CHAPTER 10

Drug Delivery

S. DELLEANI[a], E. LACROCE[a] AND F. ROSSI[*a]

[a]Department of Chemistry, Materials and Chemical Engineering, "Giulio Natta", Politecnico di Milano, via Mancinelli 7, 20131 Milano, Italy
[*]E-mail: filippo.rossi@polimi.it

10.1 Passive Drug Delivery Systems

The complex wound healing process is divided into the steps of haemostasis, angiogenesis and the restoration of skin barrier function (Figure 10.1). In order for this process to take place correctly, the presence of growth factors (GFs) and cytokines is necessary. However, these factors are not always present at sufficient levels, and this can lead to the derailment of the healing process from its normal success or to its complete interruption.

The speed of the various physiological processes that affect wound healing depends on therapeutic agents, such as growth factors, cytokines, antibacterial agents, proteins, small molecules and bioactive agents. In addition to the speed of healing, there are several factors that should be considered in deciding the route of administration of the therapies: (1) the dysfunction of the vascularization of the wound bed, which decreases the bioavailability of the compounds administered orally or intravenously; (2) the systematic side effects of some drugs; (3) the richness of the wound environment of various pro-inflammatory cytokines that can deactivate drugs; (4) the time of effectiveness of the various physiological processes and the fact that the drugs administered must be present during that period.

Localized controlled release in wound healing can provide spatiotemporal control over drug dosage at the wound site, protect drugs from metabolic

Drug Development and Pharmaceutical Science Series No. 4
Carrier-mediated Gene and Drug Delivery for Dermal Wound Healing
Edited by Pooyan Makvandi and Ehsan Nazarzadeh Zare

Published by the Royal Society of Chemistry, www.rsc.org

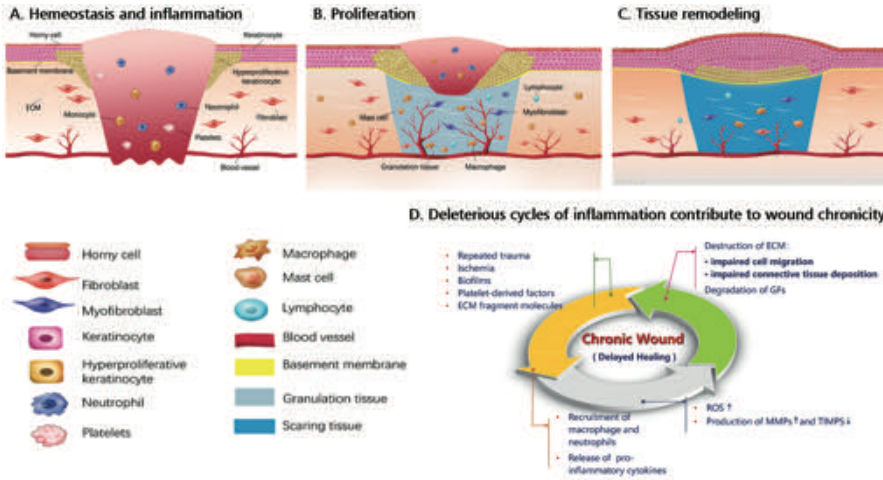

Figure 10.1 (A–C) Different phases of normal wound healing. Normal wound healing is a complicated biological process, which can be divided into: inflammatory phase (A), proliferation phase (B), and tissue remodeling phase (C). The inflammatory phase occurs shortly after injury, and is characterized by the influx of inflammatory cells. In response to inflammatory cues, neutrophils migrate to the wound first, followed by monocyte/macrophage lineages, as well as mast cells. As the inflammatory phase subsides, the proliferative phase of tissue repair begins by the migration and hyperproliferation of dermal and epidermal cells within the wound bed. This phase is marked by epithelialization, collagen deposition, angiogenesis, and formation of granulation tissue. The tissue remodeling phase is characterized by matrix remodeling and declined cellularity. During this phase, the wound undergoes contraction, resulting in the formation of a scar with reduced tensile strength. (D) Schematic to show the deleterious cycles of inflammation that contribute to wound chronicity. It is believed that persistent inflammation is a hallmark of chronic non-healing wounds. Due to repeated tissue injury, microorganisms (*e.g.* biofilms), and platelet-derived factors stimulate the influx of inflammatory cells and the prolonged release of pro-inflammatory cytokines (*e.g.* IL-1β and TNFα), leading to elevated levels of ROS and proteases (*e.g.* MMPs) in the wound bed. Particularly, the protease levels in chronic wounds exceed that of their respective inhibitors. High levels of ROS together with the imbalances between MMPs and TIMPs result in the destruction of ECM components and the degradation of growth factors. The proteolytic destruction of ECM further in turn attracts more inflammatory cells to the wound, thus promoting the inflammation into a detrimental vicious cycle and contributing to wound chronicity.

deactivation and maintain constant drug concentrations for an extended period of time. To define a delivery system as optimal it should be able to sequentially and selectively release antibacterial agents, growth factors, cytokines and other small molecules in a controlled manner so that the wound follows the natural course of healing.[2] For the purpose of this book it is useful

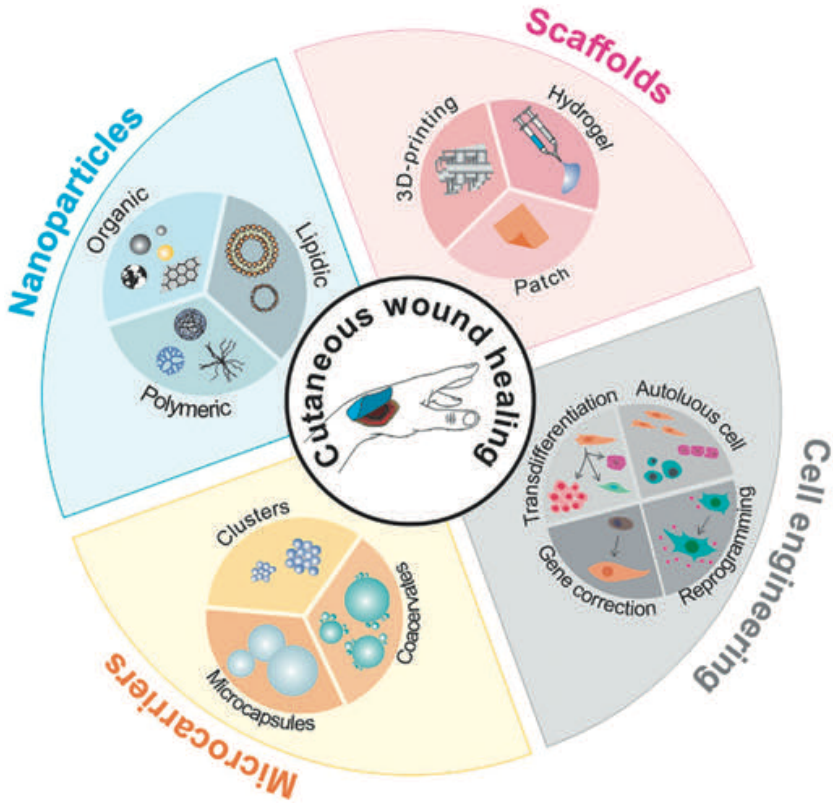

Figure 10.2 Schematic overview of drug and cell delivery systems for cutaneous wound healing. Reproduced from ref. 1 with permission from Elsevier, Copyright 2019.

to start with an overview of the most well-known drug and cell delivery systems described in the literature (Figure 10.2).

