# Chapter 2
# Soft Matter Composites Interfacing with Biomolecules, Cells, and Tissues

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## 2.1 Introduction

The parameters that affect and optimize the interactions at bio/non-biointerfaces are revised and analyzed in this chapter. We focus on soft polymeric materials starting with their critical properties that determine the viability of biological systems in contact with them. In particular, the right combination of surface chemistry, topography, and mechanical properties of the employed materials can generate the ideal interface for the target biological organism. We present the state of the art of the applications of such bio/soft matter composites interactions in tissue engineering for scaffolds and skin wound dressings.

Biocompatible, bioinert polymers are already used as implants in the human body in order to mimic the activity of a body part. The recent challenge though for the research in this field that will be discussed herein is to develop biodegradable scaffolds where specific cells can grow, adhere, proliferate, get vascularized, and eventually develop a tissue. The control of surface topography, chemistry, and mechanical properties in combination with appropriate nanofillers or biological growth factors present in the extracellular matrix can guarantee clinical success to future engineered scaffolds.

In the field of skin wound healing, active dressings that can provide the right conditions for optimized progress of the healing are gaining increasing space. We revise the most recent research efforts in the area, focusing on hydrogel type and electrospun nanofibrous dressings. These two types of materials, due to their

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particular mechanical and topographic characteristics, have demonstrated a big potentiality as active wound dressings especially combined with silver or other antimicrobial agents and antibiotics but also extracellular matrix growth factors.

At the end of the chapter, we present a special focus on nanosilver, the most common antibacterial system used so far as filler in the soft composite materials developed for interactions with biological systems.

## 2.2 Materials' Properties That Determine Bio-interactions Critical for the Ideal Scaffold Design

Two-dimensional polymeric-based surfaces have been used extensively as prototype systems for the study of the growth of diverse cell cultures. These studies are of particular importance since they clarify the influence of the materials' parameters, such as chemistry and surface topography, to the viability of the cells.

The interaction of different artificial surfaces with biomaterials is of high research interest for their utilization in biomedical applications. In fact, biointerfaces able to control the behavior of biomolecules or cells at various surfaces are promising tools for the investigation of the mechanisms taking place and are of particular interest for the development of medical devices, scaffolds, and other systems. The biointerface functionality is defined by specific physicochemical properties of the materials' surface including not only its mechanical properties and surface topography but also chemical composition, surface energy, and polarity, which together define the surface wettability.

In particular, the mechanical properties of a surface are of great importance on the cell survival, proliferation, adhesion, differentiation, and metabolism. Specifically, cells interact with a surface only if they are able to actively generate force and to transmit this force to the surroundings, sensing thus the passive properties of their environment (Schwarz and Safran 2013). This fact strongly depends on the type of the cells, and indeed, it has been shown that the differentiation of stem cells can be guided by the mechanical properties of the substrate (Fu et al. 2010; Engler et al. 2006); soft matrices can be neurogenic, stiffer matrices myogenic, and rigid matrices are proved to be osteogenic. Furthermore, the growth and movement of cells can be defined by the stiffness of the substrate. Particularly, various types of cells have the ability to migrate along gradients in stiffness of an underlying substrate and this rigidity-guided movement is called "durotaxis" (Lo et al. 2000). For this reason polymeric surfaces with controlled mechanical surface properties have been developed able to elucidate the effect of the mechanical properties on the cells behavior or differentiation, without altering the other critical properties such as surface chemistry and topography (Fu et al. 2010; Best et al. 2013; Palchesko et al. 2012). However, in most cases, it is very difficult to isolate and study exclusively such effect, since this property is often combined with other critical surface parameters (Schmidt et al. 2012; Genchi et al. 2013; Gaharwar et al. 2013).

Surface topography plays a basic role on the immobilization and activity of bioentities. To elucidate the role of surface topography in mediating cell-surface interactions, it is necessary to isolate it from all other parameters (e.g., surface chemistry). A recent study has shown that by modifying the nanotopography of a polymeric surface, the fibroblast cells attachment and spreading can be accurately controlled, and it is enhanced compared to the corresponding flat surfaces (Reynolds et al. 2013). However, the topography but also the chemical composition determine the surface wettability, a combined factor which plays a major role on the interaction with the biomaterials (Ciofani et al. 2013; Siow et al. 2006; Joy et al. 2011; Keselowsky et al. 2005). In fact, the wetting characteristics of a surface affect significantly the adsorption of proteins and the cells attachment and proliferation (Lourenço et al. 2012). Both highly hydrophilic and hydrophilic surfaces may inhibit such interactions, while surfaces with moderate wettability favor the adsorption of proteins, resulting in a positive cell response (Bacakova et al. 2011). However, all types of surface wettability can be useful for applications that deal with interactions with biomaterials, ranging from anti-biofouling materials for the fabrication of artificial blood vessels (Sun et al. 2011) to directed cells growth. In the latter case, various groups have been focused in the development of special surface architectures with controlled wettability (Zelzer et al. 2008; Oliveira et al. 2011; Ueda and Levkin 2013) for the directed cells growth on defined areas.

In particular, there has been a lot of effort for the formation of polymeric surfaces with special wetting properties ranging from superhydrophobic with ultrahigh or ultralow adhesion (Bayer et al. 2011) to superhydrophilic surfaces. This is obtained by modifying the surface roughness and/or the surface chemistry in the whole area or on specific zones using different techniques. A novel way to obtain such type of characteristics is the fabrication of smart polymeric surfaces where the wetting properties can change upon the application of an external stimulus. In most of the cases, such stimuli can be heat or light irradiation resulting thus to a localized effect. For example, surfaces with reversible wettability can be fabricated utilizing thermoresponsive or photoresponsive polymers, such as poly (isopropylacrylamides) (Sun and Qing 2011) or polymers doped with photochromic molecules (Athanassiou et al. 2006). Another way to obtain reversible surface wettability is the fabrication of nanocomposite films with polymers and titanium dioxide nanomaterials. For example, it has been recently reported the possibility to change the surface wettability of such nanocomposite surfaces from hydrophobic to hydrophilic upon UV laser light irradiation (Caputo et al. 2008), and this effect can be further enhanced by inducing a microroughness on the specific surfaces (Caputo et al. 2009). This is attributed to the unique effect of titanium dioxide to change reversibly its surface wetting properties due to oxygen vacancies formed upon UV irradiation resulting in the formation of a hydroxylated surface. The use of laser light for the tuning of the surface wettability of such materials offers the possibility to form localized patterned surfaces with controlled wetting gradients as already presented (Villafiorita Monteleone et al. 2010). An alternative method for the control of the surface chemistry and surface roughness can be also the spraying of nanomaterials of different dimensions on microrough surfaces by using masks

with controlled shape, and such process results in the formation of superhydrophobic surfaces with controlled water adhesion, making thus possible the localized interactions with biomaterials on defined areas (Milionis et al. 2013, 2014).

Although surfaces serve as model systems or as biosensors, the actual scaffolds to be implanted in the body need to be three dimensional with an interconnected porous network, since they must resemble the structure and shape of the deficient organ part that is to be regenerated. Such scaffolds are required to provide to the transplanted cells the biological environment and the 3-D support that is needed until the regenerated tissue is formed, structurally stabilized, and efficiently vascularized.

The recent advances on the fabrication of natural or synthetic polymeric materials, in the form of foams or fibrous scaffolds, as candidates for tissue regeneration able to provide directional cell attachment and promotion are discussed herein. The polymer-based materials for the fabrication of such scaffolds should be nontoxic to cells, biocompatible and biodegradable, and should interact positively with the cells to promote cell adhesion, proliferation, migration, and differentiated cell function. Furthermore, it should be highly porous in order to provide sufficient space for cellular activity, and with appropriate mechanical properties. More specifically, microscale parameters such as pore density, size, and configuration can affect the cell proliferation and differentiation (Karageorgiou and Kaplan 2005; Ng et al. 2009; Pamula et al. 2008), and therefore current research is focused on the development of porous material scaffolds that integrate with biological molecules or cells and regenerate tissues. However, one of the main challenges is the engineering of materials that can match both the mechanical properties and the biological environment of the tissue.

Synthetic and natural polymers, such as poly(α-hydroxy acids), polycarbonates, poly(fumarate)s, poly(urethane)s, polyesters, and their copolymers in the first case and collagen, polysaccharides, silk, gelatin, fibrin, and their derivatives in the second case, have been utilized for the formation of foams or fibrous matrices, for tissue engineering, e.g., bone and cartilage. Synthetic polymers can be designed in order to present desired mechanical and chemical properties compatible for scaffolds, to have appropriate biodegradation time, and they can be broadly available and cost-effective. In contrary to the synthetic, natural polymers present the appropriate affinity, making thus possible the promotion of desirable cell responses. However, they present several disadvantages, such as the poor mechanical strength and the complexities in the purification and extraction from the natural sources (Ng et al. 2012; Place et al. 2009a). The type and the properties of the foams or fibrous polymeric scaffolds are strongly dependent on the nature of the polymer utilized and on the type of tissue regenerated, while in the recent years, the scaffolds incorporate both microporous structures and nanostructures in order to better simulate the in vivo microenvironment and to enhance the cellular functions. Therefore, it is often used the combination of different methods for the fabrication of nanofeatures on the scaffolds but also the use of nanoparticles together with the polymeric materials for their fabrication (Ng et al. 2012; Dvir et al. 2011). Electrospinning, phase separation, gas foaming, 3-D printing, stereolithography, particulate leaching techniques, etc., are used, and the surface of the resulting scaffolds is sufficiently modified for the enhanced cell adhesion and proliferation.

Electrospun fibrous polymeric scaffolds have a very high surface-to-volume ratio, while a wide range of porosity with microscale interconnected pores, shape, and dimensions can be selected depending on the polymer solutions and processing parameters. One of the advantages of the electrospun fibrous scaffolds is the ability to orient the fibers but also to precisely control the porosity rendering thus such method ideal for a controlled and optimized solution for each type of scaffold application such as in vascular, neural, bone, cartilage, and tendon/ligament scaffolds (Moffa et al. 2013; Polini et al. 2013; Lee et al. 2010; Wang et al. 2011a; Nandakumar et al. 2010; Choi et al. 2008). Gas foaming techniques utilize the formation of gas bubbles by a chemical reaction or the expansion of $CO_2$ in polymer viscous matrices (Kim et al. 2012; van der Pol et al. 2010). In thermally induced phase separation process, a homogeneous polymer solution turns into a multiphase system characterized by a polymer-rich and a polymer-poor phase, under certain temperature conditions. After removal of the solvent, the polymer-rich phase solidifies to form a matrix, while the rest becomes pores. If the temperature is low enough, the solid–liquid demixing occurs with a frozen solvent and the concentrated polymer phase. Depending on the type of polymer solution and the process parameters, various types of scaffolds are formed for specific applications (Wei and Ma 2008; Mandoli et al. 2010; Jack et al. 2009). Particulate leaching is an easy and straightforward method to generate a porous polymeric structure and deals with the mixing of a polymer or prepolymer viscous melt or solution with a granular template of desired shape and size such as sugar or salt. After the polymerization or the solidification of the polymer, the template is leached remaining with the porous polymeric structure. Such method can be used for the fabrication of foams structures (Pamula et al. 2009; Mou et al. 2013) or in combination with other methods (e.g., phase separation, electrospinning) in order to form scaffolds with multiscale porosity (Liu and Ma 2009; Wei and Ma 2009; Kim et al. 2008a; Guarino et al. 2008). On the other hand, rapid prototyping methods such as 3-D printing (Seyednejad et al. 2011, 2012) and stereolithography offer the opportunity to prepare structures with precise complex and reproducible geometries that can help in the growth of the implanted cells but also in the biodegradability of the scaffolds in an accurate and highly manageable way. The 3-D design can be done via specific computer softwares, and therefore it can be tuned and modified according to specific needs. In the case of stereolithography, lasers have been used to successfully produce microstructured biomaterials for tissue engineering scaffolds, and a suite of different laser techniques has been reported to produce structures with a range of microstructure resolutions (Beke et al. 2012, 2013; Sušec et al. 2013; Johnson et al. 2013).

## 2.3 Bioactivated Cell-Instructive Scaffolds

The next generation of scaffolds requires the encoding of complex arrays of biofunctional signals to control and guide cellular events and tissue remodeling. The concept of tissue and cell guidance is rapidly evolving as more information on the biological control of the extracellular microenvironment on cellular function and tissue morphogenesis becomes available. These findings have burst a novel concept in bioactive material design based on nanometric control of structural and functional features to recapitulate the spatiotemporal molecular regulatory program and the three-dimensional architecture of the native extracellular matrix. Micro- and nanostructured scaffolds able to sequester and deliver biomolecular moieties in a tightly spatial and temporal controlled manner have been proposed as highly effective in tissue repairing, in guiding functional angiogenesis, and in controlling stem cell differentiation. Although these materials are a first attempt to mimic the complex and dynamic microenvironment presented in vivo, an increased symbiosis among material engineering, micro- and nanotechnology, drug delivery, and cell and molecular biology is needed to fabricate biomaterials that encode the whole array of biosignals to guide and control developmental processes in tissue- and organ-specific differentiation and morphogenesis.

Scaffold design concept has been constantly evolving during the last two decades passing from the original notion of an inert temporary material permissive to cell and tissue growth to the modern concept of proactive cell-instructive material, able to control and guide tissue morphogenesis (Hacker and Mikos 2006) (Fig. 2.1).

Originally, scaffolds were envisaged as provisional constructs that could only provide geometrical guidance to cell and tissue growth. According to the modern concept, instead, the ideal scaffold should provide an active guidance to the whole process of tissue repairing by displaying a series of chemical, biochemical, and biophysical cues to elicit specific events at the cellular and tissue level. Spatio-temporal presentation of biological signals must be combined with microstructural and mechanical properties to provide a proper cell-instructive environment not only within the scaffolds but also at the interface with the native tissues. The typical approach is to reestablish the essential features of the extracellular matrix (EMC) environment in a natural or synthetic material. Along this line, purified ECM components or decellularized ECMs derived from animals have been widely used in tissue engineering. Decellularized ECM has been successfully used as a scaffold for soft tissue applications (Voytik-Harbin et al. 1998), and single purified ECM components, such as collagen, hyaluronic acid, and fibrin, have been combined to create controlled and standardized materials with structure similar to native ECM (Battista et al. 2005; Chan and Mooney 2008). Albeit biological tissue-derived materials have some advantages, such as biocompatibility and cell receptors recognition, synthetic materials are chemically programmable and reproducible; moreover, they display a high degree of control of their properties offering the possibility to tailor their performance on the specific application. In order to improve the

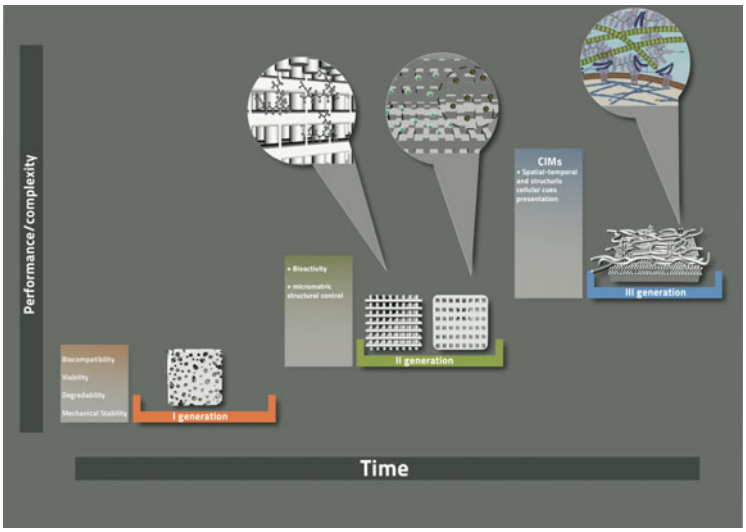


**Fig. 2.1** Evolution of scaffold concept: first scaffold generation was designed to fulfill basic properties like biocompatibility, viability, mechanical stability, degradability, and porosity. They were conceived as an inert ancillary frame to temporarily replace the function of damaged tissue, while the cells seeded within its structure could deposit novel tissue that would progressively restore the original status. The appreciation of the central role of the microenvironment on tissue morphogenesis has stirred research direction along the design of a second generation of scaffolds that encode biological signals able to control and guide cell and tissue processes. Scaffolds of this generation were enriched with bioactive moieties, either physisorbed or chemically conjugated, that could be presented at cell surface to trigger specific events. However, to elicit specific events and correctly instruct a cell to perform a specific task, signals must be presented at the right time, at the right dose, and at the right site. Therefore, next scaffold generation should provide a tight control of presentation at nanometric scale of physical and biomolecular cues to recapitulate the spatiotemporal regulatory program and the three-dimensional architecture of the native extracellular matrix. This new generation of scaffolds should be able to provide the suitable instructive microenvironment for the cell to activate the correct morphogenic pathway

interaction with cells, biological active molecules have been incorporated within synthetic materials obtaining hybrid proactive materials to promote and control cell interaction and functions. The development of synthetic material designed to present a complex array of bioactive signals with a defined time and space program is at the frontier of biomaterials science for the realization of artificial replica of the extracellular matrix.

