic and hypoxic conditions. pFlt23K–PLGA nanoparticles are not cytotoxic and are of potential value in treating wound disorders in which VEGF levels are elevated [124].

Other materials largely applied for this purpose are polysaccharides and other cationic polymers. They have been recently used in pharmaceutical research and industry to control the release of antibiotics, DNA, proteins, peptide drugs and vaccines. They have also been extensively studied as nonviral DNA carriers for gene delivery and therapy. Chitosan is one of the most used since it can promote long-term release of incorporated drugs. Chitosan micro- and nanospheres can be prepared with different manufacturing processes (nanofabrication). Moreover, the preparation of chitosan and chitosan/DNA nanospheres using a novel and simple osmosis-based method has been recently reported [125]. Masotti described a novel nanofabrication method that may be useful for obtaining small DNA-containing nanospheres (38 ± 4 nm) for biomedical applications [126]. Their reported method has general applicability to various synthetic or natural biopolymers [126]. Solvent, temperature and membrane cut-off are the physicochemical parameters that control the overall osmotic process, resulting in nanostructured systems of different size and shape that may be used in several biotechnological applications.

To target enzymatic activity during the remodeling phase, Chellat F et al. investigated the effects of chitosan–DNA nanoparticles on a human macrophage cell line [127]. Cytokine (TNF-α, IL-1β, IL-6 and IL-10), MMPs (MMP-2 and MMP-9) and MMP inhibitor (TIMP-1 and TIMP-2) release were assessed after incubation with different amounts of nanoparticles. Their secretion was quantified by enzyme-linked immunoadsorbent assay. Gelatinolytic activity of MMP-2 and MMP-9 was determined by zymography in cell supernatants and lysates. Cytokine secretion was not detected even in the presence of high amount of nanoparticles. Conversely, the secretion of MMP-9 in cell supernatants increased significantly after 24 and 48 h in comparison with nontreated cells. MMP-2 secretion was augmented only after 48 h with incubation of the highest concentrations of nanoparticles (10 and 20 µg/ml DNA content). However, zymography studies showed that secreted MMPs were in their proactive form, while in the presence of 10 and 20 µg/ml DNA containing nanoparticles, the active form of MMP-9, but not MMP-2, was detected in cell lysates. In conclusion, exposure of THP-1 macrophages to chitosan–DNA nanoparticles did not induce release of proinflammatory cytokines, but ensured the presence of active MMP-9 within the macrophages. This event could possibly be related to nanoparticle phagocytosis and degradation rather than to inflammatory reactions [128].

Stem cells

Stem cells hold great potential as cell-based therapies to promote vascularization, hair follicle reconstruction and tissue regeneration. Yang et al. have developed transiently modified stem cells that highly express VEGF for the purposes of promoting angiogenesis [129]. Nonviral, biodegradable polymeric nanoparticles were developed to deliver the hVEGF gene to human mesenchymal stem cells and human embryonic stem cell-derived cells. Treated stem cells demonstrated markedly enhanced hVEGF production, cell viability and engraftment into target tissues. Implantation of scaffolds seeded with VEGF-expressing stem cells (hMSCs and hESdCs) led to a two- to four-fold higher vessel densities 2 weeks after implantation, compared with control cells or cells transfected with VEGF through Lipofectamine 2000, a leading commercial reagent. A total of 4 weeks after intramuscular injection into mouse ischemic hindlimbs, genetically modified human mesenchymal stem cells substantially enhanced angiogenesis and limb salvage while reducing muscle

degeneration and tissue fibrosis. These results indicate that stem cells engineered with biodegradable polymer nanoparticles may be therapeutic tools for vascularizing tissue constructs and treating ischemic disease [128].

Conclusion & future perspective

Recently, tremendous progress has been made in discovering the cellular and molecular mechanisms underlying the wound healing process. An efficient and complete process of wound healing is critical for the general wellbeing of any patient. Traditional clinical treatments of wounds/ulcers are still relevant, but more

overlap between novel highly technological approaches, incorporating knowledge on cellular and subcellular events occurring during the normal healing process, could markedly improve future therapeutic interventions.