10.2 Substrate-mediated Drug Delivery for Wound Healing: Dressings

The fundamental prerogatives for wound dressings are to show good biological compatibility, biodegradability, water absorption and retention properties, low cytotoxicity, non-stick capacity and antibacterial effects. Furthermore, some properties of great importance for this type of systems are the prevention of wound infection, the possibility of obtaining gas exchange, the ease of removal and the absorption of part of the excrescent exudate of the wound so that it remains part of the exudate itself to maintain local wound moisture, thus accelerating wound healing. There are testimonies of crude applications of plant herbs, animal fat and honey for the realization

of tissue engineered scaffolds. In fact, especially in the tradition of medical plants in African culture, many were used for the treatment of wounds thanks to their antibacterial properties.[3–5] The problem with this type of dressing obviously lies in the fact that crude plant extracts also contain other types of chemical compounds, which can be potentially harmful and toxic when tissues and wounds are exposed to them during the healing process. In more recent years we have been confronted with dressing techniques such as natural or synthetic bandages, wadding, lint and gauze manufactured to have different degrees of absorbency. The main purpose of these devices was to ensure a dry wound environment, thus facilitating the evaporation of exudates from the wound itself and at the same time avoiding the possible appearance of harmful bacteria. It has also recently been shown that keeping the wound in a warm environment helps healing, making it faster and with fewer complications. The various dressings can be classified: (1) according to their function inside the wound (debridement, antibacterial, occlusive, absorbent, adherence), (2) according to the type of material used to produce the treatment (*e.g.* hydrocolloid, alginate, collagen) and (3) the characteristic physical form of the dressing (ointment, film, foam, gel). For completeness, there are also further classifications based on the type of dressing (primary, secondary and insular) and the type of dressing method (traditional, modern and advanced).

10.2.1 Traditional Dressings

Cotton wool, natural or synthetic bandages and gauze and composite dressings of woven cotton with gauze are characterized as traditional dressings, generally used as primary or secondary dressings to protect the wound from contamination.[6,7] Their low cost, easy use and cost-effective manufacture define the main advantageous properties of these products. Unlike topical pharmaceutical formulations, these dressings are dry and do not provide a moist wound environment. As mentioned above, they can be used as primary or secondary dressings, performing a specific function. For the packaging of open wounds, gauze pads are used, which with their fibres are able to absorb liquids and exudates and act as a filter to suck fluid from the wound. However, the disadvantage of these gauze dressings is that they need to be changed regularly to prevent maceration of the healthy underlying tissue and are therefore less convenient than more modern dressings. It is also necessary to consider the other common disadvantages of these systems including ischemia and necrosis and adherence to the wound bed, which limit their use in managing healing. In fact, although dressings of this type have been designed to provide sufficient bacterial protection, this is negated when the external surface of the dressing is moistened due to wound exudate or the presence of external fluids. Additionally, gauze dressings tend to become more adherent to wounds as fluid production decreases and are painful to remove, thus causing patient discomfort.[8] An effort to address these

disadvantages has been to graft a gauze-cotton composite with a non-adhesive inner surface, fabricated to relieve pain or minimize damage to renewed skin during dressing removal.

10.2.2 Modern Dressings

Modern dressings are the result of improvement over the traditional dressings described in the previous paragraph. Retaining and creating a moist environment near the wound so that they can facilitate healing is their main property and evolution. Modern dressings can be classified according to the material from which they are made, in particular: hydrocolloids, alginates and biological. These dressings then come, in most cases, in the form of gels, thin films and foam sheets.

10.2.2.1 Hydrocolloid Dressings

Hydrocolloid dressings are among the most used dressings.[9,10] The term "hydrocolloid" refers to that group of products obtained from colloidal materials (gelling agents) and other materials (elastomers, adhesives) aimed at wound management.[11,12] These systems come in the form of thin films and sheets or as composite dressings in combination with other materials, such as alginates. The real advantage of this type of dressings lies in their clinical use, in fact these products are able to adhere to both wet and dry sites. They also possess properties of being impermeable to water vapor, and with the absorption of the exudate from the wound there is a change in the physical state of the dressing with the formation of a gel capable of covering the wound. The permeability to water and air then increases as the gel formation progresses.[13] Finally, thanks to the absence of pain at the time of removal, they are the ideal choice in the case of paediatric wounds, both acute and chronic.

10.2.2.2 Alginate Dressings

Alginate dressings are produced by the union of calcium and sodium salts of alginic acid, a polysaccharide comprising mannuronic and guluronic acid units. These products can be presented as freeze-dried porous sheets (foams) or as flexible fibres, which are more suitable in the case of cavity wounds. The relevant properties of these systems in medical use derive from the ability to form a gel in contact with wound exudates, thus acquiring a high absorption capacity. The latter takes place through the formation of a hydrophilic gel capable of limiting wound secretions and at the same time reducing possible bacterial contamination. The mechanism of formation of the protective gel film is based on the exchange of the ions present in the alginate fibres with those present in the exudate when these dressings are applied to the wounds. This phenomenon allows the wound to be kept under optimal humidity and

temperature conditions. In detail, the gelling action of alginates is due to the presence of calcium ions, able to induce slow degradation in cross-linked polymeric gels. These cross-links that are formed between the calcium ions and the alginic acid polymer justify the use of these materials as scaffolds for tissue engineering.[14,15] In addition to the formation of the gel, this type of dressing has a pharmacological function that exploits the presence of calcium ions. The effects of the dressing could therefore occur through the calcium ions released by the alginate, making the calcium alginate able to improve some of the cellular aspects of healing. In practice, the calcium ions of alginate dressings, when released into the wound, help the coagulation mechanism physiologically during the first phase of wound healing. Moreover, alginate dressings in the form of fibres are biodegradable and, therefore, very useful in the case of surgical wound closures, when trapped in a wound[16] and through saline irrigation they can be easily rinsed. Therefore, no damage to the granulation tissue occurs during removal, and this leads to the painless success of a possible dressing change. A limitation of these alginate dressings is that, since they require moisture to function satisfactorily, they cannot be used for dry wounds covered with hard necrotic tissue.

10.2.2.3 Biological Dressings

The name of these dressings derives from the fact that they are made with biomaterials that play an active role in the wound healing process. Among these wound healing dressings, there are also tissue engineering products derived from natural tissues or artificial sources.[17] In making these products, polymers such as collagen,[18] hyaluronic acid,[19] chitosan,[20] alginates and elastin are generally combined. The main advantages of these biomaterials reside in the properties of being part of the natural tissue matrix, of being biodegradable and of being active in wound healing and in the formation of new tissues.[21] In addition to being one of the natural constituents of connective tissue, collagen is one of the main structural proteins of any organ. This polymer is mainly used for its vital role in the natural wound healing process from the induction of coagulation to the final scar formation.[22] In fact, collagen is able to stimulate the formation of fibroblasts and to accelerate the migration of endothelial cells in contact with damaged tissue. Starting from these systems it is also possible to produce lyophilized collagen biomatrices capable of collecting liquids, debris and cells.[23] The real key point of this matrix is the possibility of being medicated, thus becoming a real reservoir for the administration of the drug.[24] Hyaluronic acid is a glycosaminoglycan component of the extracellular matrix that is biocompatible, biodegradable, devoid of immunogenicity and capable of lubricating joints and inflammatory processes.[25] Thanks to these properties, cross-linked hyaluronic acid hydrogel films have been made for drug delivery.[26] Chitosan is a linear polysaccharide known to be able to accelerate granulation during the proliferative phase of wound healing.[27] Its application is also considered for wound healing.[28]

10.2.3 Dressings for Intracellular Delivery

The cells in the field of dressings can also be programmed to obtain a desired phenotype, the most important phenotype in wound healing is represented by macrophages, which are fundamental for tissue regeneration. More specifically, during the inflammatory phase, the polarization of the M1 macrophages (the pro-inflammatory phenotype) causes the removal of debris and pathogens. Subsequently, during the proliferation phase, the phenotype will then be biased towards the M2 anti-inflammatory phenotype.

However, it must be specified that in the case of a chronic wound this change in phenotype does not occur and causes continuous inflammation. It is for this reason that various tools have been developed for the transfer of genes, plasmids and active molecules directly into cells. These drug delivery systems tend to be active, but some passive systems also exist.