ECM is the natural medium in which cells grow, differentiate, and migrate and represents the gold standard material for tissue regeneration (Bosman and Stamenkovic 2003). The cell–ECM interaction is highly specific and reciprocal. Cells produce, organize, and eventually degrade the macromolecular components of the ECM, and, in turn, ECM sequesters and presents molecular signals that control and guide cell response. ECM is a dynamic environment in which several proliferation–adhesion–differentiation motifs are continuously generated,

sequestered, and released often according to cellular stimuli (Katz and Streuli 2007; Fittkau et al. 2005; Stupack and Cheresh 2002). Moreover, solid-state, structural ECM molecules, such as heparin, act as reservoirs for secreted signaling molecules for their on-demand release (Rapraeger 2000; Wijelath et al. 2002; Taipale and KeskiOja 1997). Growth factors (GFs), for instance, are locally stored in insoluble/latent forms through specific binding with glycosaminoglycans (e.g., heparins) and released upon demand to elicit their biological activity. The sequestration of GFs within the ECM in inert form is necessary for rapid signal transduction, allowing extracellular signal processing to take place in time frames similar to those inside cells. Moreover, spatial gradients of GFs play a major role in ECM maintenance and equilibrium because they are able to direct cell adhesion, migration, and differentiation deriving from given progenitor cells and organize patterns of cells into complex structures, such as vascular networks and the nervous system (Gurdon et al. 1994; Tanabe and Jessell 1997; Burgess et al. 2000). Thus, spatial patterns in tissues are dictated by both the architectural features of the ECM and concentration profiles/gradients of diffusible bioactive factors (Kong and Mooney 2007).

The development of modern scaffolds has been driven by biomimicry-inspired design to recapitulate in a simplified form the essential features of the molecular and structural microenvironment existing in the ECM. For instance, several micro- and nanofabrication strategies, including molecular and nanoparticulate self-assembly, micro and nanoprinting, electrospinning, and molecular and nano-templating (Hutmacher 2001; Sachlos and Czernuszka 2003; Teo et al. 2006; Guarino et al. 2007; Beniash et al. 2005; Place et al. 2009b; Mehta et al. 2012), have been used in an attempt to reproduce the spatial organization of the fibrillar structure of the ECM that provides essential guidance for cell organization, survival, and function (Sachlos and Czernuszka 2003; Guarino et al. 2007). Topographic and stereomorphological cellular cues can be provided by controlling fiber dimension and arrangement (Teo et al. 2006); chrono- and spatial-programmed presentation of bioactive moieties can be encoded by placing morphogenic factor-loaded degradable microparticles in predefined regions of the scaffold (Mehta et al. 2012; Luciani et al. 2008); finally, the exposition of matricellular cues can be controlled, even dynamically, by grafting integrin adhesive motifs (Causa et al. 2007) (Fig. 2.2).

The necessity to control the presentation of microenvironmental cues at cell level denotes the key shift from the concept of shape to cell guidance that accompanies modern scaffold design strategies. However, the attainment of tight control over space, time, and molecular arrangement of the cascade of signals required to control and guide the process of tissue or organ repair is, albeit theoretically achievable, practically and economically non-pursuable (Place et al. 2009b). The recapitulation of the complex molecular events occurring within the extracellular space during the process of tissue repair and regeneration should be reproduced in the most essential features using simplified strategies. For instance, within its fibrillar components, ECM has a vast range of integrin-binding motifs, each of them with a specific function and activity (Causa et al. 2007; Ventre et al. 2012). Most of these motifs have been identified and their corresponding short sequence

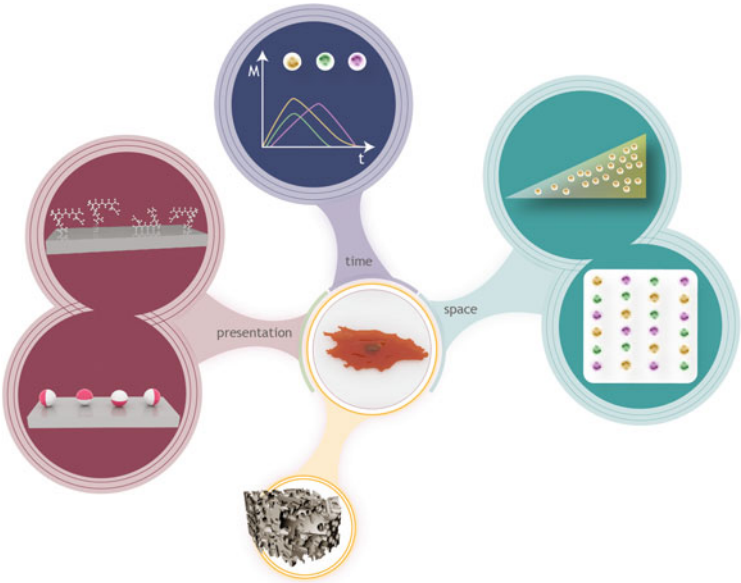


**Fig. 2.2** Schematic of bioactivated scaffolds—next generation of scaffolds should control the presentation of bioactive signals in time, space and configuration/conformation. Molecular conformation must be suitable for cell interaction to elicit the desired response and can be tailored with the aid of molecular spacer. Time and space presentation of the signal must be arranged and accorded with specific profiles. The synchronization over time of a specific signal can be achieved by the use of engineered GFs-encapsulated microdepots (Biondi et al. 2008). Embedding microdepots releasing GFs at known release rates in a defined spatial distribution within the scaffold it is virtually possible to recreate any, even complex, molecular microenvironment (Sun et al. 2004; Whitesides et al. 2001). The combination of micropositioning systems and mathematical modeling describing the complex and multiple mechanisms governing the release kinetics from single microspheres within the scaffold can be of help in realizing scaffolds with highly controlled architecture by computer-aided scaffold design (CASD) (Sun et al. 2004; Hutmacher et al. 2004)

peptides synthetically reproduced (Ventre et al. 2012). The accessibility to an entire library of integrin-binding peptides makes it possible to reproduce the integrin-mediated cross talk realistically and in a simplified manner by inserting small molecular units within the scaffold instead of the whole fibrillar protein such as collagen, fibronectin, or laminin. However, the insertion of all possible matricellular cues mimicking peptides in a scaffold would certainly make a more realistic replica of the natural molecular niche for cells but would make scaffold production impractical and perhaps unnecessarily complex. Furthermore, since during any tissue repairing process GFs are continuously produced within the extracellular space and dynamically presented at cell surface, the use of these generally labile proteins within the scaffold requires sophisticated technologies to preserve their activity for a medium–long time period (Borselli et al. 2007). The use of peptides capable of eliciting comparable morphogenic activity allows a dramatic

reduction in the level of scaffold complexity. Even if these peptides generally elicit cell response at a higher dose compared to their natural or recombinant counterparts, they provide a viable alternative in terms of cost and handling. QK peptide, for instance, has been proved to be effective in eliciting angiogenic response and has been already exploited as an alternative to VEGF in promoting scaffolds (Finetti et al. 2012). Analogously, BMP mimetic peptide already proved a potent osteogenic active molecule (Zouani et al. 2010). These small molecules, as their natural counterparts, often impart a potentiated biological response if bound to a solid substrate. It has been proved that materials with grafted peptides enhance tissue formation; such a result points to provide a better integration of the scaffold with the neoforming tissue (Wang et al. 2007). In natural ECM, GAGs (glycosaminoglycans) provide binding domains for GFs (growth factor), and this mechanism of action could be encoded within artificial ECM by introducing a specific binding domain for the mimicking peptides. Alginate and poly(acrylamide) gel, for instance, have been sulfated to enhance the binding affinity to some GFs, including VEGF, PDGF, and HGF, potentiating the angiogenic activity and extending the flexibility of the scaffold for growth factor presentation and preservation (Merkel et al. 2002; Rouet et al. 2005; Chaterji and Gemeinhart 2007). Furthermore, the modulation of binding affinity within the scaffolds structure provides a viable strategy to control stable gradients of GFs (Fig. 2.2) or their mimicking peptides, which are proved to be essential in controlling and guiding morphogenetic processes (Griffith and Swartz 2006).

In natural ECM, there is a continuous production of GFs that are sequestered within molecular recess and eventually used upon cell request. Sources of GFs or their mimicking peptides, at a specific location within a synthetic scaffold, can be provided with the use of micro- or nanoparticles loaded with bioactive moieties and programmed to deliver according to a specific profile (Fig. 2.2). Integration of GF-loaded microparticles engineered to release sequentially various GFs has been already discussed in the literature (Luciani et al. 2008; Richardson et al. 2001; Saltzman and Olbricht 2002). According to this approach, it is possible to control the spatial distribution and the gradients of bioactive agents at different locations within the scaffold (Luciani et al. 2008; Borselli et al. 2007; Chen et al. 2007). A more advanced method to manufacture microsphere-integrated scaffolds able to regulate GFs release kinetics both temporally and spatially may take advantage of micromanipulation-based techniques. Possible developments and advancement include the control over the presentation of relevant signals, not only within the physical domain of the scaffolds but also within the host surrounding tissues. Microdepot acting as a single point source may be micropositioned by 3-D printing and soft lithography to obtain highly regulated structures able to trigger the extent and possibly the architecture/structure of tissue formation (Sun et al. 2004; Whitesides et al. 2001). The combination of micropositioning systems and mathematical modeling describing the complex and multiple mechanisms governing the release kinetics from single microspheres within the scaffold can be of help in creating scaffolds with a highly controlled architecture using computer-aided scaffold design programs (CASD) (Whitesides et al. 2001; Hutmacher et al. 2004).

Over the past decades, the concept of scaffolds has been strongly redefined: from the original definition of a space-filling material to the most modern vision of programmable bioactive material, able to guide and control complex cellular processes. Thanks to a sapient integration of cellular and molecular biology combined with the advancement in material science and nanotechnology, future scaffolds can be envisaged as a simplified, yet effective, replica of the natural ECM—with the potentiality to make tissue engineering a real clinical success.

## 2.4 Introduction to Skin Wound Active Dressing Materials

The skin is the largest organ in the human body and the one interfacing with the external environment, keeping protected the rest of the body but also receiving sensory stimuli. It consists of three layers. The outermost layer is the bloodless epidermis and is bonded to the underlying dermis layer. In the dermis are included blood vessels, collagen and elastin fibers, glands, hair follicles, and nerves' endings. The innermost layer is the subcutis, an energy reservoir and impact protective layer, composed of mainly fat tissue. When the skin layers are damaged, an acute wound is formed. The treatment of acute wounds should involve first the cleaning, to reduce the risk of infection, and then the closure, where appropriate dressings are involved. The wound closure helps in the reduction of the infections risks, brings the separated tissues close together, and promotes the healing process. Such healing process goes through four phases: (1) hemostasis, (2) inflammation, (3) proliferation, and (4) maturation remodeling. As the healing progresses, the different phases often overlap, as schematically demonstrated in Fig. 2.3. When the healing of an acute wound does not go through the usual phases, failing to close in the expected time due to intrinsic or extrinsic causes, then a chronic wound can develop. In such wound, the septic infections are common, but resolving their origin can help in restarting the healing process.

The dressing of a skin wound has a crucial role in the healing process since it provides the right conditions that assure its optimized progress till the final closure. There are already numerous wound dressings in the market, each one tackling diverse types of wounds, acute or chronic. These dressings can be categorized according to their interactions with the wound: (1) passive dressings that just cover the wound (i.e., gauzes), (2) interactive dressings that promote the healing by being oxygen and water vapor permeable but not permeable to bacteria (i.e., transparent films, foams, gels), and (3) active dressings that deliver substances that contribute to the healing process (alginates, chitosan, hydrocolloids). Usually, the skin wound dressing types that are available in the market are traditional gauzes and tulles, transparent films, and foams that cannot absorb the exudates but are moisture permeable and dressings that upon contact with the wound exudates form a gel creating a moist environment (hydrocolloids, alginates, hydrofibers). Lately, the availability of active dressings containing silver or other antimicrobial agents or antibiotics is progressively increasing.

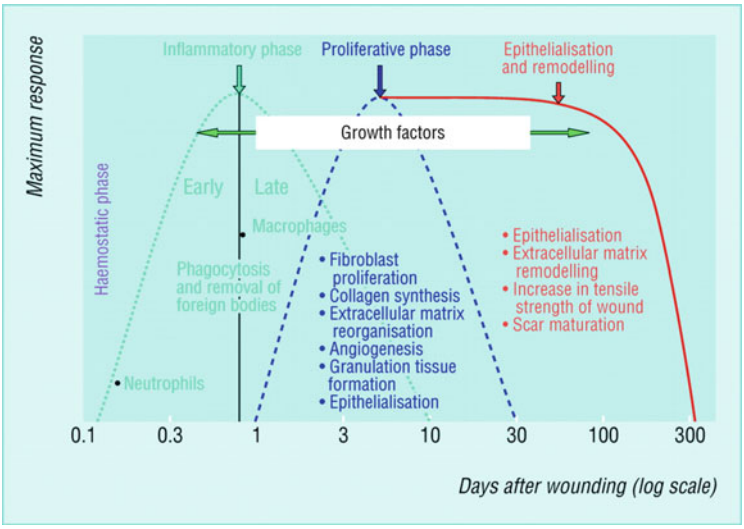


**Fig. 2.3** The four overlapping phases of acute wound healing (Enoch et al. 2006)

The majority of the research efforts in this field are concentrated to hydrogel type and nanofibrous, especially electrospun, materials that have a big potentiality as active wound dressings, providing new possibilities for the wound-healing market.

## 2.5 Hydrogel Materials with Antibacterial Agents for Skin Regeneration

Hydrogels are hydrophilic polymers which can be swollen by water and have found biomedical applications for tissue engineering and drug delivery. For instance, alginate- and chitosan-based materials can uptake large amounts of water compared to their initial weight when in contact with moist media such as open tissue wounds. As a result, they gelatinize. During the formation of alginate- or chitosan-based gels, they exchange or loose ions and eventually can become structurally unstable. Hence, various methods have been developed to maintain structural longevity in hydrogels obtained from natural polymers (Malmsten 2011). Natural polymer-based hydrogels are generally classified into various categories depending on the preparation method, the charge, and the mechanical and structural characteristics. In this chapter, we will present the state of the art developed in recent years on hydrogels based on natural polymers and their various applications in the field of wound healing and treatment.