Nanotechnology offers great opportunity in improving wound healing treatments. The nanometer scale opens the way for the development of novel materials for use in highly advanced medical technology. Future developments depend indeed on identification of clinically relevant targets and on raising targeting efficiency of the multifunctional nanocarriers. As researchers develop the ever-expanding body

---

Executive summary

Inflammation

- Skin damage
  - Increasing life time of the fibrin clot: one of the first products of the hemostatic response is thrombin, which is responsible for the aggregation of blood platelets in the formation of the 'platelet plug' and for increasing vascular permeability, allowing cells and fluid to enter the wounded tissue. In human plasma, the half-life of thrombin is shorter than 15. In order to provide drugs with long-term protection it has been suggested that therapeutic drugs be conjugated to nanoparticles, thereby enhancing their effectiveness. In Ziv-Polat laboratory they have conjugated thrombin to maghemite (γ-Fe$_2$-O$_3$) nanoparticles (iron oxide nanoparticles) for the treatment of incisional wounds on rat skin.
  - Bacterial infection and sepsis: the presence of bacteria in the healing bed exaggerates the tissue damaging processes.
  - Nanoparticles bearing antibiotics against methicillin-resistant *Staphylococcus aureus* (MRSA): one of the most human pathogens commonly found in skin abrasions and open wounds is represented by MRSA, an opportunistic microbe. Several nanoparticles bearing vancomycin or *N*-methylthiolated β lactams against MRSA have been developed to this regard.
  - Silver-based nanoparticles: silver shows multilevel antibacterial effect on cells that considerably reduce the chances of developing resistance. Jain *et al.* synthesized silver nanoparticles by a proprietary process that involves photoassisted reduction of Ag$^+$ to metallic nanoparticles and their biostabilization. Tian *et al.* using silver nanoparticles in an *in vivo* model, confirmed that silver promote wound healing by decreasing inflammatory through cytokine modulation.
  - Nitric oxide (NO)-delivering nanoparticles: NO, a reactive free radical produced by inflammatory cells, posses broad spectrum antibacterial properties against both Gram-positive and Gram-negative bacteria. Small molecules of NO donor based on several materials (e.g., silica) shows speed healing by killing bacteria and overcoming the general NO deficiency.
- Gastrointestinal ulcers
  - Bacterial infection: *Helicobacter pylori* is the major cause of gastrointestinal infection. In this view, Ramteke *et al.* prepared clarithromycin- and omeprazole-containing gliadin nanoparticles; polystyrene nanoparticles have been instead employed by Hasani *et al.* for a detailed analysis of their ability to adhere on the ulcerate tissue of the stomach. A complete study of drug release and attachment behavior of nanoparticles has been performed by Lin *et al.* in which pH-responsive chitosan/heparin nanoparticles for stomach-specific anti-*H. pylori* therapy have been developed and characterized.

Proliferation

- Growth factors: the aim of growth factors is to attract cells into the wound and to promote cell migration into the wound area, stimulate the growth of epithelial cells and fibroblast, start the formation of new blood vessels, and have profound influence on the matrix formation and remodeling of the scars. One way of enhancing the *in vivo* efficacy of growth factors is to facilitate the sustained release of bioactive molecules by their incorporation into polymer nanocarriers. Hyaluronan/gelatin/poly(D,L-lactic-co-glycolic) acid (PLGA) nanoparticles embedded with different growth factors have been successfully applied on skin wounds.
- Opioids: recently it has been showed that opioids enhance wound healing process by the improving of keratinocytes migration. Wolf *et al.,* using solid lipid nanoparticles and dentritic core-multishell nanotransporter both embedded with several opioids, confirmed the influence of these drugs on keratinocytes migration and local tolerability.

Remodeling

- The last step of normal acute wound healing is remodeling, during which the extracellular matrix components will be modified by the balanced mechanisms of proteolysis and new matrix secretion, and during which the wound becomes to be gradually less vascularized.
  - Gene therapy: nonviral polymeric gene delivery systems offer increased protection from nuclease degradation, enhanced plasmid DNA uptake and controlled dosing to sustain the duration of plasmid DNA administration. Such gene delivery systems can be formulated from biocompatible and biodegradable polymers, such as PLGA, polysaccharides and chitosan.
  - Stem cells: stem cells hold great potential as cell-based therapies to promote vascularization and tissue regeneration. Nonviral, biodegradable polymeric nanoparticles were developed by Yang F *et al.* to deliver the hVEGF gene to human mesenchymal stem cells and human embryonic stem cell-derived cells, confirming the engraftment of the graft tissue.

of nanoparticles for use as drug or gene delivery vehicles, there is a growing need to understand how a given nanoparticle's physical and chemical properties affect biological activity and toxicity. Despite the many advantages given by their size (i.e., they can easily pass through the skin barrier), some unpredictable events could occur as well. For example, nanoparticles may interfere with some functions of proteins on the surface of cells, or be taken up into cells and bind to intracellular molecules.

Furthermore, as of today, there is no specific European, American or international standard on the toxicology and biocompatibility of nanoparticles. Thus, while these products are already in use, further research on these medical devices and development of standards on biocompatibility must be extended to the applied science of nanotechnology.