10.3 Substrate-mediated Drug Delivery for Wound Healing: Scaffolds

10.3.1 Hydrogel Scaffolds

Hydrogels are insoluble and swellable hydrophilic 3D materials based on synthetic or natural polymers. Hydrogels stand as new materials in the scaffold design scenario that promote wound healing.[29,30] The porous, hydrophilic and transparent architecture that characterizes them allows the monitoring of regeneration, gaseous exchange and fluid balance by controlling the evaporation of water and the absorption of exudate, ensuring humidity in the area of the lesion. The application of the hydrogels can take place both in the case of amorphous gels and solid elastic films. In the second case, the production of elastic films requires the presence of cross-linked polymeric components in order to physically trap the water.[9] These films have the remarkable characteristic of being able to absorb and retain significant volumes of water in contact with suppurating wounds. Generally, hydrogel dressings, in addition to requiring secondary coverage in the case of application to the wound in the form of a gel, also require frequent changing. Films, on the other hand, do not need a secondary dressing as a support and, thanks to their flexible nature, can be adapted to any type of wound as they can be cut. It is logical to use products in the form of gel in the case of primary dressings and products in the form of film in the case of primary or secondary dressings. For both types, the absorption of exudate is limited since the hydrogel naturally contains significant quantities of water (70–90%) and consequently they are used for light to moderately exuding wounds. Furthermore, this accumulation of liquids can lead to various complications, such as bacterial proliferation resulting in the onset of infections and bad odour. Furthermore, hydrogels demonstrate low mechanical resistance making them very difficult to handle,[31] a disadvantage that can affect patient compliance.[9]

On the other hand, hydrogels, boast many of the characteristics of an "ideal dressing".[9] They are in fact suitable for cleansing dry, sloughing or necrotic wounds, also improving autolytic debridement. The hydrogel dressings are non-reactive with biological tissue, permeable to metabolites and non-irritating. Wet healing is promoted by these systems, the latter being non-adherent and able to cool the wound surface itself with consequent reduction of pain and therefore high acceptance by patients. Hydrogels are also excellent products because they do not leave residues, are malleable and improve the re-epithelialization of wounds.[9]

10.3.2 Foam and Spongy Scaffolds

Foam and spongy scaffolds are made of porous polyurethane foam or polyurethane foam film, with or without adhesive edges. Some foam scaffolds have additional wound contact layers to prevent adhesion when the wound is dry and an occlusive polymer backing layer to prevent excessive fluid and bacteria loss. These systems are able to keep the environment around the wound moist, provide thermal insulation and are easy to apply. They exhibit important characteristics of high absorbency, which can also be controlled by the properties of consistency, thickness and pore size of the foams. In particular, in the case of an open-pore structure, a high water-vapour-transmission speed is also obtained.[32] This porous structure makes these systems suitable for partial or full thickness wound types with minimal to moderate drainage and highly absorbent structures with heavy wound exudation.[9] Finally, foam is the preferred choice over gauze in terms of pain reduction and patient satisfaction.[33]

10.3.3 Bi-layered Scaffolds

Chronic wounds generally affect different layers of the skin, both dermal and subcutaneous. The dermis, located under the epidermis, characterized by an extremely vascularized and innervated connective tissue, has a low cell density and is maintained by fibroblasts capable of supporting the vascular, lymphatic and nervous systems. Given the structure of the dermis, its regeneration is less efficient and more complicated than the regeneration of the epithelium, since the structure of the latter, in contrast, is mainly cellular.[34]

Here lies the reason for the development of double-layered scaffold systems capable of combining both the epithelial and dermal layers.[35] The structure identified to be the most effective is composed of a dense surface layer and a porous lower layer, this is because this alternative would be the most satisfactory for complete regeneration of the skin at full thickness. In detail, the epithelial layer should prevent bacterial infiltration and dehydration in the wound area, while the ideal dermal layer should have great liquid absorption properties and should favour the penetration of fibroblasts. That said, a new double-layered acellular scaffold composed of chitosan hydrogels obtained through a low-energy physical cross-linking method has been

produced, while also trying to avoid any additional chemical agents. The final result was a top layer of hydrogel optimized to be rigid and dense to ensure protection, gas exchange and adequate mechanical properties and a bottom layer of soft hydrogel designed to be flexible and able to adapt and adhere to the wound site. This type of scaffold thus promoted dermal–epidermal interphase regeneration and wound healing of full-thickness skin tissue.[35]

10.3.4 Physical Drug Encapsulation in a Scaffold for Slow Drug Release

These scaffolds are used as repositories for bioactive molecules to ensure their stability and function and to allow sequential release for prolonged periods. The physical encapsulation of the drug in a scaffold is done in such a way as to protect the drug from degradation in the hostile environment that can be created in chronic wounds and, at the same time, provides a more sustained and localized administration of the drug than the administration of a bolus drug.[1]

10.3.5 Tuning Drug Release From Scaffolds for Stage-wise Drug Delivery

An encapsulation of multiple growth factors into a single scaffold with a different pattern of release for gradual delivery of growth factors was developed to synchronize the wound healing process. In detail, a programmable release of multiple growth factors *via* scaffolds would allow not only the gradual delivery of the growth factor but also a synergistic effect with the same structure characteristic of the scaffolds, leading to greater efficiency in the healing of chronic wounds. Thus, this type of sequential release of growth factors would efficiently simulate the physiological course of wound healing.[36]

Examples of degradable synthetic scaffolds are then able to regulate drug release according to the desired dose and time by optimally combining the degradation rates of the scaffold with tissue internal growth. In addition, the degradation and drug release of hydrolytically degradable scaffolds can be manipulated by working on the cross-linking of the density and porosity of the systems.[1]

10.4 Controlled-release Drug Delivery Systems

In the case of passive drug administration, diffusion occurs through the carrier matrix to reach the surrounding medium.[37] Drug carriers can be of the inorganic type (mesoporous particles, metal–organic structures, ceramic or carbon-based nanotubes) and of the organic type (lipid-based systems, layer-by-layer systems and hydrogels). The first type provides adequate encapsulation for poorly soluble drugs, the second type is used as a passive transdermal drug delivery tool since they are degradable and can overcome the natural epidermal barrier.[38,39] The characteristics of vector size, shape,

porosity, degradability and electrostatic charge can influence the rate and efficacy of drug release.[40]

10.4.1 Synthetic-based Drug Delivery Systems

These systems are widely used due to their ability to be customized through the physico-chemical properties of the polymers that compose them (non-degradable or biodegradable) and thanks to the various possible encapsulation methods.[41] The drug release for this type of systems depends on several parameters: molecular weight (Mw), glass transition temperature (Tg), crystallinity, solubility and degradation rate of the polymer.[42,43] The molecular weight of the polymer has a direct effect on the glass transition temperature, viscosity, crystallinity, mechanical properties and degradation rate. In other words, in the case of low molecular weight polymers, a faster degradation rate and a higher elastic modulus are obtained. This results in greater deformation and expansion of the pores after deformation, leading to higher release. In contrast, in the case of high molecular weight polymers a lower elastic modulus, a lower deformability to degradation and a more limited drug release are obtained.[44] The glass transition temperature (Tg) defines the temperature at which amorphous regions pass from the glassy to the rubbery state. For temperatures below Tg, amorphous regions are glassy and have a more limited diffusivity and therefore release, while for temperatures above Tg, amorphous regions have greater mobility and significantly greater diffusivity, leading to higher release. Since the release depends on the permeation that occurs through amorphous regions, the crystallinity of the polymer is also a harmful parameter, especially in the case of low molecular weight polymers, and therefore the crystallinity of the polymer is a parameter to be taken into consideration for these systems.[45,46]

Furthermore, it should be emphasized that the hydrophobic polymeric particles undergo surface erosion while the hydrophilic polymeric particles swell and degradation occurs within the mass of the polymer, this means that the hydrophilic: hydrophobic ratio of the polymer has an effect on release.[47] Parameters such as the chemical composition of the polymer, the molecular weight and the degree of crystallinity are able to modify the solubility of the polymer in the aqueous system. The release mechanism of these systems is mainly controlled diffusion and two types of release systems: matrix and reservoir, can be considered. In the first case the diffusion rate is defined by the diffusion distance, by the degree of swelling of the polymer and by the drug concentration gradient; while in the second case, the release is regulated by the thickness and permeability of the polymer particles.[48] Finally, the release of drugs from biodegradable polymeric particles can occur through two methods of erosion: surface and bulk. In surface erosion systems the degradation involves only the outer surface of the particle, while in bulk erosion systems the degradation affects the entire polymer particle homogeneously.[49,50] Therefore, the degradation and release phenomena can be

optimized by regulating mixtures composed of hydrophilic and hydrophobic polymers.