Hydrophilic and organic antimicrobial agents and drugs can be readily incorporated into sodium alginate- and chitosan-based polymers in aqueous solutions.

Sodium alginate solutions containing hydrophilic antibacterial agents can be turned into films or beads which will swell and gelatinize upon contact with, for instance, hydrated skin wounds. A recent work combined povidone–iodine (PVPI) and sodium alginate into antimicrobial calcium cross-linked films and beads which released PVPI in a controlled manner in moist media or in water (Liakos et al. 2013). PVPI is encapsulated in the films forming circular microdomains. Upon immersion into moist media such as bacteria- or fungi-laden agar or aqueous media or contaminated water, these films swell, gelatinize, and start releasing the antimicrobial agent (PVPI) slowly inhibiting the growth. The films also display antimicrobial and antifungal activity when exposed to agar media heavily populated by *E. coli* and *Candida albicans* fungi as seen in Fig. 2.4. This was achieved by natural gelation and swelling of alginate in such media. These alginate films can also encapsulate hydrophobic antimicrobial agents such as natural essential oils (EOs) (Liakos et al. 2014). The process differs from direct mixing in aqueous solution in that surfactant stabilized emulsions need to be prepared. Namely, elicriso italic, chamomile blue, cinnamon, lavender, tea tree, peppermint, eucalyptus, lemongrass, and lemon oils were encapsulated in the films as potential active substances. Glycerol was used to induce plasticity and surfactants were added to improve the dispersion of EOs in the sodium alginate matrix.

The topography, chemical composition, mechanical properties, and humidity resistance of the films were studied. Antimicrobial tests were conducted on films containing different percentages of EOs against *E. coli* bacteria and *Candida albicans* fungi (Liakos et al. 2014). Such diverse types of essential oil-fortified alginate films can find many applications mainly as disposable wound dressings but also in food packaging, medical device protection and disinfection, and indoor air quality improvement applications, to name a few. Not all essential oils encapsulated in alginic matrices present similar effects against inhibiting bacterial or fungal growth as exemplified in Fig. 2.5.

There has been much interest in forming 3-D hydrogel structures from natural polymers containing large amounts of water but being structurally stable and functional (antimicrobial and drug releasing). In order to enhance their mechanical robustness, researchers use physical or chemical cross-linking procedures to create three-dimensional (3-D) polymeric networks. The most common biopolymer hydrogels are obtained from alginic acid polymers, chitosan, gelatin, and β-cyclodextrin (enzymatically obtained from starch). Due to their high water content and soft, porous 3-D structure (see Fig. 2.6), they can easily simulate in vivo extracellular matrix (ECM) microenvironment in biomedical applications. Hydrogels can be applied externally or can be injectable and can carry cells or drugs into the body in a minimally invasive manner. Hydrogels have also been used for the creation of 3-D scaffolds for cell culture and transplantation and as carriers for local release of proteins and drugs (Lee et al. 2013; Fonseca et al. 2014).

Alginic acid-based natural polymers are linear polysaccharides with homopolymeric blocks of (1,4)-linked β-D-mannuronate and α-L-guluronate that are extracted from seaweed and shrimp shells (see Fig. 2.7). They are widely used in making various forms of biomedical materials. In general, alginate forms a hydrogel via

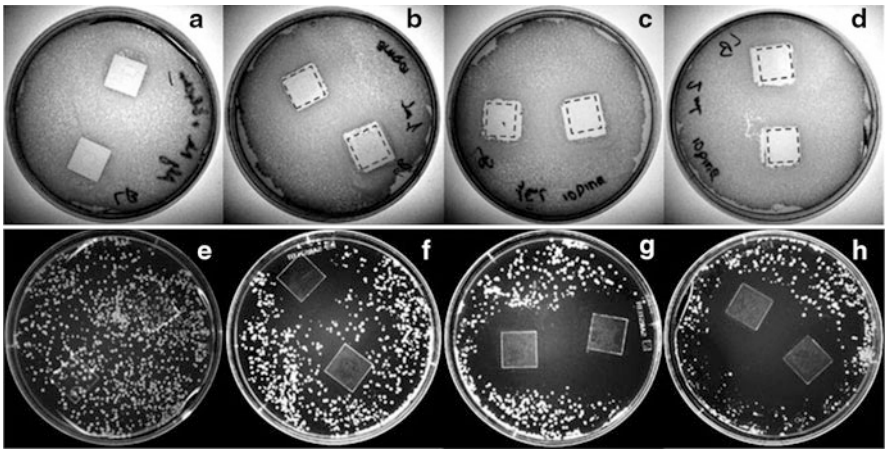


**Fig. 2.4** Photographs of Petri dishes containing heavily populated *E. coli* bacteria after 48 h incubation in the presence of (**a**) 70 wt% NaAlg/30 wt% glycerol film (control film), (**b**) film 1, (**c**) film 2, and (**d**) film 3. The *red noncontinuous line* indicates the borders of the glass slide. Photographs of Petri dishes containing *C. albicans* after 48 h incubation in the presence of (**e**) 70 wt% NaAlg/30 wt% glycerol film, (**f**) film 1, (**g**) film 2, and (**h**) film 3. Film 1, film 2 and film 3 indicate increasing concentrations of PVPI (Liakos et al. 2013)

ionic interactions between carboxylic acids and divalent cations such as $Ca^{2+}$, $Mg^{2+}$, and $Ba^{2+}$ as depicted in Fig. 2.8. Applications of alginate hydrogels range from injecting cells and drugs to wound dressings and dental implants due to their low toxicity, low cost, and gelling ability by the action of divalent cations.

There is still an active research interest in rendering alginate-based hydrogels more robust by controlling their mechanical and biophysical properties such as elastic modulus, swelling ratio, and degradation rate, although control over rapid ionic cross-linking and rapid loss of ions is still highly challenging. Therefore, intermolecular cross-linking methods such as conjugating various types of cross-linkers to the alginate backbone have been developed, but such reagents and reaction conditions for conjugation and cross-linking are typically toxic to encapsulated cells and can cause denaturation of growth factors or complications in wound treatment and healing.

The structure and mechanical behavior of gelatin gels have already been widely studied in the past (Wan et al. 2008; Van Vlierberghe et al. 2011; Mazzitelli et al. 2013). Gelatin normally dissolves in aqueous solutions at temperatures around body temperature where it exists as flexible single coils. On cooling down, transparent gels are formed, if the concentration is higher than the critical gelation concentration. These gels are formed by physical cross-links, also called "junction zones," originating from a partial transition to "ordered" triple-helical collagen-like sequences, separated by peptide residues in the "disordered" conformation. Because of its unique gelation and biomimetic properties, gelatin is interesting to use as a hydrogel for biomedical applications (Van Vlierberghe et al. 2011). There exist several methods to cross-link gelatin hydrogels. The disadvantage of most

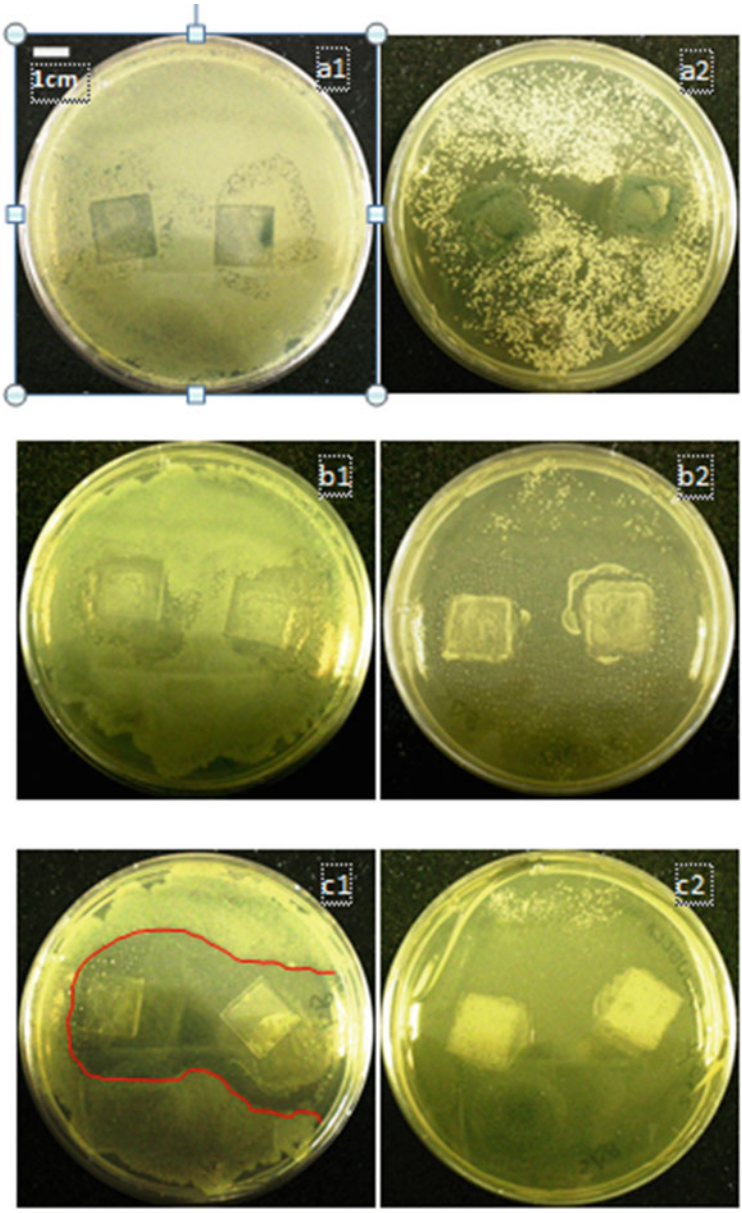


**Fig. 2.5** EO-encapsulated calcium cross-linked alginate films incubated in agar media containing bacteria and fungi. (**a1**) Chamomile blue incubated with *E. coli* and (**a2**) chamomile blue incubated with *C. albicans*. (**b1**) Peppermint incubated with *E. coli*, (**b2**) peppermint with *C. albicans*, (**c1**) cinnamon incubated with *E. coli* (the *continuous red line* represents the inhibition zone to guide the eye), and (**c2**) cinnamon incubated with *C. albicans* (Liakos et al. 2014)

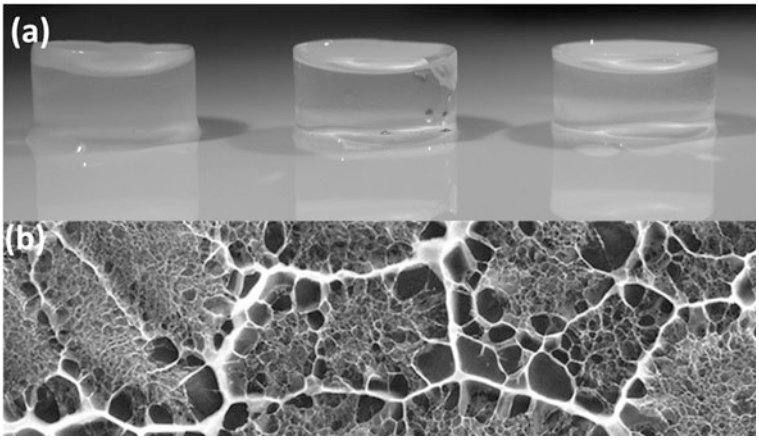


**Fig. 2.6** (**a**) Photograph of alginate hydrogels. (**b**) Cryo-scanning electron microscopy image of alginate hydrogel (Lee et al. 2013)

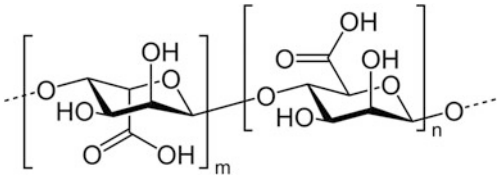


**Fig. 2.7** Chemical structure of alginic acid polymer

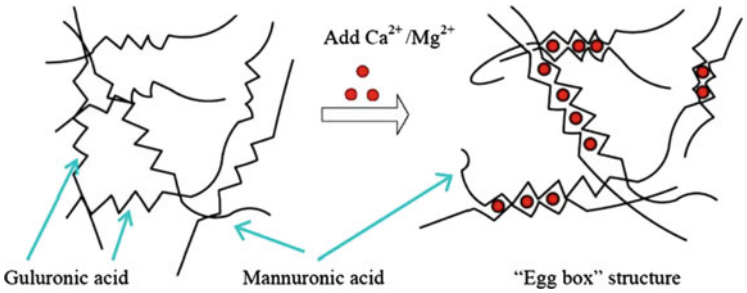


**Fig. 2.8** Cross-linking of alginic acid with calcium or magnesium ions (Fonseca et al. 2014)

chemical cross-linking procedures is the fact that they are irreversible and can contain chemical traces that can hinder wound healing. Recent works demonstrated that cross-linking via disulfide bond formation by oxidation of thiolated compounds could offer a solution for this problem, since this process is reversible. Cleavage of the disulfide linkages via reducing agents (e.g., dithiothreitol) results in thiolated, soluble macromolecules. The mechanical properties of thiolated gelatin hydrogels as depicted in Fig. 2.9 depend on the contributions of both the physical

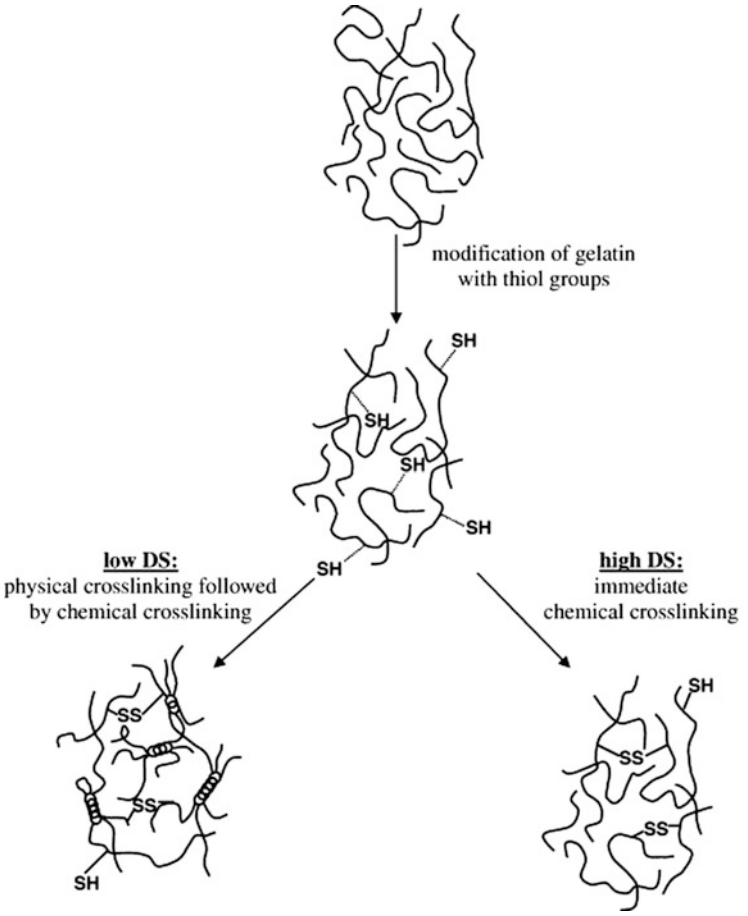


**Fig. 2.9** Schematic representation of the influence of the synthesis route on the formation of the gelatin network (Wan et al. 2008)

cross-linking and the chemical cross-linking by disulfide formation. Above the sol–gel temperature, the gel strength only depends on the chemical network, due to the thermoreversibility of the physical entanglements. Common cross-linking agents are *N*-acetylhomocysteine thiolactone and Traut's reagent.