Financial & competing interests disclosure

Bibliography

Papers of special note have been highlighted as:
▪ of interest
▪▪ of considerable interest

1   Freitas JRA: Nanotechnology, nanomedicine and nanosurgery. *Biol. Med.* 3(4), 243–246 (2005).

2   Seetharam RN: Nanomedicine – emerging area of nanobiotechnology research. *Curr. Sci.* 3(91), 260 (2006).

3   Sandhiya S, Dkhar SA, Surendiran A: Emerging trends of nanomedicine – an overview. *Fundam. Clin. Pharmacol.* 23(3), 263–269 (2009)

▪   A good starting point to gain an understanding of the fundamentals of nanomedicine. It discusses in detail the applications of nanotechnology in medicine with more emphasis on drug delivery and therapy.

4   Suri SS, Fenniri H, Singh B: Nanotechnology-based drug delivery systems. *J. Occup. Med. Toxicol.* 2, 16 (2007).

5   Medintz IL, Mattoussi H, Clapp AR: Potential clinical applications of quantum dots. *Int. J. Nanomed.* 3, 151–167 (2008)

6   Debbage P: Targeted drugs and nanomedicine: present and future. *Curr. Pharm. Des.* 15(2), 153–172 (2009).

▪   Focuses on the biological mechanisms of the nanoparticles targeted to a disease site.

7   Rizzi SC, Upton Z, Bott K, Dargaville TR: Recent advances in dermal wound healing: biomedical device approaches. *Expert Rev. Med. Devices* 7(1), 143–154 (2010).

8   Adamson R: Role of macrophages in normal wound healing: an overview. *J. Wound Care* 18(8), 349–351 (2009).

9   Wainwright DJ: Burn reconstruction: the problems, the techniques, and the applications. *Clin. Plast. Surg.* 36(4), 687–700 (2009).

10   Shaw TJ, Martin P: Wound repair at a glance. *J. Cell. Sci.* 15, 122(Pt 18), 3209–3213 (2009).

▪   A good review of wound healing process summarizing the various cell behaviors and their contributions at the wound site

11   Adams PD: Healing and hurting: molecular mechanisms, functions, and pathologies of cellular senescence. *Mol. Cell.* 36(1), 2–14 (2009).

12   Cohen JL, Jorizzo JL, Kircik LH: Use of a topical emulsion for wound healing. *J. Support Oncol.* 5(10 Suppl. 5), 1–9 (2007).

13   Poon IK, Hulett MD, Parish CR: Molecular mechanisms of late apoptotic/necrotic cell clearance. *Cell Death Differ.* 17(3), 381–397 (2009).

14   Spiekstra SW, Breetveld M, Rustemeyer T, Scheper RJ, Gibbs S: Wound-healing factors secreted by epidermal keratinocytes and dermal fibroblasts in skin substitutes. *Wound Repair Regen.* 15(5), 708–717 (2007).

15   Nurden AT, Nurden P, Sanchez M, Andia I, Anitua E: Platelets and wound healing. *Front. Biosci.* 1(13), 3532–3548 (2008).

▪   The authors review the ways in which platelets participate in clinical situations requiring rapid healing.

16   Janmey PA, Winer JP, Weisel JW: Fibrin gels and their clinical and bioengineering applications. *JR Soc. Interface* 6(30), 1–10 (2009).

17   Amara U, Rittirsch D, Flierl M, Bruckner U *et al.:* Interaction between the coagulation and complement system. *Adv. Exp. Med. Biol.* 632, 71–79 (2008).

18   Khoo CW, Tay KH, Shantsila E, Lip GY: Novel oral anticoagulants. *Int. J. Clin. Pract.* 63(4), 630–641 (2009).

19   Ziv-Polat O, Topaz M, Brosh T, Margel S: Enhancement of incisional wound healing by thrombin conjugated iron oxide nanoparticles. *Biomaterials* 31(4), 741–747 (2010).

▪   A well-detailed paper on preparation and *in vivo* application of thrombin conjugated iron oxide nanoparticles.

20   Hurlow J, Bowler PG: Clinical experience with wound biofilm and management: a case series. *Ostomy Wound Manage.* 55(4), 38–49 (2009).

21   Qadan M, Cheadle WG: Common microbial pathogens in surgical practice. *Surg. Clin. North Am.* 89(2), 295–310 (2009).

22   Panuncialman J, Falanga V: The science of wound bed preparation. *Surg. Clin. North Am.* 89(3), 611–626 (2009).

23   Scheurich D, Woeltje K: Skin and soft tissue infections due to CA-MRSA. *Mo. Med.* 106(4), 274–276, (2009)

24   Kobayashi SD, DeLeo FR: An update on community-associated MRSA virulence. *Curr. Opin. Pharmacol.* 9(5), 545–551 (2009).