10.4.2 Lipid-based Drug Delivery Systems

These systems are a further class of drug carriers chosen for drug delivery due to their affinity with cell membranes and their ability to pass through biological barriers, as in the case of the skin.[51] Liposomes are the most frequently used carriers of lipid-based drugs.[52,53] The reason for this choice lies in the fact that liposomes present themselves as excellent drug carriers, of a biocompatible nature and capable of delivering drugs both in the intracellular and extracellular environment. These characteristics make them powerful drug carriers for wound healing applications.[54] The disadvantage of these, however, is that they have a shorter drug release than all polymeric systems, as well as having a significantly lower load capacity than the others. To overcome the limitations associated with liposomal structures, solid lipid nanoparticles and nanostructured lipid carriers have been developed.[55]

10.4.3 Transdermal Delivery Systems

The selection of an adequate point of administration is an important factor in the outcome of localized administration of drugs. Chronic wounds are covered with a layer of non-viable tissue, which separates the external environment from the underlying tissue. Therefore, at the time of topical administration of drugs and factors, they must first pass through dead tissue to access the cells that really should receive therapy.

It is easy to understand how significant amounts of drugs or factors can be deactivated before reaching the growing tissue. Furthermore, a hypothetical significant production of exudate in chronic wounds can further reduce the penetration rate of topically administered drugs. In this context hypodermic injections, which are the traditional way of administering the drug through the skin, are rather unfavourable as they are painful, need professional assistance and capable of transmitting disease when hypodermic needles come into contact with different patients. A significant push was therefore required to develop tools capable of administering drugs transdermally. These transdermal tools include microcarriers and/or nanocarriers capable of passing through the skin barrier and stratum corneum and microneedles capable of painlessly penetrating through the barrier and delivering drugs to the underlying vital tissue. Microneedles are arrays of short needles used for the painless administration of drugs *via* the transdermal route.[56] The dimensions of these are small enough to allow the passage of the stratum corneum without affecting the underlying nerves[56] (Figure 10.3). It is possible to classify these microneedles as: (1) solids, able to break the epidermal barrier and allow the penetration of drugs administered topically,[57] (2) coated with drugs, capable of penetrating into the tissue and providing their internal payload,[58] (3) soluble, which penetrate the tissue and release their payload gradually during

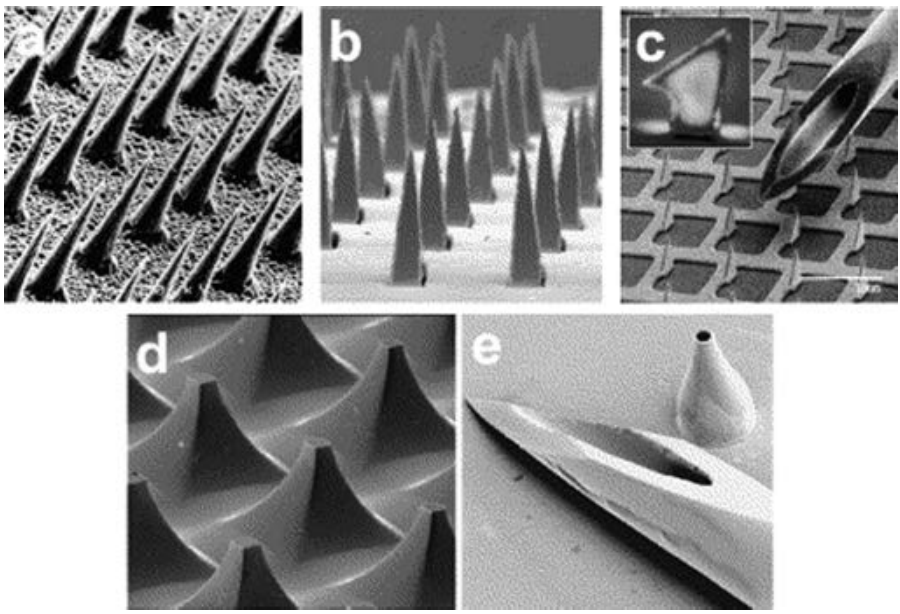

Figure 10.3 Images of microneedles used for transdermal drug delivery. (a) Solid microneedles (150 μm tall) (b) Solid microneedles (1000 Am tall) (c) Solid microneedles ("microprojection array", 330 Am tall) (d) Solid microneedles ("microenhancer array", 200 Am tall) (e) Hollow microneedles (500 Am tall). Reproduced from ref. 56 with permission from Elsevier, Copyright 2004.

their degradation[59–61] and (4) cables, which, once penetrated into the tissue, facilitate the active administration of drugs to the interior of the region of interest.[62] An interesting application of microneedles for the treatment of skin lesions has been proposed due to the development of microneedle arrays with swellable tips.[63] In practice, the system has been designed in such a way that, once they penetrate the skin, the needles swell so as to remain locked in position. The main application of these needles was the improvement of the adhesion of skin flaps in the case of the treatment of burns and chronic wounds. In general, microneedles are an interesting tool that can be made from polymers known for their excellent drug protection and gradual release of their payload. In these cases, the drug can be released over time for the completion of physiological processes. These microneedles can be made as composites of different materials and can also be developed in a multilayer manner to allow for the release of drugs required for late stage wound healing at a later time.

10.4.4 Inorganic Materials

This last class of material is also widely used in drug carrier engineering, although this type of inorganic materials does not possess proteins present in natural systems and consequently are generally considered less

immunogenic. Among these inorganic materials, gold nanoparticles (GNPs), mesoporous ones and carbon nanotubes have certainly received considerable interest in various drug delivery applications due to their stability and anti-inflammatory properties[64] (Figure 10.4).

In wound healing, gold nanoparticles, in particular, have been used for the delivery of active compounds, such as antioxidants and nucleic acids. The choice of these materials stems from their ability to increase the absorption of antioxidant and anti-inflammatory components added to the blend to accelerate wound healing.[65] Mesoporous particles, on the other hand, have excellent drug carrying capacity and the electrostatic interaction between the solid matrix and the encapsulated compounds can significantly increase the release time.[66,67] Furthermore, these particles are one of the few cases of drug vectors capable of offering an almost linear release profile and, thanks to their biocompatibility, have been considered for various biomedical applications.[68] It is therefore logical that the selection of the appropriate material for the design of the drug carrier is essential for the success of its