Moreover, recent works demonstrated fabrication of gelatin-based hydrogel patches (Mazzitelli et al. 2013). The effect of different preparation parameters were analyzed with respect to the rheological and pharmaceutical characteristics of hydrogel blend patches as transdermal delivery formulation. Mixtures of pectin and gelatin were employed for the production of patches, with adjustable properties, following a two-step gelation procedure. The first gelation, a thermal one, is trigged by the presence of gelatin, whereas, the second gelation, an ionic one, is due to the formation of the typical egg box structure of pectin. In particular, the patch structural properties were assessed by oscillation stress sweep measurements which

provided information concerning their viscoelastic properties. In addition, different modalities for drug loading were analyzed with respect to drug homogeneous distribution; testosterone was employed as model drug for transdermal administration. Finally, the performances of the produced transdermal patches were studied, in terms of reproducibility and reliability, by determination of in vitro drug release profiles.

Transdermal patches are usually formulated to assure a sustained systemic drug release, from a few days up to a couple of weeks. In this respect, the accurate rheological characterization performed on patches with different formulations had the aim to select those presenting the best viscoelastic properties, allowing an easy administration (i.e., skin application) and duration of use. As further objective aimed to evaluate the drug product performance, specific tests for determining the drug release from the produced patches were performed. Determination of drug or metal ion (in the case of silver- or copper-based patches) release profiles from transdermal medicines, although does not represent a measure of bioavailability, gives important information on the drug release characteristics that have the potential to alter the biological performance of the drug in the dosage form. As an example to nanoparticle-laden hydrogels, silver nanocomposite hydrogels were recently developed by using acrylamide and biodegradable gelatin (Reddy et al. 2013). Silver nanoparticles were generated throughout the hydrogel networks using in situ method by incorporating $Ag^+$ ions and the subsequent treatment with sodium borohydride as shown in Fig. 2.10. The effect of gelatin on the swelling studies was investigated. The hydrogel synthesized silver nanocomposites were also characterized. The biodegradable gelatin-based silver nanocomposite hydrogels were tested for antibacterial properties and exhibited a strong antibacterial activity against bacillus. These agents can easily find applications in wound and burn dressings. Moreover, cross-linked gelatin–chondroitin sulfate hydrogels exhibit excellent properties for the controlled release of small cationic antibacterial proteins into wounds (Kuijpers et al. 2000). Combining chondroitin sulfate with gelatin in a cross-linked gel increases the interaction between the cationic protein and the hydrogel, causing an increased loading capacity and an extended release time for wound treatment. As two different antibacterial proteins, recombinant thrombocidin, rTC-1, and lysozyme, were used resulting in similar release results, it is, hence, expected that such release systems can be used for a broad range of cationic antibacterial proteins without major adaptations. The effectiveness of these hydrogels in skin treatment was demonstrated on polyester films (Dacron) coated with a skin-like tissue as seen in Fig. 2.11. Finally, these hydrogels are biocompatible and degrade almost completely within several weeks of application, thus allowing tissue integration, which is advantageous for the healing characteristics of porous biomaterials, and may improve the long-term infection resistance of these materials.

Similar to alginic acid polymers, chitosan-based hydrogels have also received a great deal of attention due to their well-documented biocompatibility, low toxicity, and degradability by human enzymes and their natural antibacterial properties (Giri et al. 2012; Bhattarai et al. 2010; Fiejdasz et al. 2013). These and other properties

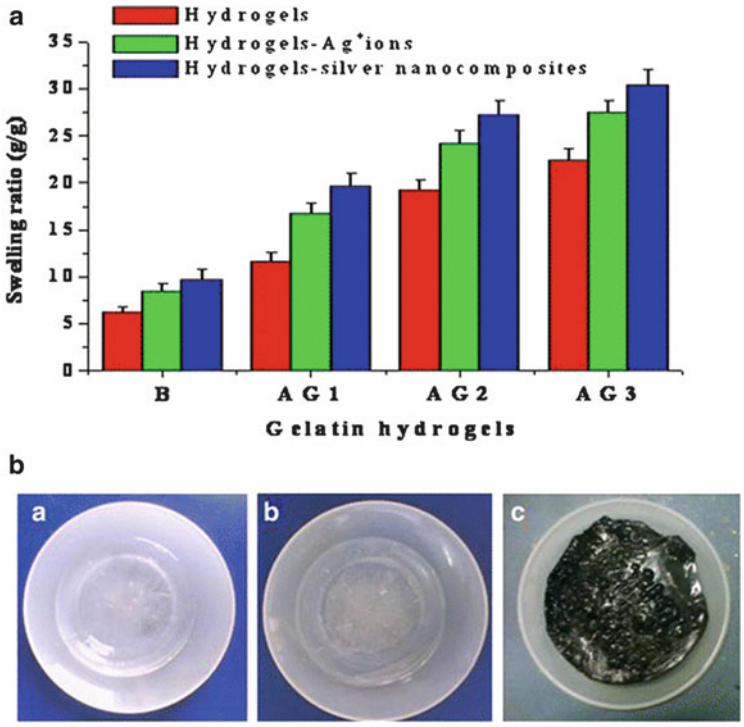


**Fig. 2.10** (**a**, *upper panel*) swelling behavior of pure hydrogel, ions-loaded hydrogel, and nanohydrogels. (**b**, *lower panel*) (a) plain hydrogel. (b) Ag+ ions-loaded hydrogel. (c) Ag nanocomposite hydrogels (Reddy et al. 2013)

such as hydrophilicity, existence of functional amino groups, and a net cationic charge have made chitosan a suitable polymer for the intelligent delivery of macromolecular compounds, such as peptides, proteins, antigens, oligonucleotides, and genes. Chitosan hydrogels have been prepared with a variety of different shapes, geometries, and formulations that include liquid gels, powders, beads, films, tablets, capsules, microspheres, microparticles, sponges, nanofibrils, and inorganic composites. In each preparation, chitosan is either physically associated or chemically cross-linked to form the hydrogel. As a hydrogel, chitosan networks should satisfy the following: (1) interchain interactions must be strong enough to form semipermanent junction points in the network and (2) the network should promote the access and residence of water molecules within. Gels that meet these demands may be prepared by non-covalent strategies that rely on electrostatic, hydrophobic, and hydrogen bonding forces. Figure 2.12 shows the schematics of four major physical interactions (i.e., ionic, polyelectrolyte, interpolymer complex, and hydrophobic associations) that lead to the gelation of a chitosan solution (Bhattarai et al. 2010). Because the network formation by all of these interactions is purely physical, gel formation can be reversed. Due to cationic amino groups of

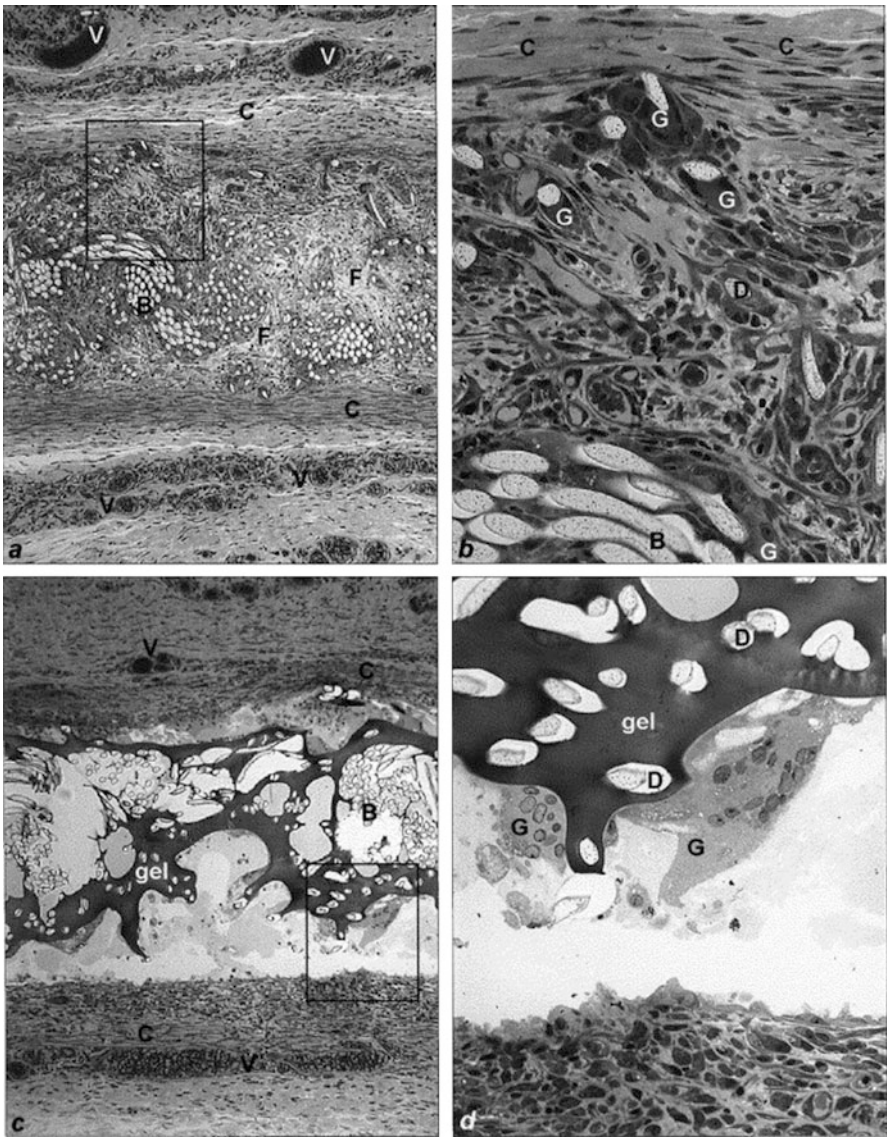


**Fig. 2.11** Polyester film coated with soft tissue. Dacron-pt (**a**; 5×), Dacron-pt (**b**; 20×), Dacron-pt-gel-0.8 (**c**; 5×), and Dacron-pt-gel–ChS-0.8 (**d**; 20×) after 10 days of implantation [B = Dacron fiber bundles, V = blood vessels, F = fibrin, D = Dacron fiber, C = capsule, G = giant cell, gel = cross-linked gel (gelatin or gelatin–ChS)] (Kuijpers et al. 2000)

chitosan, ionic interactions can occur between chitosan and negatively charged molecules and anions. Ionic complexation of mixed charge systems can be formed between chitosan and small anionic molecules, such as sulfates, citrates, and phosphates or anions of metals like Pt (II), Pd (II), and Mo (VI). While

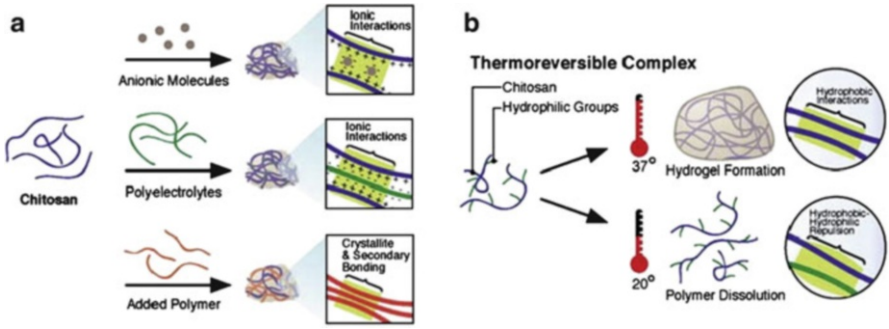


**Fig. 2.12** Schematic representation of chitosan-based hydrogel networks derived from different physical associations: (**a**) networks of chitosan formed with ionic molecules, polyelectrolyte polymer, and neutral polymers; (**b**) thermoreversible networks of chitosan graft copolymer resulting semisolid gel at body temperature and liquid below room temperature (Bhattarai et al. 2010)

polyelectrolytes form electrostatic interactions with chitosan, they are different from the ions or ionic molecules used in ionic complexation in that they are larger molecules with a broad molecular weight range, such as polysaccharides, proteins, and synthetic polymers (Fig. 2.12). They are complexed without the use of organic precursors, catalysts, or reactive agents, alleviating the concern about safety in the body or cross-reactions with a therapeutic payload. In addition, because PECs consist of only chitosan and the polyelectrolyte, their complexation is straightforward and reversible.

Chitosan–alginate blend-based nanocomposite hydrogels containing sugars (see Fig. 2.13) were also shown to be highly effective in wound treatment (Travan et al. 2009). The role of chitosan is fundamental in the formation and stabilization of well-dispersed small silver nanoparticles, for instance. Reproducibility of size distribution together with a demonstrated stability of the nanoparticles over time can be achieved in chitosan-based polymer matrices. Moreover, the use of sugar-based additives adds a considerable appeal to the results obtained. The simultaneous presence of a sugar-based bioactive polymer for cell stimulation (Fig. 2.13) and of silver nanoparticles in the gel for antibacterial activity represents a major achievement in would treatment. Such approaches can bridge the gap between nanotechnology and glycobiology (Travan et al. 2009; Morais et al. 2013).

In open-wound treatment, there is the major risk of infection that presents serious consequences, which can compromise the recovery success. To prevent those infections, several approaches are used such as sterility protocols and antibiotics administration. However, these protocols are not always effective, and the antibiotic activity can even fail due to pathogenic resistance development. Therefore, recently, studies were made to ascertain the use of certain ions as antimicrobial agents, such as cerium (Ce), which has revealed antimicrobial properties against several microorganisms (Morais et al. 2013). This way, it can be incorporated in different biomaterials to grant them antimicrobial ability, contributing to a better

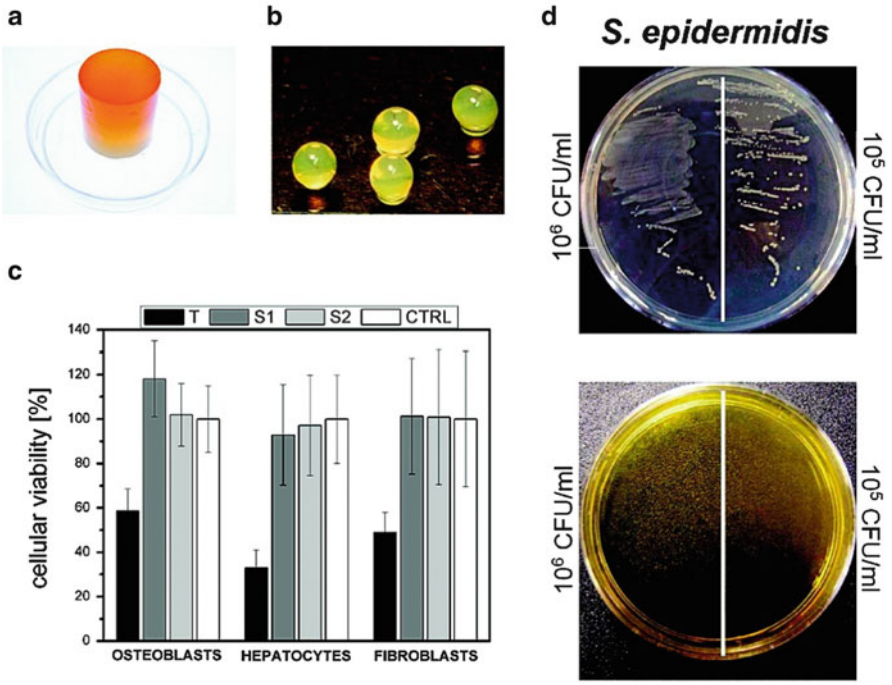


**Fig. 2.13** (**a**) Mixed alginate – chitosan–sugar cylindrical hydrogel containing silver nanoparticles. (**b**) Alginate–silver microspheres. (**c**) Cytotoxicity analysis (MTT assay) on mouse fibroblast (NIH-3T3), human hepatocarcinoma (HepG2), and human osteosarcoma (MG63) cell lines of functional gel microspheres external solutions (S1 and S2, external solutions not diluted and 1:10 diluted, respectively; T, cytotoxicity positive control, cells treated with Triton 1 %; CTRL, cytotoxicity negative control, cells treated with 0.015 M NaCl solution). (**d**) Growth of *S. epidermidis* on 20 % Mueller – Hinton AC gel (*upper Petri dish*) and on 20 % Mueller – Hinton AC-nAg gel (*lower Petri dish*) (Travan et al. 2009)

wider spectrum wound sterilization and healing performance. A recent study evaluated the biological performance of hydrogels based on alginate, chitosan, and hyaluronic acid blends, which were found to enhance tissue generation. Furthermore, in order to obtain a hydrogel not only with a tissue generation enhancement ability but also with an antimicrobial ability to avoid infections, Ce(III) ions were incorporated in one of these hydrogels, and its biological performance was also studied and effectiveness of Ce(III) was demonstrated (Morais et al. 2013).