25   Liu PF, Lo CW, Chen CH *et al.* Use of Nanoparticles as therapy for methicillin-resistant *Staphylococcus aureus* infections. *Curr. Drug Metab.* 10(8), 857–884 (2009).

▪   One of the most recent review emphasizing the potential of nanoparticles in the targeted antibiotics for therapy of staphylococcal infections.

26   Levin TP, Suh B, Axelrod P, Truant AL, Fekete T: Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant *Staphylococcus aureus*: report of a clinical failure. *Antimicrob. Agents Chemother.* 49, 1222–1224 (2005).

27   Chang S, Chang S, Sievert DM *et al.*; Vancomycin-Resistant *Staphylococcus aureus* Investigative Team: Infection with vancomycin-resistant *Staphylococcus aureus* containing the vanA resistance gene. *N. Engl. J. Med.* 348 1342–1347 (2003).

28 Centers for Disease Control Prevention: *Staphylococcus aureus* with reduced susceptibility to vancomycin – United States, 1997. *Morb. Mortal. Wkly Rep.* 46, 765–766 (1997).

29 Hazlewood KA, Brouse SD, Pitcher WD, Hall RG: Vancomycin-associated nephrotoxicity: grave concern or death by character assassination? *Am. J. Med.* 123(2), 182, E1–E7 (2010).

30 Kell A J, Stewart G, Ryan S *et al.*: Vancomycin-modified nanoparticles for efficient targeting and preconcentration of Gram-positive and Gram-negative bacteria. *ACS Nano* 2, 1777–1785 (2008).

31 Hachicha W, Kodjikian L, Fessi H: Preparation of vancomycin microparticles: importance of preparation parameters. *Int. J. Pharm.* 324 176–182 (2006).

32 Chakraborty SP, Sahu SK, Mahapatra SK *et al.*: Nanoconjugated vancomycin: new opportunities for the development of anti-VRSA agents. *Nanotechnology* 21(10), 105103 (2010).

▪ **References** [29–32] **define nanoparticles vancomycin preparations against methicillin-resistant** *Staphylococcus aureus* **(MRSA).**

33 Turos E, Shim JY, Wang Y *et al.*: Antibiotic-conjugated polyacrylate nanoparticles: new opportunities for development of anti-MRSA agents. *Bioorg. Med. Chem. Lett.* 17(1), 53–56 (2007).

34 Greenhalgh K, Turos E: *In vivo* studies of polyacrylate nanoparticle emulsions for topical and systemic applications. *Nanomedicine* 5(1), 46–54 (2009).

35 Garay-Jimenez JC, Gergeres D, Young A, Lim DV, Turos E: Physical properties and biological activity of poly(butyl acrylate-styrene) nanoparticle emulsions prepared with conventional and polymerizable surfactants. *Nanomedicine* 5(4), 443–451 (2009).

36 Greenhalgh DG: Topical antimicrobial agents for burn wounds. *Clin. Plast. Surg.* 36(4), 597–606 (2009).

37 Wolf SE: The year in burns 2007. *Burns* 34(8), 1059–1071 (2008).

38 Zilberman M, Elsner JJ: Antibiotic-eluting medical devices for various applications. *J. Control. Release* 130(3), 202–215 (2008).

39 Edwards-Jones V: The benefits of silver in hygiene, personal care and healthcare. *Lett. Appl. Microbiol.* 49(2), 147–152 (2009).

40 Lo SF, Hayter M, Chang CJ, Hu WY, Lee LL: A systematic review of silver-releasing dressings in the management of infected chronic wounds. *J. Clin. Nurs.* 17(15), 1973–1985 (2008).

▪ **Clearly highlights the need for well-**
designed, methodologically standardized outcome measurement research into the effectiveness of silver-releasing dressings. It also points to the need for a comprehensive assessment of wound bed status in further studies.

41 Monteiro DR, Gorup LF, Takamiya AS, Ruvollo-Filho AC, de Camargo ER, Barbosa DB: The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. *Int. J. Antimicrob. Agents* 34(2), 103–110 (2009).

42 Singer M, Nambiar S, Valappil T, Higgins K, Gitterman S: Historical and regulatory perspectives on the treatment effect of antibacterial drugs for community-acquired pneumonia. *Clin. Infect. Dis.* 47(Suppl. 3), S216–S224 (2008).

43 Albright CF, Nachum R, Lechtman MD: Electrolytic silver ion generator for water sterilization in Apollo spacecraft water systems. Apollo applications program. *NASA Contract. Rep.* Report Number: NASA-CR-65738 (1967).