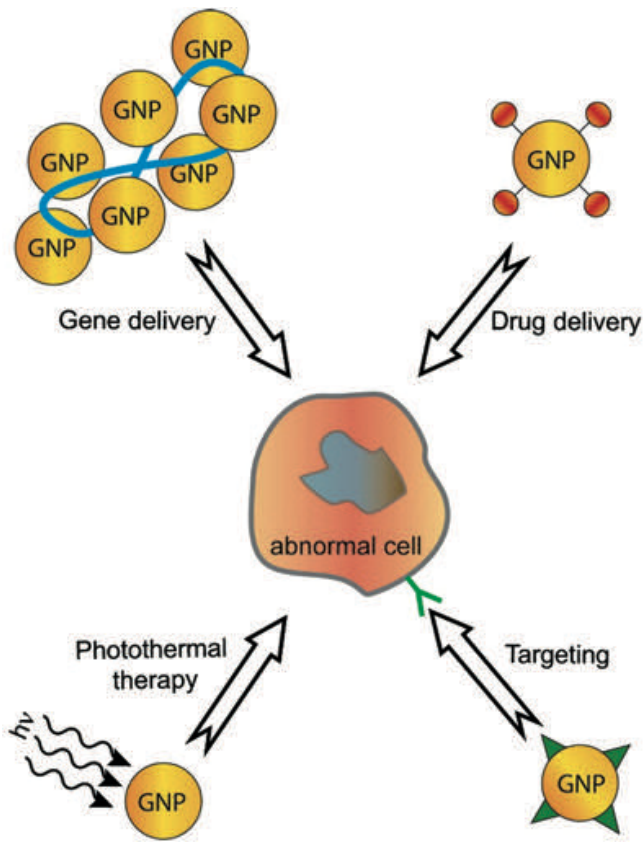

Figure 10.4 Various applications of gold nanoparticles. Reproduced from ref. 64 with permission from Elsevier, Copyright 2008.

use; in particular, the charge and the water solubility of the product play an important role in the selection of the drug carrier. As for the drug, it is then necessary to consider its stability, as well as the potential effects of the environment on the wound. It is then possible to customize the release profile by exploiting the shape, size and microstructure of the carriers.

10.5 Carriers for Wound Healing: Nano and Micro Carriers

10.5.1 Nanocarriers

Nanoparticulate drug delivery systems provide controlled drug delivery for therapeutic purposes. Nanocarriers also have the interesting properties of prolonging the drug's half-life, improving bioavailability, optimizing pharmacokinetic profiles and reducing the frequency of drug administration. Nanocarriers cover a wide category of systems, in particular, polymeric, lipid and inorganic nanoparticles can be considered because they are the most used types in the administration of drugs in wound healing.[1]

10.5.1.1 Inorganic Nanoparticles

Various inorganic nanoparticles, such as gold, silica, iron oxide and quantum dots, are used as attractive drug carriers due to their characteristics of high surface area, adjustable size with small dispersion, functionalization and multifunctional capabilities. In particular, some nanoparticles (metal, silver oxide, copper oxide, zinc oxide and titanium dioxide), thanks to their high intrinsic antimicrobial activity, are considered valid alternatives for the treatment of drug-resistant bacterial infections.[69,70] Gold nanoparticles, in detail, are widely used due to their ease of synthesis, their tuneable size and shape, their flexible surface modification, bioconjugation and tuneable optical and electronic properties.[71] In the specific case of drug administration, particles of this type act as a nucleus in which the drug is immobilized at a high concentration per surface area. To improve wound healing, gold nanoparticles are chosen for their property of administering antimicrobial agents, which is very useful in the case of infected wounds. In fact, given the small size and large surface area, these systems are able to provide a large contact area with bacteria with the consequent destruction of the permeability and respiration functions of the bacterial membranes.[64,65] Thanks to their high surface area, their biocompatibility and degradability even silicon-based nanoparticles (Si NP) are promising drug carriers. Thanks to the highly porous structure of these silicon nanoparticles, it is possible to trap a variety of therapeutic loads. This porous structure of these particles also has the advantage of being able to be regulated as regards the size of the pores, covering a range that goes from a few nanometres to a few microns.[72]

Furthermore, it is possible to regulate the degradation of these particles depending on the pore size and the chemistry, on which non-toxic silicic acid is produced.[73,74] As for the type of drug that can be loaded at high efficiency, the

best choice includes low molecular weight molecules and these loads will then be released when the nanoparticles degrade.[75,76] Storage takes place inside the pores and this position allows effective protection of the loaded drugs from enzymatic degradation at the wound site. Silane hydrogel nanoparticles are a type of silicon-based nanoparticles, generally used for drug delivery using the sol–gel nanoparticle preparation process.[77] Furthermore, using organic additives it is possible to manipulate the surface and the loading–release profile of the drug within the nanoparticle.[78,79] Inorganic nanoparticles can therefore act simultaneously as both drug carriers and therapeutic agents thanks to their intrinsic therapeutic properties useful for wound repair.[1]

10.5.1.2 Polymeric Nanoparticles

Polymer nanoparticles are used for drug delivery due to their biocompatibility, biodegradability, injectability and intracellular delivery capacity. A further reason for their extensive use stems from the physicochemical properties of these nanoparticles, which can be precisely tailored at the molecular level to increase drug load and control drug release.

A significant example of this type of systems are poly (lactic-*co*-glycolic acid) (PLGA)-based nanoparticles, which are very interesting thanks to their versatile degradation kinetics and controlled drug release properties. The preparation of PLGA nanoparticles exploits the double emulsion water–oil–water method, thus designing a product with high drug encapsulation efficiency and with a relatively uniform size distribution. In the context of wound healing, it is interesting to note that PLGA can act by providing lactate by-products upon degradation because exogenous lactate can accelerate angiogenesis, activation of procollagen factors and the recruitment of endothelial progenitor cells into wounds.[80]

10.5.1.3 Lipid-based Nanocarrier Systems

Lipid-based nanoparticles are attractive for the topical treatment of skin diseases because the small particle size and lipid composition of the nanoparticles means that close contact between nanoparticles and wound sites can be achieved. This approach is advantageous since it leads to an increase in the residence time of the nanoparticles in the wound bed. Examples of these nanoparticles are solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs). The first type of particle, in particular, shows a slower drug release because the ordered molecular conformation and the condensed structure that characterizes it tends to retain a part of the drug incorporated within the lipid nucleus. This behaviour is due to the strong hydrophobic interactions between the lipid and the drug. However, even though both SLNs and NLCs are prepared by the emulsification–ultrasonication method, the difference between the two nanoparticles is in the fact that the preparation of SNLs requires an organic solvent while the formulation of NLCs uses a liquid lipid (oil). In conclusion, although the wound healing efficacy of both SNL and NLC incorporating growth factors are similar,[81] the NLC

formulation is often chosen over SLN because the use of organic solvents is not required for the preparation of NLC.[82]

10.5.1.4 Applications: Nanocarriers in Hydrogels

To overcome the problems of low mechanical strength, inadequate flexibility and the inability to allow long-term drug release of hydrogels, the possibility of incorporating ceramic, metal and polymeric nanoparticles in both hydrogels and electro-spun yarns has been investigated.[83,84] In this regard, it is also possible to encapsulate nanoparticles, such as zinc oxide, titanium oxide and silver particles as antibacterial agents in the hydrogel scaffolds and electrospinning in order to avoid bacterial colonization, local and internal infections and depositions of disorganized collagen.[85,86] Incorporating these nanoparticles improved adhesion, function, diffusion and proliferation of fibroblasts, thus promoting wound healing.[87] In addition, in the case of incorporation of biodegradable polymeric nanoparticles, the system is able to load, protect and modulate the release of bioactive molecules such as growth factors, drugs and proteins. It is therefore possible to combine polymers suitable for drug carriers or with relevant micro-characteristics so as to be able to design scaffolds capable of meeting the various physical and biological requirements necessary for rapid wound healing.

10.5.2 Microcarriers

Microspheres are a valid choice thanks to their excellent control of the drug release profile, especially in the case of low burst, in cases in which intracellular release is not necessary. The main advantage of these systems lies in being able to obtain, thanks to the encapsulation of the microsphere, a long-term release of an effective concentration of antibiotics, thus reducing the risk of bacterial infection.[88] The microencapsulation of highly hydrolatable drugs is also particularly useful because it improves their bioavailability at the wound site. In addition, the administration of exogenous H_2S donors also greatly improves the angiogenesis of diabetic wound healing. An example of an H_2S donor is sodium hydrosulphide (NaHS), a highly hydrolatable compound that begins to generate H_2S during the encapsulation process when exposed to water.[89] Poly (ethylene glycol) (PEG) microspheres containing vascular endothelial growth factor-binding peptides (VBP) can also be cited as a significant example for the control of wound angiogenesis, which are able to regulate angiogenesis by varying the rate of degradation of the microspheres.[90] Hence, adjustment of the microspheres can be beneficial to improve the angiogenesis of the wound healing process and also to reduce scar formation.