Fluorinated chitosan hydrogels were shown to be highly effective in wound treatment (Wijekoon et al. 2013). Recently, series of novel, biocompatible hydrogels able to repeatedly take up and deliver oxygen at beneficial levels have been developed by conjugating various perfluorocarbon (PFC) chains to methacrylamide chitosan via Schiff base nucleophilic substitution, followed by photopolymerization to form hydrogels. This new class of fluorinated and biologically derived chitosan materials can be formed into injectable or moldable photo-cross-linked hydrogels

allowing controlling both the capacity and rate of oxygen delivery, providing beneficial oxygen levels for days in a wound (Wijekoon et al. 2013). Since these systems are capable of reloading oxygen more than once, they can be utilized for long periods of time potentially weeks for treatment and cell regeneration. Fibroblast cells were shown to respond favorably to such enhanced oxygen environments even without supplemental oxygen which should directly translate to accelerated wound healing in vivo.

Another material of choice for construction is cyclodextrins (CD) towards biopolymer hydrogels with antimicrobial properties (Glisoni et al. 2013). For instance, two types of hydrophilic networks with conjugated beta-cyclodextrin (β-CD) were recently developed with the aim of engineering useful platforms for the localized release of an antimicrobial 5,6-dimethoxy-1-indanone N4-allyl thiosemicarbazone (TSC) in the soft and moist tissue such as the eye and its potential application in ophthalmic diseases. Poly(2-hydroxyethyl methacrylate) soft contact lenses (SCLs) coated with β-CD, namely, pHEMA-co-β-CD, and superhydrophilic hydrogels (SHHs) of directly cross-linked hydroxypropyl-β-CD were synthesized and characterized regarding their structure (ATR/FT-IR), drug loading capacity, swelling, and in vitro release in artificial lacrimal fluid. Incorporation of TSC to the networks was carried out both during polymerization (DP method) and after synthesis (PP method). The first method led to similar drug loads in all the hydrogels, with minor drug loss during the washing steps to remove unreacted monomers, while the second method evidenced the influence of structural parameters on the loading efficiency (proportion of CD units, mesh size, swelling degree). Both systems provided a controlled TSC release for at least 2 weeks, TSC concentrations (up to 4000 μg/g dry hydrogel) being within an optimal therapeutic window for the antimicrobial ocular treatment. Microbiological tests against *P. aeruginosa* and *S. aureus* confirmed the ability of TSC-loaded pHEMA-co-β-CD network to inhibit bacterial growth as demonstrated in Fig. 2.14.

As we exemplified in this section, hydrogels are playing an increasing role in regenerative medicine and wound care owing to their growing functional sophistication. This is being fortified by advances in hydrogel synthesis, particularly through molecular and genetic engineering, which provide greater control of hydrogel structure and hence the emergence of hydrogels with new functionalities particularly existence of multifunctional aspects such as antimicrobial properties as well as cell proliferation and structural stability. In order to exploit and expand biomedical uses of hydrogels based on biopolymers, it is necessary to fully understand the relationship between hydrogel structure and function. This section is by no means a comprehensive review of such materials but aimed to highlight the key attributes of biopolymer hydrogels that modulate their function, with discussions and examples on the link between these attributes and hydrogel behavior, and identifying possible future applications to elucidate them.

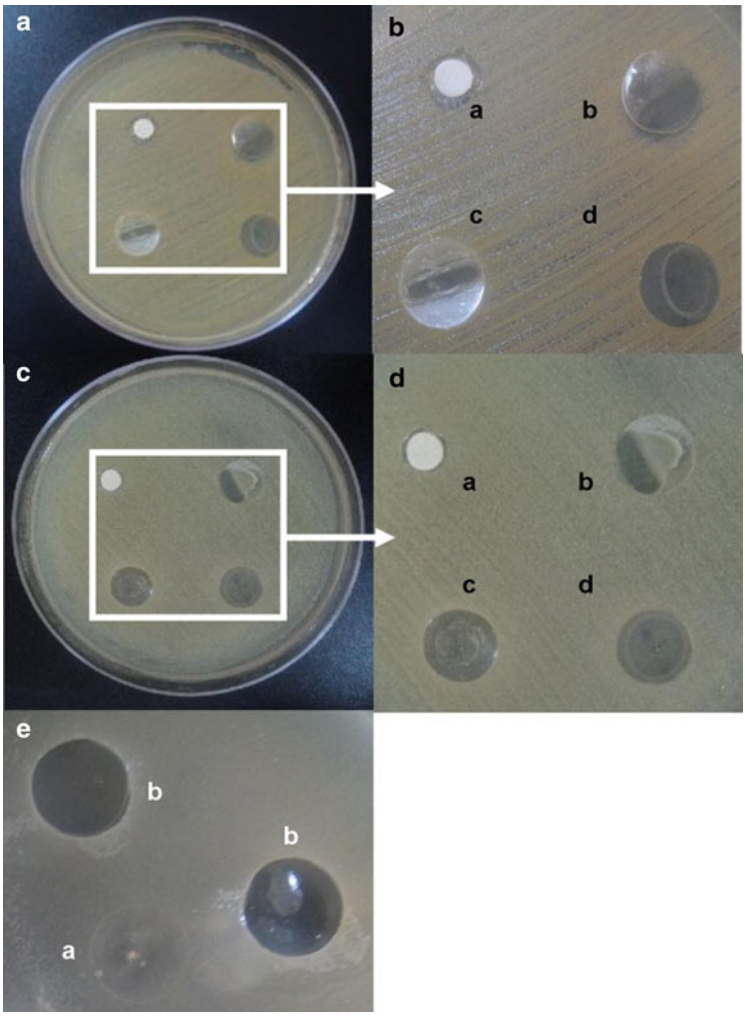


**Fig. 2.14** Antibacterial activity in (**a**, **b**, and **e**) against *S. aureus* ATCC 6538 and (**c** and **d**) against *P. aeruginosa* ATCC 9027 cultures after 24 h. (a) Paper disk loaded with 200 μg of TSC (positive control), (b) SCL without TSC (negative control), (c) TSC-loaded SCL obtained by the PP method with stirring of 24 h and (d) TSC-loaded SCL obtained by the DP method. All the SCLs are pHEMA-co-β-CD with 10 % (w/v) of mono-MA-β-CD. (**b** and **d**) The magnification of (**a**) and (**c**), respectively. (**e**) Bacterial growth beneath the surface of (a) TSC-free and (b) TSC-loaded SCLs (Glisoni et al. 2013)

## 2.6 Fibrous Materials with Antibacterial and Tissue Regenerating Activity for Skin Wound Healing

The use of hydrogel materials described in Sect. 2.5 as wound dressings has some drawbacks, such as swelling and low active surface, that can be overcome using fibrous composite materials. These materials can be conventional fibrous dressings, such as woven cotton, properly modified to attain also antibacterial properties, or nonwoven synthetic or natural polymeric fibers made with conventional methods or by electrospinning for fibers of nanometric dimensions. Such fibers can incorporate various active nutrient or antibacterial and antimicrobial compounds that can aid the wound-healing process. In order to develop fibrous wound dressings, various methods such as dry spinning, wet spinning, spinning with viscose-type spinnerets, or even functionalization of textiles have been presented so far in the literature. In the majority of these approaches, natural polymers were used, like chitin, chitosan (Pillai et al. 2009; Notin et al. 2006), alginates (Qin 2008; Neibert et al. 2012), cellulose, silk fibroin, combination of those (Fan et al. 2005, 2006), or also their combination with synthetic polymers.

Over the last years, these techniques have been progressively replaced by electrospinning. Electrospinning is a highly versatile, effective, easily scalable, and low-cost technique to fabricate ultrathin fibers that cannot be produced with any other technique, with diameters in the submicron and nanometer range depending on the polymeric materials used and the processing conditions. Another advantage of electrospun nanofibrous wound dressing with respect to all the other dressing types is based to the fact that they can mimic the architecture of EMC due to the nanometer scale of the fibers' diameter and to their overall nanotopography. As reported above, wound healing is a complex and dynamic process of restoring cellular structures and tissue layers through interactions of cells, growth factor, and EMC (Calne 2011). The biological functionality of EMC has not yet being fully reproduced in wound dressing materials, possibly due to its complexity and multifunctionality. Indeed, the EMC is the main constituent of the dermal skin layer containing proteoglycans, collagen, hyaluronic acid, fibronectin, and elastin, all components essential for skin regeneration. A recent review on electrospun materials used for wound healing makes a very complete synopsis on the activity of the EMC components during the wound-healing process (Rieger et al. 2013). Nevertheless, although the complete biological activity of the EMC is not replicated, the use of specific polymers in combination with the particular topography of the electrospun mats can provide to the tissues the ideal environment to promote wound healing. By loading them with active principles present in the EMC, exactly like in the case of the bioactivated cell-instructive scaffolds described in Sect. 2.3, the tissue regeneration activity can be greatly assisted when such active dressings are put in the proximity of the wound. In tissue engineering, electrospun mats were proved ideal scaffolds for cells adhesion, growth, proliferation, and differentiation (Moffa et al. 2013; Polini et al. 2013). Here, we focus on the research efforts made in the field of active wound dressings.

In the electrospinning technique, a high electric field is applied in order to create fibers with a diameter ranging from a few nanometers to larger than 1 μm. A basic electrospinning apparatus consists of a syringe filled with the target polymer solution, a syringe pump, a high voltage supply, and a collector. The metallic needle of the syringe serves as electrode to induce electrical charges within the solution, under the influence of a strong electrostatic field. When the charge repulsion overcomes the surface tension of the polymeric solution, a charged polymeric jet is formed and is accelerated towards the collector. During the flight of the jet, the solvent evaporates and polymeric nanofibers are collected.

The electrospun fiber mats have high surface area, much higher than films of hydrogel materials or even that other fibrous dressing made in conventional ways. In this way, that can assure optimized exude absorption, moisture permeation, and gas transport (Zhang et al. 2005). On the top, this technique is highly versatile in terms of used materials. Indeed, electrospun fibers can be made of natural or synthetic polymers or different combinations of both. Finally, the electrospun mats can attain different functionalities by tuning the different polymer concentrations and by incorporating in the nanofibers different drugs, active biological molecules, antibacterial agents, etc.

## 2.7 Electrospun Mats Without Active Agents

Natural polymers, such as polysaccharides and proteins are the most common electrospun materials used for treatment of skin wounds due to their inherent properties that assist the process of healing. In the majority of the cases, they are used in combination with synthetic polymers due to their intrinsic low processability (e.g., poor solubility and high surface tension) (Lee et al. 2009) but also in order to enhance the mechanical properties and tune the morphological features of the produced mats. In particular, the polysaccharide chitosan has demonstrated intrinsic hemostatic and antibacterial properties, and for this reason many research works have been focused on its electrospinning. Since it cannot be electrospun alone, it is used in combination with other polymers. For example, an electrospun matrix of chitosan, collagen, and polyethylene oxide was fabricated followed by further cross-linking using glutaraldehyde vapor. Animal studies showed increased wound-healing rate using this matrix as wound dressing compared to gauzes and commercial collagen sponges (Chen et al. 2008). Also the electrospun combination of chitosan and silk fibroin has demonstrated good antibacterial activity and biocompatibility using murine fibroblasts. Although only in vitro tests were performed, the results suggest that such composite nanofibrous membranes can be used in wound healing (Cai et al. 2010). A successful combination of electrospun nanofibers includes chitosan, hydroxybenzotriazole, and polyvinyl alcohol blend. This underwent cytotoxicity tests and was found nontoxic to human fibroblast cells, suggesting its suitability as wound dressing material (Charernsriwilaiwat et al. 2010). A natural polysaccharide that has shown great potentiality for the

regeneration of tissues is the alginate, but few works have been done on its electrospinning since it easily forms fibers using the wet spinning technique of sodium alginate in a calcium salt aqueous solution. Uniform electrospun nanofibers were obtained by a blend of a cell adhesive peptide modified alginate, unmodified alginate, and PEO and demonstrated good human dermal fibroblast cells adhesion (Jeong et al. 2010). Another study has better demonstrated the potentiality of blended PVA-sodium alginate electrospun nanofibrous mats as wound dressings by in vivo experiments. The healing performances of wounds using the prepared electrospun dressings were compared with commercially available dressings with promising results (Üstündağ et al. 2010). A polysaccharide that is the main component of the natural extracellular matrix in connective tissues is the hyaluronic acid and as such is expected to play an important role in the wound-healing process. Indeed, electrospun mats of hyaluronic acid compared in a preclinical study with five commercial gauzes and antibiotic dressings showed increased performances in the healing of wounds (Uppal et al. 2011). Among the natural protein polymers that have been electrospun for wound dressing applications, collagen is possibly the most popular since it is an important extracellular matrix component that promotes wound healing. Electrospun membranes of polylactide–polyglycolide/collagen were found to be very effective as wound-healing accelerators of open wounds in rats especially in the early-stage healing (Liu et al. 2010a). The effects of polycaprolactone/collagen electrospun nanofibers in aligned and random arrangements on phenotypic expression of human adipose stromal cells in vitro were studied recently. The cells demonstrated higher synthesis capacity for critical extracellular matrix molecules in the aligned nanofibers, demonstrating the potentiality of the latter for accelerated wound repair (Xiaoling and Wang 2012). Electrospun nanofibrous membranes of modified polycaprolactone–collagen were found suitable for the attachment and proliferation of fibroblast, suggesting the potential to be used for the treatment of skin defects and burn wounds (Venugopal et al. 2006). Finally, electrospun silk has been evaluated in terms of conformational and biocompatible characteristics related to wound dressings. Six distinct electrospun silk material groups in the hydrated state exhibited absorption, water vapor transmission, oxygen permeation, and enzymatic biodegradation, essential characteristics for dressings of wounds. In the dry state, three of the electrospun silk materials were found to be the best potential candidates for wound dressings (Wharram et al. 2010). Another protein, the gelatin has been electrospun with poly(vinyl alcohol) starting from their aqueous solutions. The produced nanofibers fibers were subsequently cross-linked with glutaraldehyde vapor and heat treated. Due to the cytocompatibility of the mats, proved through test in vitro with fibroblasts, they were suggested as potential wound dressings (Yang et al. 2011). Blends of low-molecular-weight fish scale collagen peptides and chito-oligosaccharide with polyvinyl alcohol were electrospun to form nanofibrous membranes. The membranes showed good antibacterial activity especially against gram-positive *Staphylococcus aureus* and a bit less against gram-negative Escherichia coli, indicating that the membranes released intracellular materials, particularly with *S. aureus*. The electrospun membranes showed also good biocompatibility using

in vitro measurements with human skin fibroblasts. The authors claimed that low-molecular-weight fish scale collagen peptides are superior to mammalian collagen for wound repairing (Wang et al. 2011b). Finally, electrospun fibrinogen nanofibers were proposed as wound dressing, since fibrinogen is a protein present in the blood plasma with important role in wound healing (Wnek et al. 2003).