44 Conrand AH, Tramp CR, Long CJ, Wells DC, Paulsen AQ, Conrand GW: Ag⁺ alters cell growth, neurite extension, cardiomyocyte beating, and fertilized egg constriction. *AViat. Space Environ. Med.* 70, 1096–1105 (1999).

45 Melaiye A, Youngs WJ: Silver and its application as an antimicrobial agent. *Expert Opin. Ther. Pat.* 15, 125–130 (2005).

46 Silvestry-Rodriguez N, Sicairos-Ruelas EE, Gerba CP, Bright KR: Silver as a disinfectant. *Rev. Environ. Contam. Toxicol.* 191, 23–45 (2007).

47 Atiyeh BS, Costagliola M, Hayek SN, Dibo SA: Effect of silver on burn wound infection control and healing: review of the literature. *Burns* 33(2), 139–148 (2007).

48 Neal AL: What can be inferred from bacterium-nanoparticle interactions about the potential consequences of environmental exposure to nanoparticles? *Ecotoxicology* 17(5), 362–371 (2008).

49 Jain J, Arora S, Rajwade JM, Omray P, Khandelwal S, Paknikar KM: Silver nanoparticles in therapeutics: development of an antimicrobial gel formulation for topical use. *Mol. Pharm.* 6(5), 1388–1401 (2009).

50 Arora S, Jain J, Rajwade JM, Paknikar KM: Interactions of silver nanoparticles with primary mouse fibroblasts and liver cells. *Toxicol. Appl. Pharmacol.* 236(3), 310–318 (2009).

51 Tian J, Wong KK, Ho CM *et al.*: Topical delivery of silver nanoparticles promotes wound healing. *ChemMedChem* 2(1),
129–136(2007).

52 Haag SM, Hauck EW, Eickelberg O, Szardening-Kirchner C, Diemer T, Weidner W: Investigation of the antifibrotic effect of IFN-γ a on fibroblasts in a cell culture model of Peyronie's disease. *Eur. Urol.* 53(2), 425–430 (2008).

53 Nascimento EG, Sampaio TB, Medeiros AC, Azevedo EP: Evaluation of chitosan gel with 1% silver sulfadiazine as an alternative for burn wound treatment in rats. *Acta Cir. Bras.* 24(6), 460–465 (2009).

▪ **References** [50–53] **regard silver nanoparticles effects on cellular behavior in wound healing.**

54 Rice LB: The clinical consequences of antimicrobial resistance. *Curr. Opin. Microbiol.* 12(5), 476–481 (2009).

55 Peterson LR: Bad bugs, no drugs: no ESCAPE revisited. *Clin. Infect. Dis.* 49(6), 992–993 (2009).

56 Spellberg B, Guidos R, Gilbert D *et al.*: The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. *Clin. Infect. Dis.* 46(2), 155–164 (2008).

57 Wilson DN: The A-Z of bacterial translation inhibitors. *Crit. Rev. Biochem. Mol. Biol.* 44(6), 393–433 (2009).

58 Weller RB: Nitric oxide-containing nanoparticles as an antimicrobial agent and enhancer of wound healing. *J. Invest. Dermatol.* 129(10), 2335–2337 (2009).

59 Gusarov I, Shatalin K, Starodubtseva M, Nudler E: Endogenous nitric oxide protects bacteria against a wide spectrum of antibiotics. *Science* 325(5946), 1380–1384 (2009).

60 DeRosa F, Kibbe MR, Najjar SF, Citro ML, Keefer LK, Hrabie JA: Nitric oxide-releasing fabrics and other acrylonitrile-based diazeniumdiolates. *J. Am. Chem. Soc.* 129(13), 3786–3787 (2007).

61 Miller C, McMullin B, Ghaffari A *et al.*: Gaseous nitric oxide bactericidal activity retained during intermittent high-dose short duration exposure. *Nitric Oxide* 20(1), 16–23 (2009).

62 Barraud N, Schleheck D, Klebensberger J *et al.*: Nitric oxide signaling in *Pseudomonas aeruginosa* biofilms mediates phosphodiesterase activity, decreased cyclic di-GMP levels, and enhanced dispersal. *J. Bacteriol.* 191(23), 7333–7342 (2009).

63 Hetrick EM, Shin JH, Paul HS, Schoenfisch MH: Anti-biofilm efficacy of nitric oxide-releasing silica nanoparticles. *Biomaterials* 30(14), 2782–2789 (2009).

64 Hetrick EM, Schoenfisch MH: Reducing

implant-related infections: active release strategies. *Chem. Soc. Rev.* 35(9), 780–789 (2006).

65 Soneja A, Drews M, Malinski T: Role of nitric oxide, nitroxidative and oxidative stress in wound healing. *Pharmacol. Rep.* 57(Suppl.) 108–119 (2005).