10.6 Active Drug Delivery Systems

The traditional methods of constant passive release of drugs over time described so far often result in a high plasma concentration of the drug which, being outside the therapeutic window, can cause side effects and reduce the

effectiveness of the treatment. Passive drug control allows the system to contain larger quantities of drug, always ensuring a drug concentration in the blood within the limits of the therapeutic window. By doing so, it is possible to use the drug for a longer period of time and more efficiently.[91] In the dynamic environment of wounds, it is important to ensure the correct timing of administration of the active compounds. However, there are cases of treatments for some pathophysiological complications, such as infections, which may require the release of the drug only at the right time. Treatment of infections is done by systemic or topical administration of antibiotics once the infection has been detected. A system is needed for treating infected wounds that can deliver antibiotics only when needed and with the correct dosage.

This situation is achieved through the use of two types of systems: (1) systems that can be activated externally and (2) systems that respond autonomously to changes in physiological conditions, such as pH, temperature or other microenvironmental changes in the tissue (Figure 10.5).

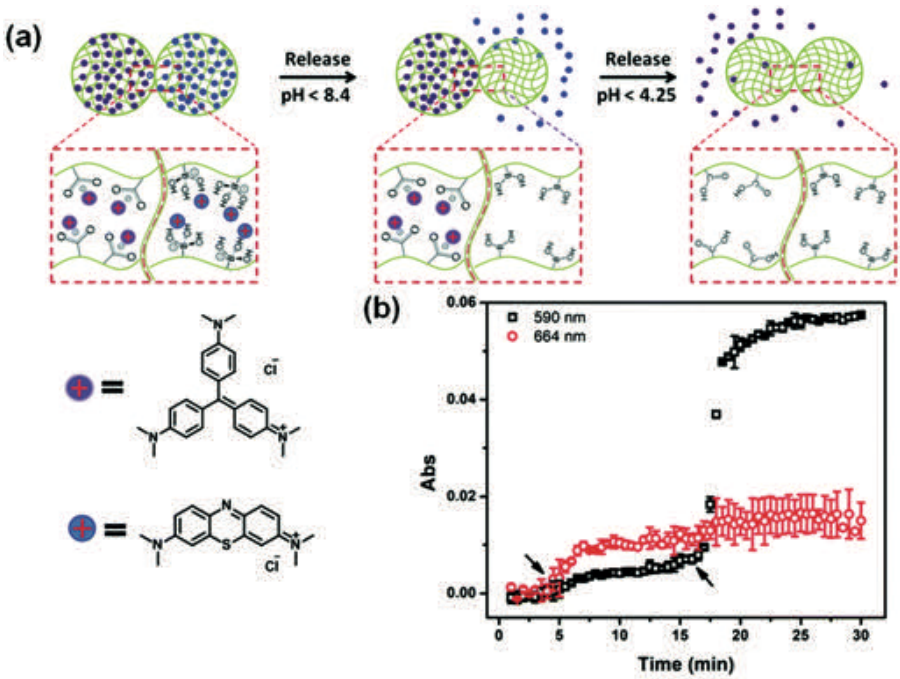

Figure 10.5 (a) Schematic illustration of pH triggered MB and CV release from APBA-MG and AAc-MG, respectively. As each microgel is neutralized, the electrostatic interactions between the microgel and the charged molecules are diminished, and the MB and CV are released from the microgel sequentially. (b) The release profile for a device made of APBA-MG and AAc-MG loaded with MB and CV, respectively. The arrows indicate the times that the solution pH was changed to 7 (at ~5 min) and 3 (at ~17 min). Two different wavelengths were monitored (590 nm and 664 nm, for CV and MB, respectively) that correspond to the absorbance of the two different model drugs.

In this section only systems of the second type will be analysed. For these, polymers are the most used materials thanks to their tuneable character, which allows precise control over the mechanical and physico-chemical characteristics of the material.[92] Compared with passive systems, active systems have several advantages. In particular, their capacity for spatial, temporal and dosage control in drug release, in addition to the fact that these systems require a lower drug load than passive ones. This makes drug delivery therapies more efficient, cheaper and safer.[93]

10.6.1 Self-responding Drug Delivery Systems

In the case of drug delivery systems, the design of systems capable of responding to their environment and changing their state is very interesting. Particularly in the wound environment, parameters such as temperature and pH are indicators of the state of the wound that change as the level of inflammation, oxygenation and infection varies. The skin temperature is generally between 32 °C and 34 °C; however, it can reach higher values locally due to inflammation. The exposure to blood and body fluids following an injury temporarily increases the local pH to about 7. This value in the healing process will then be reduced to a slightly acidic value of 4–5. In any case, the pH can be changed at the time of the onset of a bacterial infection.[94] In infected wounds we find an extremely acidic or slightly alkaline pH depending on the classes of bacteria and the wound environment. The pH also depends on the oxygenation level of the wound itself. It is therefore necessary to consider changes in temperature and pH in the environment in the development of drug delivery systems. Examples of temperature sensitive systems are thermoreactive polymers, which can be divided into two classes based on how they respond to heat: lower critical solution temperature polymers (LCST) and higher critical solution temperature polymers (UCST). The first type of polymers undergo desolvation with exposure to heat, while the second become soluble when heated.[95] The characteristic critical temperatures, upper and lower, can be modulated thanks to factors such as the molecular weight and the concentration of the polymer.[96] An example of an ideal range for the critical temperature is between 35 °C and 45 °C.[97,98]

Examples of pH-sensitive systems are ionizable polymers, which are weak acids or bases and function thanks to the change in their ionization state with consequent change in the conformational state of the polymer.[99] To develop materials that respond to changes in both pH and temperature, copolymers of materials reactive to both parameters have been developed and used to design better drug delivery systems. One class of these materials that affects wound healing is that composed of reactive systems, *i.e.* materials that respond to the level of chemokines and cytokines in the wound bed.[100,101] Therefore, by using separable protease peptides that can connect suitable drugs to the polymeric structures, it is possible to obtain the formation of polymers with a drug release rate proportional to the concentration of the targeted chemicals.