## 2.8 Electrospun Mats Loaded with Active Agents

In the skin wound healing, active agents are considered substances that intervene in the course of one or more phases of the process facilitating its finalization. A variety of electrospun mats loaded with active agents have been proposed for topical antimicrobial, drug, antibiotic, or bioactive molecules delivery.

Loading in the course of electrospinning Silk–PEO electrospun mats containing epidermal growth factor were fabricated, from a common solution, for the promotion of wound-healing processes. The incorporated epidermal growth factor was slowly released (25 % release in 170 h). Using a human three-dimensional model, the authors demonstrated that the biofunctionalized silk mats, when used as dressings, aid the healing of wounds by increasing the time of wound closure by the epidermal tongue by 90 %. On the top, the mats were preserving their structural integrity during the healing time (Schneider et al. 2009). Among the wound dressing fibrous materials, the ones that contain silver nanoparticles for antibacterial activity are quite popular. In a work on gelatin nanofibers, silver nanoparticles were formed in situ in the gelatin solution starting from their $AgNO_3$ precursor at least 12 h after the preparation of the solution, with the amount of nanoparticles increasing with increasing time. Electrospinning of the nanoparticles-containing solutions lead to nanocomposite fibers that were further cross-linked with moist glutaraldehyde vapor to improve their stability in an aqueous medium. The fibrous mats showed good antibacterial activity with decreasing strength against *Pseudomonas aeruginosa*, followed by *Staphylococcus aureus*, *Escherichia coli*, and methicillin-resistant *S. aureus* (Rujitanaroj et al. 2008). In another work, silver nanoparticles were synthesized in situ in the spinning formic acid solution of chitosan or *N*-carboxyethyl chitosan and PEO. The nanoparticles were uniformly dispersed in the nanofibers, and 15 % wt. of them was decorating the fibers' surface. The composite electrospun mats were proposed for antibacterial wound dressing materials (Penchev et al. 2009). Instead of silver, also $TiO_2$ nanoparticles have been used as antibacterial fillers. Indeed, in situ generated $TiO_2$ in electrospun polyurethane fibers was efficient against *Ps. aeruginosa* and *S. aureus*. The membranes also showed water vapor transmission and immediate adherence to L929 cells, all properties essential for wound dressing applications (Yan et al. 2011). In other research works, plant extracts have been used as fillers in electrospun nanofibers either to promote cell proliferation or to induce an antibacterial activity to the developed dressings. In particular, cellulose acetate fiber mats containing either asiaticoside (from the plant *Centella asiatica*) or

curcumin (from the plant *Curcuma longa* L.) were successfully prepared. Normal human dermal fibroblasts were attached and proliferate better on the electrospun mats when asiaticoside was included, whereas the presence of curcumin imparted their antioxidant activity (Suwantong et al. 2010). Moreover, the crude bark extract of the plant *Tecomella undulate* was loaded in PCL/PVP electrospun fibers that were found to inhibit the growth of *P. aeruginosa*, *S. aureus*, and *E. coli* (Suganya et al. 2011). Lysozyme, an enzyme found in abundance in egg white and a natural form of protection from gram-positive pathogens, was used as an additive in electrospun mats of chitosan–ethylenediaminetetraacetic acid and polyvinyl alcohol. The rate of wound healing of the composite mats was found to be accelerated compared to gauze controls, in experiments performed in vivo using male Wistar rats, indicating that lysozyme-loaded nanofibers have a potential for wound healing (Charernsriwilaiwat et al. 2012). Furthermore antibiotics were successfully electrospun in combination with the right polymers. In particular, electrospun nanofibrous membranes of PEG–PLA incorporating the hydrophilic antibiotic drug, tetracycline hydrochloride, were found to preserve the bioactivity. The antibiotic was released over 6 days and was found to be effective in inhibiting growth of *S. aureus*. Such a local sustained delivery of antibiotics makes these membranes promising as wound dressings for ulcers caused by diabetes or other diseases (Xu et al. 2010). Finally, the group of Xiaohong Li has used emulsion electrospinning to embed fibroblast growth factor into ultrafine poly(ethylene glycol)-based fibers with a core–sheath structure to promote the wound-healing process. In vivo tests in the dorsal area of diabetic rats showed that the gradual growth factor release increased the wound recovery rate with improved vascularization, enhanced collagen deposition and maturation, complete re-epithelialization, and formation of skin appendages. The authors suggest the use of such electrospun fibrous mats to accelerate the healing of diabetic skin ulcers (Yang et al. 2012).

In few cases of electrospun mats developed for wound dressings, the active agents are loaded to the fibers after the mats preparation, a method that has given also promising results. As an example, we mention silk fibroin mats that were prepared by electrospinning and subsequently coated with silver nanoparticles. The composite mats were fabricated as prototypic wound dressings and demonstrated good antimicrobial properties against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Uttayarat et al. 2012). Another example of postproduction functionalization is the loading of a cationic drug neomycin onto the cationic exchange nanofibers of poly(styrene sulfonic acid-co-maleic acid) and polyvinyl alcohol. Prior to loading, the fibers were subjected to thermal cross-linking to produce ion exchange nanofiber mats. In vivo, wound-healing tests performed in Wistar rats revealed that the functionalized mats decreased the acute wound size during the first week after tissue damage better than gauze and blank nanofiber mats. On the top, neomycin-loaded nanofiber mats demonstrated satisfactory antibacterial activity against both gram-positive and gram-negative bacteria (Nitanan et al. 2013).

## 2.9 Multicomponent Electrospun Mats

The ideal wound dressing materials should follow the different phases of the wound-healing process by providing to the wound the right substances at the right time in order to optimize the wound-healing processes and times. A few works have demonstrated the use of multicomponent electrospun mats, for a simultaneous or a stepwise release of the active agents in specific stages of the wound-healing process. In particular, composite electrospun mats of poly(lactic-co-glycolic acid) with mesoporous silica nanoparticles were used for the co-encapsulation and prolonged simultaneous release of the hydrophilic model drug rhodamine B and the hydrophobic model drug fluorescein. The codrug delivery system can be very useful for wound dressings that require combined therapy of several kinds of drugs (Song et al. 2012a). Further work of the group on the same system showed that the release of the two drugs can be monitored separately. Most of the fluorescein was released rapidly during the 324 h of the trial, but the rhodamine B showed a sustained release behavior (Song et al. 2012b).

Multicomponent systems can be also considered all the core–shell electrospun fibers. Especially for wound dressing applications, the use of electrospun membranes that consist of core–shell fibers is gaining increasing interest. To prepare such fibers using electrospinning, two different polymers can be separately delivered to the inner and outer channel of a coaxial-tube spinneret. Different active agents can be loaded to the core and to the shell of the fibers in order to obtain their sustainable delivery to the wound. Wang et al. used poly(DL-lactic acid) and poly (3-hydroxybutyrate), two biodegradable polymers, for the production of core–shell nanofibers with the possibility to swap the material for the core and the shell. Using poly(3-hydroxybutyrate) as the shell, the loaded dimethyloxalylglycine drug could be released in a controllable manner. Whereas the single component fibers showed an immediate release, the core–shell fibers showed two-stage release kinetics when the drug was embedded in the core. The amount released in the first stage was 25 % within 60 h, independent from the shell thickness. In the second stage, the release rate was controlled by the thickness of the shell and was linear (Wang et al. 2010). Another very recent work demonstrated the use of core–shell nanofibers of gelatin and poly(L-lactic acid)-co-poly-(ε-caprolactone) to encapsulate multiple epidermal induction factors such as the epidermal growth factor, insulin, hydrocortisone, and retinoic acid. When the same fibers were blend spun, an initial 44.9 % burst release of the active agents was observed during the first 15 days, whereas no burst release was detected from the core–shell nanofibers. Moreover, the proliferation and differentiation to epidermal lineages of stem cells on the core–shell nanofibers were higher with respect to the blended fibers (Jin et al. 2013). In a similar way, the antibiotic gentamicin was encapsulated in coaxial fibers containing a skin of PLA and a core of collagen using electrospinning in order to provide to wounds a strong and time-controllable antibacterial release (Torres-Giner et al. 2012).

## 2.10 A Special Focus on Antibacterial Silver Nanoparticles

Infectious diseases by human pathogens have been considered one of the first causes of mortality and disability since the last century. The discovery and global commercialization of antibiotics in the second half of the twentieth century was a milestone of modern medicine. However, together with the development of effective antibacterial drugs, the issue of antimicrobial resistance is also raising concerns worldwide, due to an almost indiscriminate abuse in the last decades (Powers 2004; Spellberg 2008; Spellberg et al. 2008; Morens et al. 2010). Bacteria, in fact, may rapidly evolve specific molecular determinants able to interact with the drug in an unpredictable way, leading to its inactivation, degradation, or expulsion (Andersson and Hughes 2010; Schwaber et al. 2004; Levy and Marshall 2004). As a consequence, several active molecules discovered in the last decades are now rather inadequate also for the treatment of pathologies commonly considered as weakly hazardous. Thus, new effective solutions are required through innovative, multidisciplinary approaches, which should include the design and development of new antibacterial compounds meeting the requirements of low cost of production, specificity, and long-term efficacy (to avoid the significant limitation of bacterial resistance to classical drugs). In this regard, nanotechnology may provide some previously unexplored methods and techniques to develop innovative antimicrobial drugs and devices. In particular, silver-based nanomaterials in the form of colloidal nanoparticles (AgNPs) are emerging as promising candidates for the next generation of systemic drugs, thanks to broad-spectrum efficacy and their intrinsic ability to reach even very peripheral body districts and to cross biological barriers. On the other side, nanoengineered silver-based nanocomposites are increasingly explored for the realization of safe intracorporeal implants to avoid the formation of localized infections.

Although silver has been considered a "poisoning metal" for microorganisms since antiquities (Liau et al. 1997; Klasen 2000a, b), the current advancement of nanotechnology is enabling the realization of different types of AgNPs and silver nanocomposites with high controlled and tuned physicochemical characteristics at nanoscale level (e.g., in terms of size, shape, and surface chemistry) (Dahl et al. 2007). Herein, we aim to provide to the readers the most recent knowledge about the use of AgNPs as antimicrobial agent, a topic that is increasingly attracting great interest, as also confirmed by the annual worldwide production of nanosilver of more than 300 tons (Nowack et al. 2011; Kumar et al. 2008). In particular, we review the biocidal effects of AgNPs, with a special focus on both the advantages and open issues rising in this topic and on the molecular mechanisms of nanosilver action. In addition, we discuss the limits and drawbacks in the methods exploited for the antibacterial tests, providing useful guidelines for the design of efficient antibacterial nanosystems. Although there is a huge and increasing number of studies available on this subject (Eckhardt et al. 2013; Chernousova and Epple 2013; Hajipour et al. 2012; Lemire et al. 2013), it should be considered that literature data are rather contrasting (especially regarding the role played by the

physicochemical properties of NPs and the actual dose), mainly because of the general lack of standardized nanomaterials and assays employed for characterizing the biocidal effects.

The effect of the physicochemical properties of AgNPs (e.g., size, shape, and surface chemistry) on their antimicrobial activity is discussed next. Concerning the size, smaller AgNPs demonstrate a stronger bactericidal activity compared to bigger particles. For instance, Choi and collaborator demonstrated that AgNPs in the range of 5–20 nm are more effective compared to bigger NPs, to AgCl (in the form of colloids), and to free $Ag^+$ ions (from a silver salt) (Choi and Hu 2008). In this case, the toxicity effects have been related to production of reactive oxygen species (ROS) combined to strong membrane damage. This was later confirmed by Sondi et al. (Sondi and Salopek-Sondi 2004). The strong efficiency of small AgNPs was also demonstrated by other works, who reported that AgNPs with a diameter of c.a. 10 nm have a stronger tendency to bind the membrane of gram-negative bacteria, as compared to bigger nanoparticles, thus leading to a more pronounced damage (Morones et al. 2005). The authors also stated that the release of $Ag^+$ ions from the particles surface represents a major contribution to the overall bactericidal effects.

Apart from size, also surface charge has been demonstrated to have an important role. In particular, positively charged AgNPs were found to elicit a strong activity against microorganisms, while negatively charged particles were less toxic (El Badawy et al. 2011). This behavior was explained in terms of electrostatic interactions between the negative membrane of bacteria and the positive charge covering the surface of particles. In particular, the electrostatic interactions may increase the dose of silver in the close proximity of microorganisms.

With respect to the AgNPs shape, truncated triangular silver nanoplates, with a (111) lattice plane, were observed to elicit a strong antibacterial activity, compared to both rod- and spherical-shaped AgNPs and to $Ag^+$. However, despite these experimental data highlighted a direct correlation between the NPs shape and the biological outcomes, a crucial role was again ascribed to the NPs surface charge, since truncated silver nanoplates had a positive charge (Pal et al. 2007). The above studies concluded that the toxicity mechanisms may be related to both AgNPs and $Ag^+$ ions released from their surfaces, though not providing a definite conclusion. Only recently, an elegant work solved this problem, demonstrating that the bactericidal effects are mainly due to the silver ions (Xiu et al. 2012). The authors fabricated AgNPs of ~5 and ~11 nm and stored them under anaerobic conditions, where the release of $Ag^+$ is completely prevented (Liu et al. 2010b; Liu and Hurt 2010). Interestingly, the viability assays on *E. coli* showed that AgNPs have no detectable effects under anaerobic conditions (in which there is no $Ag^+$ release), also using NPs concentrations higher than the minimum lethal concentration (MLC). On the other side, incubation of *E. coli* and AgNPs under aerobic conditions showed significant toxicity. It is thus evident that the physicochemical characteristics of AgNPs do not play a crucial role in determining the toxicity, rather than are important in terms of influencing the rate of $Ag^+$ release from the nanoparticle surfaces. For instance, the specific surface area (per mass unit) is higher in

smaller AgNPs, thus allowing a higher rate of silver ion release. There are several other works that explored the bactericidal effects of AgNPs (Smetana et al. 2008; Panacek et al. 2006, 2009; Vertelov et al. 2008; Kim et al. 2008b, 2009; Navarro et al. 2008). However, it should be considered that there is a general level of data disagreement, especially regarding the final dose of AgNPs required for eliciting a strong bactericidal effect, the preferential molecular targets of NPs, and the real molecular mechanisms underlying toxicity.

The most acknowledged theory regarding the bactericidal effect of silver indicates a mechanism of direct membrane damage, due to chemical interaction between $Ag^+$ and bacterial membrane proteins. In particular, $Ag^+$ is a soft cation and, according to the hard–soft acid–base theory (HSAB) of Pearson, it may strongly bind soft ligand, such as the sulfur groups of proteins. In addition, other coordination complexes have been proposed between $Ag^+$ and all the different amino acids, in which the binding affinity was theoretically and experimentally calculated (Nomiya et al. 2000; Jover et al. 2008, 2009; Kasuga et al. 2012). As a consequence of the interaction event, bacterial membrane may undergo a general loss of function, especially regarding the impairment of the respiratory chain, followed by dissipation of proton motive force and ATP production, and increased permeability which does not allow the membrane to compensate the external osmotic pressure (Eckhardt et al. 2013; Dibrov et al. 2002). The decrease in ATP level, combined with membrane loss of activity, may then generate further metabolic concerns, especially for crucial enzyme-dependent metabolic pathways. Additionally, another possible mechanism of membrane damage-related toxicity includes the formation of breaks or pits (Li et al. 2011; Mirzajani et al. 2011). In particular, silver ions have been proposed to destroy the $\beta - 1 \rightarrow 4$ glycosidic bonds connecting the main building blocks of the peptidoglycan, namely, the *N*-acetylglucosamine and *N*-acetylmuramic acid, which are consequently released into the media (Mirzajani et al. 2011). In this scenario, positively charged AgNPs situated in close proximity to the cell membrane may be also subjected to a strong pH decrease (down to values of 3, due to the bacterial proton motive force) that, in turn, might promote localized $Ag^+$ release, which further increases the NPs toxicity.