▪ **Cohomprehensive review on the role of NO, and focus on O$_2^-$ and ONOOH (major components of oxidative and nitroxidative stress, respectively) in the normal and impaired process of wound healing.**

66 Furuta T, Delchier JC: *Helicobacter pylori* and non-malignant diseases. *Helicobacter* 14(Suppl. 1), 29–35 (2009).

67 Bornschein J, Rokkas T, Selgrad M, Malfertheiner P: *Helicobacter pylori* and clinical aspects of gastric cancer. *Helicobacter* 14(Suppl. 1), 41–45 (2009).

68 O'Connor A, Gisbert J, O'Morain C: Treatment of *Helicobacter pylori* infection. *Helicobacter* 14(Suppl. 1), 46–51 (2009).

69 Egan BJ, O'Connor HJ, O'Morain CA: What is new in the management of *Helicobacter pylori*? *Ir. J. Med. Sci.* 177(3), 185–188 (2008).

70 Graham DY, Shiotani A: New concepts of resistance in the treatment of *Helicobacter pylori* infections. *Nat. Clin. Pract. Gastroenterol. Hepatol.* 5(6), 321–331 (2008).

71 McColl KE: Effect of proton pump inhibitors on vitamins and iron. *Am. J. Gastroenterol.* 104(Suppl. 2) S5–S9 (2009).

72 Lai SK, Wang YY, Hanes J: Mucus-penetrating nanoparticles for drug and gene delivery to mucosal tissues. *Adv. Drug Deliv. Rev.* 61(2), 158–171 (2009).

73 Behrens I, Pena AI, Alonso MJ, Kissel T: Comparative uptake studies of bioadhesive and non-bioadhesive nanoparticles in human intestinal cell lines and rats: the effect of mucus on particle adsorption and transport. *Pharm. Res.* 19(8), 1185–1193 (2002).

74 Islam T, Harisinghani MG: Overview of nanoparticle use in cancer imaging. *Cancer Biomark.* 5(2), 61–67 (2009).

75 Campo SM, Zullo A, Hassan C, Morini S: Antibiotic treatment strategies for *Helicobacter pylori* infection. *Recent Pat. Antiinfect. Drug Discov.* 2(1), 11–17 (2007).

76 Asane GS, Nirmal SA, Rasal KB, Naik AA, Mahadik MS, Rao YM: Polymers for mucoadhesive drug delivery system: a current status. *Drug Dev. Ind. Pharm.* 34(11), 1246–1266 (2008).

77 Ramteke S, Ganesh N, Bhattacharya S, Jain NK: Amoxicillin, clarithromycin, and omeprazole based targeted nanoparticles for the treatment of *H. pylori*. *J. Drug Target* 17(3), 225–234 (2009)

78 Ramteke S, Jain NK: Clathromycin- and omeprazole-containing gliadin nanoparticles for the treatment of *Helicobacter pylori*. *J. Drug Target* 16(1), 65–72 (2008)

79 Hassani S, Pellequer Y, Lamprecht A: Selective adhesion of nanoparticles to inflamed tissue in gastric ulcers. *Pharm. Res.* 26(5), 1149–1154 (2009).

▪ **References [75–79] are related to antibiotic-embedded nanoparticles for the treatment of *Helicobacter pylori*.**

80 Virani SS, Polsani VR, Nambi V: Novel markers of inflammation in atherosclerosis. *Curr. Atheroscler. Rep.* 10(2), 164–170 (2008).

81 Agüeros M, Areses P, Campanero MA *et al.*: Bioadhesive properties and biodistribution of cyclodextrin-poly(anhydride) nanoparticles. *Eur. J. Pharm. Sci.* 37(3–4), 231–240 (2009).

82 Lamprecht A, Schäfer U, Lehr CM: Size-dependent bioadhesion of micro- and nanoparticulate carriers to the inflamed colonic mucosa. *Pharm. Res.* 18(6), 788–793 (2001).

83 Chang CH, Lin YH, Yeh CL *et al.*: Nanoparticles incorporated in pH-sensitive hydrogels as amoxicillin delivery for eradication of *Helicobacter pylori*. *Biomacromolecules* 11(1), 133–142 (2010).

84 Lin YH, Chang CH, Wu YS, Hsu YM, Chiou SF, Chen YJ: Development of pH-responsive chitosan/heparin nanoparticles for stomach-specific anti-*Helicobacter pylori* therapy. *Biomaterials* 30(19), 3332–3342 (2009).