References

1. H. S. Kim, X. Sun, J. H. Lee, H. W. Kim, X. Fu and K. W. Leong, *Adv. Drug Delivery Rev.*, 2019, **146**, 209.
2. N. S. Bhise, R. B. Shmueli, J. C. Sunshine, S. Y. Tzeng and J. J. Green, *Expert Opin. Drug Delivery*, 2011, **8**, 485.
3. A. C. Kudi, J. U. Umoh, L. O. Eduvie and J. Gefu, *J. Ethnopharmacol.,* 1999, **67**, 225.
4. K. Inngjerdingen, C. S. Nergård, D. Diallo, P. P. Mounkoro and B. S. Paulsen, *J. Ethnopharmacol.*, 2004, **92**, 233.
5. P. J. Houghton, *Phytother. Res.*, 2006, **20**, 941.
6. S. Dhivya, V. V. Padma and E. Santhini, *BioMedicine*, 2015, **5**, 24.
7. M. K. Strecker-McGraw, T. R. Jones and D. G. Baer, *Emerg. Med. Clin. North Am.*, 2007, **25**, 1.
8. I. B. Wall, R. Moseley, D. M. Baird, D. Kipling, P. Giles, I. Laffafian, P. E. Price, D. W. Thomas and P. Stephens, *J. Invest. Dermatol.*, 2008, **128**, 2526.
9. J. S. Boateng, K. H. Matthews, H. N. E. Stevens and G. M. Eccleston, *J. Pharm. Sci.*, 2008, **97**, 2892.
10. B. Mirani, E. Pagan, B. Currie, M. A. Siddiqui, R. Hosseinzadeh, P. Mostafalu, Y. S. Zhang, A. Ghahary and M. Akbari, *Adv. Healthcare Mater.*, 2017, **6**, 1700718.
11. C. Dealey, *Br. J. Nurs.*, 1993, **27**, 358.
12. A. E. Stoica, C. Chircov and A. M. Grumezescu, *Molecules*, 2020, **25**, 2699.
13. M. Uzun, S. C. Anand and T. Shah, *J. Biomed. Eng. Technol.*, 2013, **1**, 1.
14. C. K. Kuo and P. X. Ma, *Biomaterials*, 2001, **22**, 6, 511.
15. L. Wang, R. M. Shelton, P. R. Cooper, M. Lawson, J. T. Triffitt and J. E. Barralet, *Biomaterials*, 2003, **24**, 3475.
16. T. Gilchrist and A. M. Martin, *Biomaterials*, 1983, **4**, 317.
17. R. Nathoo, N. Howe and G. Cohen, *J. Clin. Aesthet. Dermatol.*, 2014, **7**, 44.
18. J. A. M. Ramshaw, J. A. Werkmeister and V. Glattauer, *Biotechnol. Genet. Eng. Rev.*, 1996, **13**, 335.
19. C. J. Doillon and F. H. Silver, *Biomaterials*, 1986, **7**, 3.
20. M. Ishihara, K. Nakanishi, K. Ono, M. Sato, M. Kikuchi, Y. Saito, H. Yura, T. Matsui, H. Hattori, M. Uenoyama and A. Kurita, *Biomaterials*, 2002, **23**, 833.
21. H. Ueno, H. Yamada, I. Tanaka, N. Kaba, M. Matsuura, M. Okumura, T. Kadosawa and T. Fujinaga, *Biomaterials*, 1999, **20**, 1407.
22. P. Sai and M. Babu, *Burns*, 2000, **26**, 54.
23. L. Rey and J. C. May, *Freeze Drying/Lyophilization of Pharmaceutical and Biological Products*, Taylor & Francis Group, Boca Raton, 3rd edn, 2010.
24. Z. Ruszczak and W. Friess, *Adv. Drug Delivery Rev.*, 2003, **55**, 1679.
25. Y. H. Liao, S. A. Jones, B. Forbes, G. P. Martin and M. B. Brown, *Drug Delivery*, 2005, **12**, 327.
26. S. Khunmanee, Y. Jeong and H. Park, *J. Tissue Eng.*, 2017, **8**, 2041731417726464.

27. L. C. Oliver and G. Blaine, *Br. J. Surg.*, 1950, **37**, 307.
28. H. Ueno, T. Mori and T. Fujinaga, *Adv. Drug Delivery Rev.*, 2001, **52**, 105.
29. S. Saghazadeh, C. Rinoldi, M. Schot, S. S. Kashaf, F. Sharifi, E. Jalilian, K. Nuutila, G. Giatsidis, P. Mostafalu, H. Derakhshandeh, K. Yue, W. Swieszkowski, A. Memic, A. Tamayol and A. Khademhosseini, *Adv. Drug Delivery Rev.*, 2018, **127**, 138.
30. N. Annabi, A. Tamayol, J. A. Uquillas, M. Akbari, L. E. Bertassoni, C. Cha, G. Camci-Unal, M. R. Dokmeci, N. A. Peppas and A. Khademhosseini, *Adv. Mater.*, 2014, **26**, 85.
31. H. Sashiwa and S. I. Aiba, *Prog. Polym. Sci.*, 2004, **29**, 887.
32. M. Ramos-E-Silva, M. C. Ribeiro and D. E. Castro, *Clin. Dermatol.*, 2002, **20**, 715.
33. H. Vermeulen, D. T. Ubbink, A. Goossens, R. de Vos and D. A. Legemate, *Br. J. Surg.*, 2005, **92**, 665.
34. E. J. Chong, T. T. Phan, I. J. Lim, Y. Z. Zhang, B. H. Bay, S. Ramakrishna and C. T. Lim, *Acta Biomater.*, 2007, **3**, 321.
35. N. Boucard, C. Viton, D. Agay, E. Mari, T. Roger, Y. Chancerelle and A. Domard, *Biomaterials*, 2007, **28**, 3478.
36. C. Fischbach and D. J. Mooney, *Adv. Polym. Sci.*, 2006, **203**, 191.
37. N. Kamaly, B. Yameen, J. Wu and O. C. Farokhzad, *Chem. Rev.*, 2016, **116**, 2602.
38. D. G. Ramírez-Wong, S. Saghazadeh, S. van Herck, B. G. de Geest, A. M. Jonas and S. Demoustier-Champagne, *Biomacromolecules*, 2017, **18**, 4299.
39. P. T. Hammond and D. H. Koch Chair, *Mater. Today*, 2012, **15**, 196.
40. L. Pachuau, *Expert Opin. Drug Delivery*, 2015, **12**, 1895.
41. A. Kakkar, G. Traverso, O. C. Farokhzad, R. Weissleder and R. Langer, *Nat. Rev. Chem.*, 2017, **1**, 63.
42. C. Maderuelo, A. Zarzuelo and J. M. Lanao, *J. Controlled Release*, 2011, **154**, 2.
43. N. Peppas, *J. Macromol. Sci., Part C*, 1983, **23**, 61.
44. J. Braunecker, M. Baba, G. E. Milroy and R. E. Cameron, *Int. J. Pharm.*, 2004, **282**, 19.
45 V. Karavelidis, E. Karavas, D. Giliopoulos, S. Papadimitriou and D. Bikiaris, *Int. J. Nanomed.*, 2011, **6**, 3021.
46. A. M. Kaushal, P. Gupta and A. K. Bansal, *Crit. Rev. Ther. Drug Carrier Syst.*, 2004, **21**, 133.
47. Y. Fu and W. J. Kao, *Expert Opin. Drug Delivery*, 2010, **7**, 429.
48. B. Narasimhan, *Adv. Drug Delivery Rev.*, 2001, **48**, 195.
49. B. D. Ulery, L. S. Nair and C. T. Laurencin, *J. Polym. Sci., Part B: Polym. Phys.*, 2011, **49**, 832.
50. J. P. Jain, D. Chitkara and N. Kumar, *Expert Opin. Drug Delivery*, 2008, **5**, 889.
51. S. Latifi, A. Tamayol, R. Habibey, R. Sabzevari, C. Kahn, D. Geny, E. Eftekharpour, N. Annabi, A. Blau, M. Linder and E. Arab-Tehrany, *Sci. Rep.*, 2016, **6**, 25777.
52. V. P. Torchilin, *Nat. Rev. Drug Discovery*, 2005, **4**, 145–160.