Following membrane damage/poration, external $Ag^+$ and even AgNPs (although this latter occurrence has not been clearly demonstrated) may gain direct access to the cytosol, where silver ions may induce further damage. Upon reaching the cytosol, $Ag^+$ ions may interact with a number of important enzymes, unfolding them and decreasing, for instance, the enzymatic activity of the respiratory chain dehydrogenase (Li et al. 2011). Moreover, $Ag^+$ ions can interfere with the enzymes by replacing their native metal cation from the binding site (e.g., in the case of metalloproteins). Silver ions may also strongly bind DNA, with a preferential binding site to guanine N7 and adenine N7 (Arakawa et al. 2001). This may lead, in turn, to inaccurate DNA condensation, as well as errors in DNA replication and transcriptions, that may cause random mutations.

All these considerations assume that $Ag^+$ directly induces a specific damage, due to physical/chemical interaction events. On the other side, silver ions may also

lead to indirect damages by means of reactive oxygen species (ROS) production. In this respect, singlet oxygen, hydrogen peroxide, superoxide radical anion, and hydroxyl radical are known to target lipids, DNA, RNA, and proteins, causing severe effects, including malfunction of membranes, proteins, and DNA replication machinery (Cabiscol et al. 2000). The issue of $Ag^+$ -related ROS production remains, however, quite controversial, as some research works addressed strong correlation (Choi and Hu 2008; Inoue et al. 2002; Hwang et al. 2008), while other experimental data displayed no significant trends (Sintubin et al. 2011; Xiu et al. 2011). This is likely due to the ability of microorganisms to resist oxidative stress by adopting several molecular strategies, which include direct immediate detoxification carried out by enzymes (i.e., catalase, superoxide dismutase, and peroxidase) (Fang 2004) and a long-term detoxification controlled by a transcriptional expression of several proteins (including OxyR, SoxRS, and PerR). These strategies enable bacteria a high survival probability against ROS-related stress.

However, it should be considered that a detailed and universal description of the antibacterial mechanisms of AgNPs is still not available, also due to general lack of standardized materials and protocols to be employed for the assays. In particular, the synthesis of high-quality AgNPs, in terms of narrow size and shape distribution, remained a challenge for several years, and only in the recent years some good results were achieved (Burda et al. 2005; Wennemers 2012; Liang et al. 2010; Belser et al. 2009; Upert et al. 2012). A typical reaction is governed, in fact, by different thermodynamic factors. Capturing the distinct stages of a controlled atomic nucleation around few atoms represented a serious challenge, which has been only solved recently. However, most of the data available to date about the bactericidal properties of AgNPs have been obtained with particles having almost uncontrolled physicochemical properties or particles that were not characterized. Together with the absence of an analytical approach for particles characterization and testing, this hindered the possibility to have a confident explanation of the various phenomena. In this respect, an important point is that AgNPs should be characterized by means of different techniques (e.g., dynamic light scattering, UV-visible spectroscopy, transmission electron, and/or scanning electron microscopy), both in aqueous medium and after incubation in the bacterial culture medium. The specific components of the media may, in fact, interact with the particle surface (e.g., forming a protein corona), significantly changing their original physicochemical properties and, consequently, also the observed biological outcomes (Walczyk et al. 2010; Monopoli et al. 2011a, b). In particular, the colloidal stability of AgNPs in biological growth media is an important parameter to keep under control: NPs may form aggregates/agglomerates and precipitates, consequently compromising the effective dose of silver and, also, the NPs efficacy. Furthermore, also the medium proteins and salts may bind free $Ag^+$ (released from the AgNPs surface), reducing the overall final dose available.

Another point is the kinetic of silver oxidation that may be strongly affected by the specific medium used. In this latter case, a correct procedure includes the use of different methods for quantifying, in situ, the $Ag^+$ release from the NPs surface. For instance, the inductively coupled plasma spectrometry-based techniques (i.e.,

ICP-OES and ICP-MS, which are rather sensitive but require physical separation of $Ag^+$ from AgNPs) and UV–Vis analyses (having the advantage of correlating the decrease of AgNPs surface plasmon absorption band with NPs dissolution, even in complex media) (Zook et al. 2011) can be both useful to address the $Ag^+$ release.

From the above considerations, the same batch of AgNPs may behave in a completely different way when tested in different media or at different aging. Finally, most of the available commercial kits used to address the viability of bacteria, upon AgNPs treatment, are based on the use of fluorescent/colorimetric probes. In this latter case, the probe itself may directly interact with the NPs, providing false-positive or false-negative results, and AgNPs may directly interfere with the optical readout of some assays (such as in the case of bacterial viability assays). It is thus evident that a standardized method to study the interactions of NPs with bacteria is still far to be accomplished, and that future efforts should be strongly focused in such direction.

As a final point, we would like to drive the reader's attention to some of the advantages and disadvantages of the use of AgNPs or $Ag^+$ for fabricating effective antibacterial devices. In particular, while a classical laboratory test in solution will indicate that silver salts are significantly more effective than AgNPs, these latter represent a "pool" of $Ag^+$ ions that can be finely engineered/functionalized with specific targeting molecules, in order to reach a specific body compartment. Moreover, NPs possess an intrinsic Trojan horse effect, which enable them to cross biological membranes and barriers (for instance, allowing an abundant cellular uptake). In this respect, NPs are ideal candidates for defeating intracellular pathogen-related infections, where microorganisms proliferate within host cells, hiding from both standard antibiotics and host immune system. In addition, AgNPs may offer the characteristic of localized and controlled long-term release, since they can be finely engineered in order to control the kinetics of $Ag^+$ oxidation from their surface. This topic is of crucial importance in applications such as chronic infections, medical devices, and wound healing. Nevertheless, AgNPs are not probably the best choice for the treatment of acute infections, since an immediate release of $Ag^+$ is not feasible at physiologic conditions. On the other side, silver ions may be ideal candidates for fast defeating a bacterial colony, due to the high immediate dose accessible (though silver ions are not able to cross biological membranes and have a poor targeting efficiency). Moreover, unlike the laboratory model experiments (usually carried out in solution), for in vivo assays, NPs typically lead to higher effective dose as compared to silver ions.

The data available on the bactericidal effects of AgNPs represent a good chance, for pharmaceutical companies, to develop a new category of antibiotic compounds. However, several issues should be considered. First, the capability of finely controlling the $Ag^+$ release from the particles surface is a fundamental topic to be addressed, especially regarding the possibility of long-term release. Second, research tests should be based on standardized assays, reference materials, and specific SOPs (standard operating procedures, e.g., for NPS characterization and dispersion). Third, the indiscriminate use of AgNPs may lead to several worrying effects, including the possibility of enhancing the bacterial silver resistance. In this

respect, it has been demonstrated that some particular strains of *E. coli* and *Salmonella* spp. already possess a peculiar operon, named *sil*, encoding for different proteins responsible for silver resistance (Gupta et al. 1998, 1999, 2001). In particular, the *sil* gene cluster codifies for periplasmic silver-binding proteins and molecular efflux pumps, which work in cooperation for expelling $Ag^+$ ions from the cytoplasm (or even the periplasmic space) to the extracellular space. Finally, the uncontrolled environmental release of silver (in the form of bulk, $Ag^+$, and AgNPs) is increasing the chance of exposure to humans (with unpredictable toxicity consequences), as well as it represents a serious risk from an ecological viewpoint, a topic that will be discussed with more details in the following paragraph.

### *2.10.1 Implications for the Environment and Human Risk Exposure*

The environmental release of silver, in all its forms (i.e., ions, nanoparticles, and clusters), is constantly rising, and it is actually quantified to be c.a. 20 tons per year (Gottschalk et al. 2009). Hence, several research efforts aimed to understand the potential ecological consequences of silver release, in terms of investigating the toxic effects to the different organisms populating specific ecosystems. Also in this case, there is a significant data disagreement, since several works labeled nanosilver as a potential polluting agent, while other data considered it as negligible and not dangerous (Hansen and Baun 2012; Grieger et al. 2012; Blaser et al. 2008; Musee et al. 2011; Nowack et al. 2012). For instance, the release of silver in the soil has been proved to induce a dramatic decrease in the reproduction potential of the nematode *Caenorhabditis elegans* as a consequence of increased oxidative stress (Roh et al. 2009). Other environmental model organisms, such as the green alga *Chlamydomonas reinhardtii* or *Danio rerio*, displayed toxicity effects upon AgNPs treatments (Navarro et al. 2008; Asharani et al. 2008), suggesting that nanosilver is a potential pollutant. However, it should be highlighted that the ecotoxicology assays are usually performed by means of model experiments (i.e., in laboratory), which are not similar to real conditions. Here, in fact, the physicochemical characteristics of silver are quite unpredictable, in terms of particles size, shape, and agglomeration state. Hence, understanding the real effects of nanosilver on a specific fauna could represent, most probably, an extremely difficult challenge, due to the high and complex variables characterizing the system.

The rise in environmental presence of AgNPs is also increasing the possibility of human risk exposure. For this reason, many studies focused on exploring the potential adverse effects of nanosilver on eukaryotes (nanotoxicity assessment) (Christensen et al. 2010; Ahamed et al. 2010). Inhalation of vapors, aerosols, or particulates and oral or skin adsorption are the major routes of entry of silver compounds into the body. Upon inhalation, AgNPs may deposit in the respiratory tract, causing damage through direct contact with tissues. Then, they can reach the

bloodstream and diffuse throughout all the central and peripheral body districts, causing extensive toxicity to different organs (Sue et al. 2001; Wadhera and Fung 2005; Takenaka et al. 2001). Several data indicate that AgNPs may be the cause of DNA damage and apoptosis in fibroblasts and liver cells and lead to cell death and oxidative stress in human skin carcinoma and fibrosarcoma cells (Arora et al. 2008, 2012). Also for eukaryotic cell lines, some molecular mechanisms of AgNPs action have been proposed and include increased LDH outflow, misregulation of GSH-related detoxification, reduced mitochondrial function, apoptosis, DNA fragmentation, ROS generation, and metallothionein sequestration (Arora et al. 2008; Hussain et al. 2005, 2006; Braydich-Stolle et al. 2005; Hsin et al. 2008; Ahamed et al. 2008; Park et al. 2010). It should be mentioned, as in the case of the low reproducibility of AgNPs bactericidal assays, that the data on eukaryotic toxicity are not conclusive, due to similar limitations of the lack of NP reference materials and standardized protocols for the tests, which hindered to achieve a correct risk assessment of AgNPs.

## References

Ahamed M, Karns M, Goodson M, Rowe J, Hussain SM, Schlager JJ, Hong Y (2008) DNA damage response to different surface chemistry of silver nanoparticles in mammalian cells. Toxicol Appl Pharmacol 233(3):404–410

Ahamed M, Alsalhi MS, Siddiqui MK (2010) Silver nanoparticle applications and human health. Clin Chim Acta 411(23–24):1841–1848

Andersson DI, Hughes D (2010) Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol 8(4):260–271

Arakawa H, Neault JF, Tajmir-Riahi HA (2001) Silver(I) complexes with DNA and RNA studied by Fourier transform infrared spectroscopy and capillary electrophoresis. Biophys J 81(3): 1580–1587

Arora S, Jain J, Rajwade JM, Paknikar KM (2008) Cellular responses induced by silver nanoparticles: in vitro studies. Toxicol Lett 179(2):93–100

Arora S, Rajwade JM, Paknikar KM (2012) Nanotoxicology and in vitro studies: the need of the hour. Toxicol Appl Pharmacol 258(2):151–165

Asharani PV, Lian Wu Y, Gong Z, Valiyaveettil S (2008) Toxicity of silver nanoparticles in zebrafish models. Nanotechnology 19(25):255102

Athanassiou A, Lygeraki MI, Pisignano D, Lakiotaki K, Varda M, Mele E, Fotakis C, Cingolani R, Anastasiadis SH (2006) Photocontrolled variations in the wetting capability of photochromic polymers enhanced by surface nanostructuring. Langmuir 22:2329

Bacakova L, Filova E, Parizek M, Ruml T, Svorcik V (2011) Modulation of cell adhesion, proliferation and differentiation on materials designed for body implants. Biotechnol Adv 29:739

Battista S, Guarnieri D, Borselli C, Zeppetelli S, Borzacchiello A, Mayol L, Gerbasio D, Keene DR, Ambrosio L, Netti PA (2005) The effect of matrix composition of 3D constructs on embryonic stem cell differentiation. Biomaterials 26:6194

Bayer IS, Fragouli D, Martorana PJ, Martiradonna L, Cingolani R, Athanassiou A (2011) Solvent resistant superhydrophobic films from self-emulsifying carnauba wax–alcohol emulsions. Soft Matter 7:7939

Beke S, Anjum F, Tsushima H, Ceseracciu L, Chieregatti E, Diaspro A, Athanassiou A, Brandi F (2012) Towards excimer-laser-based stereolithography: a rapid process to fabricate rigid biodegradable photopolymer scaffolds. J R Soc Interface 9:3017

Beke S, Anjum F, Ceseracciu L, Romano I, Athanassiou A, Diaspro A, Brandi F (2013) Rapid fabrication of rigid biodegradable scaffolds by excimer laser mask projection technique: a comparison between 248 and 308 nm. Laser Phys 23:035602

Belser K, Vig Slenters T, Pfumbidzai C, Upert G, Mirolo L, Fromm KM, Wennemers H (2009) Silver nanoparticle formation in different sizes induced by peptides identified within split-and-mix libraries. Angew Chem Int Ed Engl 48(20):3661–3664

Beniash E, Hartgerink JD, Storrie H, Stendahl JC, Stupp SI (2005) Self-assembling peptide amphiphile nanofiber matrices for cell entrapment. Acta Biomater 1(4):387–397

Best JP, Javed S, Richardson JJ, Cho KL, Kamphuis MMJ, Caruso F (2013) Stiffness-mediated adhesion of cervical cancer cells to soft hydrogel films. Soft Matter 9:4580

Bhattarai N, Gunn J, Zhang M (2010) Chitosanbased hydrogels for controlled, localized drug delivery. Adv Drug Deliv Rev 62(1):83–99

Biondi M, Ungaro F, Quaglia F, Netti PA (2008) Controlled drug delivery in tissue engineering. Adv Drug Deliv Rev 60(2):229–242

Blaser SA, Scheringer M, Macleod M, Hungerbuhler K (2008) Estimation of cumulative aquatic exposure and risk due to silver: contribution of nano-functionalized plastics and textiles. Sci Total Environ 390(2–3):396–409

Borselli C, Oliviero O, Battista S, Ambrosio L, Netti PA (2007) Induction of directional sprouting angiogenesis by matrix gradients. J Biomed Mater Res A 80A(2):297–305

Bosman FT, Stamenkovic I (2003) Functional structure and composition of the extracellular matrix. J Pathol 200(4):423–428