85 Johnstone CC, Farley A: The physiological basics of wound healing. *Nurs. Stand.* 19(43), 59–65 (2005).

86 Hantash BM, Zhao L, Knowles JA, Lorenz HP: Adult and fetal wound healing. *Front. Biosci.* 1(13), 51–61 (2008).

87 Murphy G, Nagase H: Progress in matrix metalloproteinase research. *Mol. Aspects Med.* 29(5), 290–308 (2008).

88 Williams DF: On the nature of biomaterials. *Biomaterials* 30(30), 5897–5909 (2009).

89 Maham A, Tang Z, Wu H, Wang J, Lin Y: Protein-based nanomedicine platforms for drug delivery. *Small* 5(15), 1706–1721 (2009).

90 Silva AK, Richard C, Bessodes M, Scherman D, Merten OW: Growth factor delivery approaches in hydrogels. *Biomacromolecules* 10(1), 9–18 (2009).

91 Singh R, Singh S, Lillard JW Jr: Past, present, and future technologies for oral delivery of therapeutic proteins. *J. Pharm. Sci.* 97(7), 2497–2523 (2008).

92 Corrigan OI, Li X: Quantifying drug release from PLGA nanoparticulates. *Eur. J. Pharm. Sci.* 37(3–4), 477–485 (2009).

93 Anglin EJ, Cheng L, Freeman WR, Sailor MJ: Porous silicon in drug delivery devices and materials. *Adv. Drug Deliv. Rev.* 60(11), 1266–1277 (2008).

94 Ungaro F, De Rosa G, Miro A, Quaglia F, La Rotonda MI: Cyclodextrins in the production of large porous particles: development of dry powders for the sustained release of insulin to the lungs. *Eur. J. Pharm. Sci.* 28, 423–432 (2006).

95 Thote AJ, Gupta RB: Formation of nanoparticles of a hydrophilic drug using supercritical carbon dioxide and microencapsulation for sustained release. *Nanomedicine* 1(1), 85–90 (2005).

96 Salmaso S, Bersani S, Elvassore N, Bertucco A, Caliceti P. Biopharmaceutical characterisation of insulin and recombinant human growth hormone loaded lipid submicron particles produced by supercritical gas micro-atomisation. *Int. J. Pharm.* 379(1), 51–58 (2009).

97 Abbas KA, Mohamed A, Abdulamir AS, Abas HA: A review on supercritical fluid extraction as new analytical method. *Am. J. Biochem. Biotechnol.* 4(4), 345–353 (2008).

▪ **Recent review on supercritical fluid extraction useful for nanoparticles production.**

98 Zavan B, Vindigni V, Vezzù K *et al.*: Hyaluronan based porous nano-particles enriched with growth factors for the treatment of ulcers: a placebo-controlled study. *J. Mater. Sci. Mater. Med.* 20(1), 235–247 (2009).

99 Pandis L, Zavan B, Abatangelo G, Lepidi S, Cortivo R, Vindigni V: Hyaluronan-based scaffold for *in vivo* regeneration of the rat vena cava: preliminary results in an animal model. *J. Biomed. Mater. Res. A.* 93(4), 1289–1296 (2010) .

100 Lepidi S, Abatangelo G, Vindigni V *et al.*: *In vivo* regeneration of small-diameter (2 mm) arteries using a polymer scaffold. *FASEB J.* 20(1), 103–105 (2006).

101 Schultz GS, Wysocki A: Interactions between extracellular matrix and growth factors in wound healing. *Wound Repair Regen.* 17(2), 153–162 (2009).

102 Knighton DR, Ciresi KF, Fiegel VD, Austin LL, Butler EL: Classification and treatment of chronic nonhealing wounds. Successful treatment with autologous platelet-derived wound healing factors (PDWHF). *Ann. Surg.* 204(3), 322–330 (1986).

103 Krejci P, Prochazkova J, Bryja V, Kozubik A, Wilcox WR: Molecular pathology of the fibroblast growth factor family. *Hum. Mutat.*

30(9), 1245–1255 (2009).

104 Ikada Y, Tabata Y: Protein release from gelatin matrices. *Adv. Drug Deliv. Rev.* 31(3), 287–301 (1998).

105 Young S, Wong M, Tabata Y, Mikos AG: Gelatin as a delivery vehicle for the controlled release of bioactive molecules. *J. Control. Release* 109(1–3), 256–274 (2005).

106 Han K, Lee KD, Gao ZG, Park JS: Preparation and evaluation of poly(L-lactic acid) microspheres containing rhEGF for chronic gastric ulcer healing. *Control Release* 75(3), 259–269 (2001).

107 Laplante AF, Germain L, Auger FA, Moulin V: Mechanisms of wound reepithelialization: hints from a tissue-engineered reconstructed skin to long-standing questions. *FASEB J.* 15(13), 2377–2389 (2001).

108 Wolf NB, Küchler S, Radowski MR *et al.*: Influences of opioids and nanoparticles on *in vitro* wound healing models. *Eur. J. Pharm. Biopharm.* 73(1), 34–42(2009).