53. M. Hasan, G. ben Messaoud, F. Michaux, A. Tamayol, C. J. F. Kahn, N. Belhaj, M. Linder and E. Arab-Tehrany, *RSC Adv.*, 2016, **6**, 45290.
54. G. Gainza, W. S. Chu, R. H. Guy, J. L. Pedraz, R. M. Hernandez, B. Delgado-Charro and M. Igartua, *Int. J. Pharm.*, 2015, **490**, 404.
55. R. H. Muller, M. Radtke and S. A. Wissing, *Adv. Drug Delivery Rev.*, 2002, **54**, S131.
56. M. R. Prausnitz, *Adv. Drug Delivery Rev.*, 2004, **56**, 581.
57. L. Wei-Ze, H. Mei-Rong, Z. Jian-Ping, Z. Yong-Qiang, H. Bao-Hua, L. Ting and Z. Yong, *Int. J. Pharm.*, 2010, **389**, 122.
58. M. Ameri, S. C. Fan and Y. F. Maa, *Pharm. Res.*, 2010, **27**, 303.
59. Y. Ito, T. Yamazaki, N. Sugioka and K. Takada, *J. Mater. Sci.: Mater. Med.*, 2010, **21**, 835.
60. S. Saghazadeh, C. Rinoldi, M. Schot, S. S. Kashaf, F. Sharifi, E. Jalilian, K. Nuutila, G. Giatsidis, P. Mostafalu, H. Derakhshandeh, K. Yue, W. Swieszkowski, A. Memic, A. Tamayol and A. Khademhosseini, *Adv. Drug Delivery Rev.*, 2018, **127**, 138.
61. M. Y. Kim, B. Jung and J. H. Park, *Biomaterials*, 2012, **33**, 668.
62. Y. C. Kim, J. H. Park and M. R. Prausnitz, *Adv. Drug Delivery Rev.*, 2012, **64**, 1547.
63. M. Hasan, G. ben Messaoud, F. Michaux, A. Tamayol, C. J. F. Kahn, N. Belhaj, M. Linder and E. Arab-Tehrany, *RSC Adv.*, 2016, **6**, 45290.
64. P. Ghosh, G. Han, M. De, C. K. Kim and V. M. Rotello, *Adv. Drug Delivery Rev.*, 2008, **60**, 1307.
65. J. G. Leu, S. A. Chen, H. M. Chen, W. M. Wu, C. F. Hung, Y. der Yao, C. S. Tu and Y. J. Liang, *Nanomedicine*, 2012, **8**, 767.
66. I. I. Slowing, J. L. Vivero-Escoto, C. W. Wu and V. S. Y. Lin, *Adv. Drug Delivery Rev.*, 2008, **60**, 1278.
67. S. Meoto and M. O. Coppens, *J. Mater. Chem.*, 2014, **2**, 5640.
68. T. Asefa and Z. Tao, *Chem. Res. Toxicol.*, 2012, **25**, 2265.
69. S. M. Dizaj, F. Lotfipour, M. Barzegar-Jalali, M. H. Zarrintan and K. Adibkia, *Mater. Sci. Eng., C*, 2014, **44**, 278.
70. M. Rai, A. Yadav and A. Gade, *Biotechnol. Adv.*, 2009, **27**, 76.
71. S. S. Agasti, A. Chompoosor, C. C. You, P. Ghosh, C. K. Kim and V. M. Rotello, *J. Am. Chem. Soc.*, 2009, **131**, 5728.
72. P. Granitzer and K. Rumpf, *Materials*, 2010, **3**, 943.
73. S. D. Alvarez, A. M. Derfus, M. P. Schwartz, S. N. Bhatia and M. J. Sailor, *Biomaterials*, 2009, **30**, 26.
74. S. Watanabe, H. Nakazumi, S. Kado, K. Maeda and T. Kitao, *ChemInform*, 1990, **22**, 236.
75. J. Chhablani, A. Nieto, H. Hou, E. C. Wu, W. R. Freeman, M. J. Sailor and L. Cheng, *Invest. Ophthalmol. Visual Sci.*, 2013, **54**, 1268.
76. E. C. Wu, J. H. Park, J. Park, E. Segal, F. Cunin and M. J. Sailor, *ACS Nano*, 2008, **2**, 2401.
77. R. Gupta and A. Kumar, *Biomed. Mater.*, 2008, **3**, 034005.
78. A. J. Friedman, G. Han, M. S. Navati, M. Chacko, L. Gunther, A. Alfieri and J. M. Friedman, *Nitric Oxide*, 2008, **19**, 12.

79. M. A. Brook, Y. Chen, K. Guo, Z. Zhang and J. D. Brennan, *J. Mater. Chem.*, 2004, **9**, 1469.
80. X. Q. Dong, J. Xu, W. C. Wang, H. Luo, X. F. Liang, L. Zhang, H. J. Wang, P. H. Wang and J. Chang, *Sci. China, Ser. C: Life Sci.*, 2008, **51**, 1039.
81. G. Gainza, M. Pastor, J. J. Aguirre, S. Villullas, J. L. Pedraz, R. M. Hernandez and M. Igartua, *J. Controlled Release*, 2014, **185**, 51.
82. G. Gainza, D. C. eldran Bonafonte, B. Moreno, J. J. Aguirre, F. B. orja Gutierrez, S. Villullas, J. L. Pedraz, M. Igartua and R. M. aria Hernandez, *J. Controlled Release*, 2015, **197**, 41.
83. M. Kokabi, M. Sirousazar and Z. M. Hassan, *Eur. Polym. J.*, 2007, **43**, 773.
84. P. on Rujitanaroj, N. Pimpha and P. Supaphol, *Polymer*, 2008, **49**, 4723.
85. A. Memic, M. Aldhahri, A. Tamayol, P. Mostafalu, M. S. Abdel-Wahab, M. Samandari, K. M. Moghaddam, N. Annabi, S. A. Bencherif and A. Khademhosseini, *Nanomaterials*, 2017, **7**, 63.
86. A. Nasajpour, S. Ansari, C. Rinoldi, A. S. Rad, T. Aghaloo, S. R. Shin, Y. K. Mishra, R. Adelung, W. Swieszkowski, N. Annabi, A. Khademhosseini, A. Moshaverinia and A. Tamayol, *Adv. Funct. Mater.*, 2018, **28**, 1703437.
87. S. S. Behera, U. Das, A. Kumar, A. Bissoyi and A. K. Singh, *Int. J. Biol. Macromol.*, 2017, **98**, 329.
88. Y. Lan, W. Li, Y. Jiao, R. Guo, Y. Zhang, W. Xue and Y. Zhang, *Acta Biomater.*, 2014, **10**, 3167.
89. B. Olas, *Clin. Chim. Acta*, 2015, **439**, 212.
90. D. G. Belair, M. J. Miller, S. Wang, S. R. Darjatmoko, B. Y. K. Binder, N. Sheibani and W. L. Murphy, *Biomaterials*, 2016, **93**, 27.
91. P. Bawa, V. Pillay, Y. E. Choonara and L. C. du Toit, *Biomed. Mater.*, 2009, **4**, 022001.
92. M. Wei, Y. Gao, X. Li and M. J. Serpe, *Polym. Chem.*, 2017, **8**, 127.
93. H. Almeida, M. H. Amaral and P. Lobão, *J. Appl. Pharm. Sci.*, 2012, **2**, 1.
94. A. Tamayol, M. Akbari, Y. Zilberman, M. Comotto, E. Lesha, L. Serex, S. Bagherifard, Y. Chen, G. Fu, S. K. Ameri, W. Ruan, E. L. Miller, M. R. Dokmeci, S. Sonkusale and A. Khademhosseini, *Adv. Healthcare Mater.*, 2016, **5**, 711.
95. A. Gandhi, A. Paul, S. O. Sen and K. K. Sen, *Asian J. Pharm. Sci.*, 2015, **10**, 99.
96. D. Schmaljohann, *Adv. Drug Delivery Rev.*, 2006, **58**, 1655.
97. H. Almeida, M. H. Amaral and P. Lobão, *J. Appl. Pharm. Sci.*, 2012, **2**, 1.
98. M. Asadian-Birjand, J. Bergueiro, F. Rancan, J. C. Cuggino, R. C. Mutihac, K. Achazi, J. Dernedde, U. Blume-Peytayi, A. Vogt and M. Calderón, *Polym. Chem.*, 2015, **6**, 5827.
99. L. Peña-Parás, J. A. Sánchez-Fernández and R. Vidaltamayo, *Handbook of Ecomaterials*, 2018, 1.
100. C. Wang, W. Sun, G. Wright, A. Z. Wang and Z. Gu, *Adv. Mater.*, 2016, **28**, 8912.
101. B. Law, R. Weissleder and C. H. Tung, *Biomacromolecules*, 2006, **7**, 1261.