▪ First paper in which the activity of opioids on modulation of cell growth and survival signaling have been exploited in nanomedicine.

109 Bigliardi-Qi M, Sumanovski LT, Büchner S, Rufli T, Bigliardi PL: Mu-opiate receptor and β-endorphin expression in nerve endings and keratinocytes in human skin. *Dermatology* 209(3), 183–189 (2004).

110 Cheng B, Liu HW, Fu XB, Sheng ZY, Li JF: Coexistence and upregulation of three types of opioid receptors, μ, δ and κ, in human hypertrophic scars. *Br. J. Dermatol.* 158(4), 713–720 (2008).

111 Bigliardi PL, Büchner S, Rufli T, Bigliardi-Qi M: Specific stimulation of migration of human keratinocytes by μ-opiate receptor agonists. *J. Recept. Signal Transduct.*

Res. 22(1–4), 191–199 (2002).

112 Poonawala T, Levay-Young BK, Hebbel RP, Gupta K: Opioids heal ischemic wounds in the rat. *Wound Repair Regen.* 13(2), 165–174 (2005).

113 Bigliardi PL, Tobin DJ, Gaveriaux-Ruff C, Bigliardi-Qi M: Opioids and the skin – where do we stand? *Exp. Dermatol.* 18(5), 424–430 (2009).

114 Gill SE, Parks WC: Metalloproteinases and their inhibitors: regulators of wound healing. *Int. J. Biochem. Cell. Biol.* 40(6–7), 1334–1347 (2008).

115 Amalinei C, Caruntu ID, Balan RA: Biology of metalloproteinases. *Rom. J. Morphol. Embryol.* 48(4), 323–334 (2007).

116 Baum CL, Arpey CJ: Normal cutaneous wound healing: clinical correlation with cellular and molecular events. *Dermatol. Surg.* 31(6), 674–686 (2005).

117 Esser S, Wolburg K, Wolburg H *et al.*: Vascular endothelial growth factor induces endothelial fenestrations *in vitro*. *J. Cell. Biol.* 140, 947–959 (1998).

118 Watt FM, Hogan BL: Out of Eden: stem cells and their niches. *Science* 287, 1427–1430 (2000).

119 Spradling A, Drummond-Barbarosa D, Kai T: Stem cells find their niche. *Nature* 414, 98–104 (2000).

120 Xu G, Zhang N: Nanoparticles for gene delivery: a brief patent review. *Recent Pat. Drug Deliv. Formul.* 3(2), 125–136 (2009).

121 Ravi Kumar M, Hellermann G, Lockey RF, Mohapatra SS: Nanoparticle-mediated gene delivery: state of the art. *Expert Opin. Biol. Ther.* 4(8), 1213–1224 (2004).

122 Kim IS, Lee SK, Park YM *et al.*: Physicochemical characterization of poly(L-lactic acid) and poly(D,L-lactide-co-

glycolide) nanoparticles with polyethylenimine as gene delivery carrier. *Int. J. Pharm.* 298(1), 255–262 (2005).

123 Mayo AS, Ambati BK, Kompella UB: Gene delivery nanoparticles fabricated by supercritical fluid extraction of emulsions. *Int. J. Pharm.* 387(1–2), 278–285 (2009).

124 Nahar M, Dutta T, Murugesan S *et al.*: Functional polymeric nanoparticles: an efficient and promising tool for active delivery of bioactives. *Crit. Rev. Ther. Drug Carrier Syst.* 23(4), 259–318 (2006).

125 Masotti A, Bordi F, Ortaggi G *et al.*: A novel method to obtain chitosan/DNA nanospheres and a study of their release properties. *Nanotechnology* 19(5), 055302 (2008).

126 Masotti A, Ortaggi G: Chitosan micro- and nanospheres: fabrication and applications for drug and DNA delivery. *Mini Rev. Med. Chem.* 9(4), 463–469 (2009).

127 Chellat F, Grandjean-Laquerriere A, Le Naour R *et al.*: Metalloproteinase and cytokine production by THP-1 macrophages following exposure to chitosan-DNA nanoparticles. *Biomaterials* 26(9), 961–970 (2005).

128 Andreadis ST: Gene-modified tissue-engineered skin: the next generation of skin substitutes. *Adv. Biochem. Eng. Biotechnol.* 103, 241–274 (2007).

129 Yang F, Cho SW, Son SM *et al.*: Regenerative medicine special feature: genetic engineering of human stem cells for enhanced angiogenesis using biodegradable polymeric nanoparticles. *Proc. Natl Acad. Sci. USA* 107(8), 3317–3322 (2010).