Braydich-Stolle L, Hussain S, Schlager JJ, Hofmann MC (2005) In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. Toxicol Sci 88(2):412–419

Burda C, Chen X, Narayanan R, El-Sayed MA (2005) Chemistry and properties of nanocrystals of different shapes. Chem Rev 105(4):1025–1102

Burgess BT, Myles JL, Dickinson RB (2000) Quantitative analysis of adhesion-mediated cell migration in three-dimensional gels of RGD-grafted collagen. Ann Biomed Eng 28(1): 110–118

Cabiscol E, Tamarit J, Ros J (2000) Oxidative stress in bacteria and protein damage by reactive oxygen species. Int Microbiol 3(1):3–8

Cai Z-X, Mo X-M, Zhang K-H, Fan L-P, Yin A-L, He C-L, Wang H-S (2010) Fabrication of chitosan/silk fibroin composite nanofibers for wound-dressing applications. Int J Mol Sci 11: 3529–3539

Calne S (2011) Acellular matrices for the treatment of wounds. Wounds International, London

Caputo G, Nobile C, Kipp T, Blasi L, Grillo V, Carlino E, Manna L, Cingolani R, Cozzoli PD, Athanassiou A (2008) Reversible wettability changes in colloidal TiO2 nanorod thin-film coatings under selective UV laser irradiation. J Phys Chem C 112:701

Caputo G, Cortese B, Nobile C, Salerno M, Cingolani R, Gigli G, Cozzoli PD, Athanassiou A (2009) Reversibly light-switchable wettability of hybrid organic/inorganic surfaces with dual -micro-/nanoscale roughness. Adv Funct Mater 19:1149

Causa F, Netti PA, Ambrosio L (2007) A multi-functional scaffold for tissue regeneration: the need to engineer a tissue analogue. Biomaterials 28(34):5093–5099

Chan G, Mooney DJ (2008) New materials for tissue engineering: towards greater control over the biological response. Trends Biotechnol 26:382–392

Charernsriwilaiwat N, Opanasopit P, Rojanarata T, Ngawhirunpat T, Supaphol P (2010) Preparation and characterization of chitosan -hydroxybenzotriazole/polyvinyl alcohol blend nanofibers by the electrospinning technique. Carbohydr Polym 81:675–680

Charernsriwilaiwat N, Opanasopit P, Rojanarata T, Ngawhirunpat T (2012) Lysozyme-loaded, electrospun chitosan-based nanofiber mats for wound healing. Int J Pharm 427(2):379–384

Chaterji S, Gemeinhart RA (2007) Enhanced osteoblast-like cell adhesion and proliferation using sulfonate-bearing polymeric scaffolds. J Biomed Mater Res A 83A(4):990–998
Chen RR, Silva EA, Yuen WW, Mooney DJ (2007) Spatio-temporal VEGF and PDGF delivery patterns blood vessel formation and maturation. Pharm Res 24(2):258–264
Chen J-P, Chang G-Y, Chen J-K (2008) Electrospun collagen/chitosan nanofibrous membrane as wound dressing. Colloids Surf A Physicochem Eng Asp 313–314:183–188
Chernousova S, Epple M (2013) Silver as antibacterial agent: ion, nanoparticle, and metal. Angew Chem Int Ed 52(6):1636–1653
Choi O, Hu Z (2008) Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. Environ Sci Technol 42(12):4583–4588
Choi JS, Lee SJ, Christ GJ, Atala A, Yoo JJ (2008) The influence of electrospun aligned poly (ε-caprolactone)/collagen nanofiber meshes on the formation of self-aligned skeletal muscle myotubes. Biomaterials 29:2899
Christensen FM, Johnston HJ, Stone V, Aitken RJ, Hankin S, Peters S, Aschberger K (2010) Nano-silver—feasibility and challenges for human health risk assessment based on open literature. Nanotoxicology 4(3):284–295
Ciofani G, Genchi GG, Liakos I, Athanassiou A, Mattoli V, Bandiera A (2013) Human recombinant elastin-like protein coatings for muscle cell proliferation and differentiation. Acta Biomater 9:5111
Dahl JA, Maddux BL, Hutchison JE (2007) Toward greener nanosynthesis. Chem Rev 107(6): 2228–2269
Dibrov P, Dzioba J, Gosink KK, Hase CC (2002) Chemiosmotic mechanism of antimicrobial activity of Ag(+) in Vibrio cholerae. Antimicrob Agents Chemother 46(8):2668–2670
Dvir T, Timko BP, Kohane DS, Langer R (2011) Nanotechnological strategies for engineering complex tissues. Nat Nanotechnol 6:13
Eckhardt S, Brunetto PS, Gagnon J, Priebe M, Giese B, Fromm KM (2013) Nanobio silver: its interactions with peptides and bacteria, and its uses in medicine. Chem Rev 113(7):4708–4754
El Badawy AM, Silva RG, Morris B, Scheckel KG, Suidan MT, Tolaymat TM (2011) Surface charge-dependent toxicity of silver nanoparticles. Environ Sci Technol 45(1):283–287
Engler A, Sen S, Sweeney H, Discher D (2006) Matrix elasticity directs stem cell lineage specification. Cell 126:677
Enoch S, Grey JE, Harding KG (2006) ABC of wound healing: recent advances and emerging treatments. BMJ 332:962
Fan L, Yumin D, Zhang B, Yang J, Cai J, Zhang L, Zhou J (2005) Preparation and properties of alginate/water-soluble chitin blend fibers. J Macromol Sci A Pure Appl Chem 42(6):723–732
Fan L, Du Y, Zhang B, Yang J, Zhou J, Kennedy JF (2006) Preparation and properties of alginate/carboxymethyl chitosan blend fibers. Carbohydr Polym 65(4):447–452
Fang FC (2004) Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. Nat Rev Microbiol 2(10):820–832
Fiejdasz S, Szczubiałka K, Lewandowska-Łańcucka J, Osyczka AM, Nowakowska M (2013) Biopolymer-based hydrogels as injectable materials for tissue repair scaffolds. Biomed Mater 8:035013
Finetti F, Basile A, Capasso D, Di Gaetano S, Di Stasi R, Pascale M, Turco CM, Ziche M, Morbidelli L, D'Andrea LD (2012) Functional and pharmacological characterization of a VEGF mimetic peptide on reparative angiogenesis. Biochem Pharmacol 84(3):303–311
Fittkau MH, Zilla P, Bezuidenhout D, Lutolf MP, Human P, Hubbell JA, Davies N (2005) The selective modulation of endothelial cell mobility on RGD peptide containing surfaces by YIGSR peptides. Biomaterials 26(2):167–174
Fonseca KB, Gomes DB, Lee K, Santos SG, Sousa AF, Silva EA, Mooney DJ, Granja PL, Barrias CC (2014) Injectable MMP-sensitive alginate hydrogels as hMSC delivery systems. Biomacromolecules 15:380–390
Fu J, Wang Y-K, Yang MT, Desai RA, Yu X, Liu Z, Chen CS (2010) Mechanical regulation of cell function with geometrically modulated elastomeric substrates. Nat Methods 7:733

Gaharwar AK, Rivera C, Wu C-J, Chan BK, Schmidt G (2013) Photocrosslinked nanocomposite hydrogels from PEG and silica nanospheres: structural, mechanical and cell adhesion characteristics. Mater Sci Eng C 33:1800

Genchi GG, Ciofani G, Liakos I, Ricotti L, Ceseracciu L, Athanassiou A, Mazzolai B, Menciassi A, Mattoli V (2013) Bio/non-bio interfaces: a straightforward method for obtaining long term PDMS/muscle cell biohybrid constructs. Colloids Surf B: Biointerfaces 105:144

Giri TK, Thakur A, Alexander A, Ajazuddin, Badwaik H, Tripathi DK (2012) Modified chitosan hydrogels as drug delivery and tissue engineering systems: present status and applications. Acta Pharm Sin B 2(5):439–449

Glisoni RJ, García-Fernández MJ, Pino M, Gutkind G, Moglioni AG, Alvarez-Lorenzo C, Concheiro A, Sosnik A (2013) β-Cyclodextrin hydrogels for the ocular release of antibacterial thiosemicarbazones. Carbohydr Polym 93(2):449–457

Gottschalk F, Sonderer T, Scholz RW, Nowack B (2009) Modeled environmental concentrations of engineered nanomaterials (TiO(2), ZnO, Ag, CNT, Fullerenes) for different regions. Environ Sci Technol 43(24):9216–9222

Grieger KD, Linkov I, Hansen SF, Baun A (2012) Environmental risk analysis for nanomaterials: review and evaluation of frameworks. Nanotoxicology 6(2):196–212

Griffith LG, Swartz MA (2006) Capturing complex 3D tissue physiology in vitro. Nat Rev Mol Cell Biol 7(3):211–224

Guarino V, Causa F, Ambrosio L (2007) Bioactive scaffolds for bone and ligament tissue. Expert Rev Med Devices 4(3):405–418

Guarino V, Causa F, Taddei P, di Foggia M, Ciapetti G, Martini D, Fagnano C, Baldini N, Ambrosio L (2008) Polylactic acid fibre-reinforced polycaprolactone scaffolds for bone tissue engineering. Biomaterials 29:3662

Gupta A, Maynes M, Silver S (1998) Effects of halides on plasmid-mediated silver resistance in Escherichia coli. Appl Environ Microbiol 64(12):5042–5045

Gupta A, Matsui K, Lo JF, Silver S (1999) Molecular basis for resistance to silver cations in Salmonella. Nat Med 5(2):183–188

Gupta A, Phung LT, Taylor DE, Silver S (2001) Diversity of silver resistance genes in IncH incompatibility group plasmids. Microbiology 147(Pt 12):3393–3402

Gurdon JB et al (1994) Activin signaling and response to a morphogen gradient. Nature 371 (6497):487–492

Hacker MC, Mikos AG (2006) Trends in tissue engineering research. Tissue Eng 12:2049

Hajipour MJ, Fromm KM, Ashkarran AA, de Aberasturi DJ, de Larramendi IR, Rojo T, Serpooshan V, Parak WJ, Mahmoudi M (2012) Antibacterial properties of nanoparticles. Trends Biotechnol 30(10):499–511

Hansen SF, Baun A (2012) When enough is enough. Nat Nanotechnol 7(7):409–411

Hsin YH, Chen CF, Huang S, Shih TS, Lai PS, Chueh PJ (2008) The apoptotic effect of nanosilver is mediated by a ROS- and JNK-dependent mechanism involving the mitochondrial pathway in NIH3T3 cells. Toxicol Lett 179(3):130–139

Hussain SM, Hess KL, Gearhart JM, Geiss KT, Schlager JJ (2005) In vitro toxicity of nanoparticles in BRL 3A rat liver cells. Toxicol In Vitro 19(7):975–983

Hussain SM, Javorina AK, Schrand AM, Duhart HM, Ali SF, Schlager JJ (2006) The interaction of manganese nanoparticles with PC-12 cells induces dopamine depletion. Toxicol Sci 92(2): 456–463

Hutmacher DW (2001) Scaffold design and fabrication technologies for engineering tissues—state of the art and future perspectives. J Biomater Sci Polym Ed 12(1):107–124

Hutmacher DW, Sittinger M, Risbud MV (2004) Scaffold-based tissue engineering: rationale for computer-aided design and solid free-form fabrication systems. Trends Biotechnol 22(7): 354–362

Hwang ET, Lee JH, Chae YJ, Kim YS, Kim BC, Sang BI, Gu MB (2008) Analysis of the toxic mode of action of silver nanoparticles using stress-specific bioluminescent bacteria. Small 4 (6):746–750

Inoue Y, Hoshino M, Takahashi H, Noguchi T, Murata T, Kanzaki Y, Hamashima H, Sasatsu M (2002) Bactericidal activity of Ag-zeolite mediated by reactive oxygen species under aerated conditions. J Inorg Biochem 92(1):37–42

Jack KS, Velayudhan S, Luckman P, Trau M, Grøndahl L, Cooper-White J (2009) The fabrication and characterization of biodegradable HA/PHBV nanoparticle–polymer composite scaffolds. Acta Biomater 5:2657

Jeong SI, Krebs MD, Bonino CA, Khan SA, Alsberg E (2010) Electrospun alginate nanofibers with controlled cell adhesion for tissue engineering. Macromol Biosci 10:934–943

Jin G, Prabhakaranb MP, Kaib D, Ramakrishnaa S (2013) Controlled release of multiple epidermal induction factors through core–shell nanofibers for skin regeneration. Eur J Pharm Biopharm 85(3):689–698

Johnson DW, Sherborne C, Didsbury MP, Pateman C, Cameron NR, Claeyssens F (2013) Macrostructuring of emulsion-templated porous polymers by 3D laser patterning. Adv Mater 25:3178

Jover J, Bosque R, Sales J (2008) A comparison of the binding affinity of the common amino acids with different metal cations. Dalton Trans 45:6441–6453

Jover J, Bosque R, Sales J (2009) Quantitative structure-property relationship estimation of cation binding affinity of the common amino acids. J Phys Chem A 113(15):3703–3708

Joy A, Cohen DM, Luk A, Anim-Danso E, Chen C, Kohn J (2011) Control of surface chemistry, substrate stiffness, and cell function in a novel terpolymer methacrylate library. Langmuir 27: 1891

Karageorgiou V, Kaplan D (2005) Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 26:5474

Kasuga NC, Yoshikawa R, Sakai Y, Nomiya K (2012) Syntheses, structures, and antimicrobial activities of remarkably light-stable and water-soluble silver complexes with amino acid derivatives, silver(I) N-acetylmethioninates. Inorg Chem 51(3):1640–1647

Katz E, Streuli CH (2007) The extracellular matrix as an adhesion checkpoint for mammary epithelial function. Int J Biochem Cell Biol 39(4):715–726

Keselowsky BG, Collard DM, Garcia AJ (2005) Integrin binding specificity regulates biomaterial surface chemistry effects on cell differentiation. Proc Natl Acad Sci USA 102:5953

Kim TG, Chung HJ, Park TG (2008a) Macroporous and nanofibrous hyaluronic acid/collagen hybrid scaffold fabricated by concurrent electrospinning and deposition/leaching of salt particles. Acta Biomater 4:1611

Kim KJ, Sung WS, Moon SK, Choi JS, Kim JG, Lee DG (2008b) Antifungal effect of silver nanoparticles on dermatophytes. J Microbiol Biotechnol 18(8):1482–1484

Kim KJ, Sung WS, Suh BK, Moon SK, Choi JS, Kim JG, Lee DG (2009) Antifungal activity and mode of action of silver nano-particles on Candida albicans. Biometals 22(2):235–242

Kim HJ, Park IK, Kim JH, Cho CS, Kim MS (2012) Gas foaming fabrication of porous biphasic calcium phosphate for bone regeneration. Tissue Eng Regen Med 9:63

Klasen HJ (2000a) A historical review of the use of silver in the treatment of burns. II. Renewed interest for silver. Burns 26(2):131–138

Klasen HJ (2000b) Historical review of the use of silver in the treatment of burns. I. Early uses. Burns 26(2):117–130

Kong HJ, Mooney DJ (2007) Microenvironmental regulation of biomacromolecular therapies. Nat Rev Drug Discov 6(6):455–463

Kuijpers AJ, van Wachem PB, van Luyn MJA, Brouwer LA, Engbers GHM, Krijgsveld J, Zaat SAJ, Dankert J, Feijen J (2000) In vitro and in vivo evaluation of gelatin-chondroitin sulphate hydrogels for controlled release of antibacterial proteins. Biomaterials 21(17):1763–1772

Kumar A, Vemula PK, Ajayan PM, John G (2008) Silver-nanoparticle-embedded antimicrobial paints based on vegetable oil. Nat Mater 7(3):236–241

Lee KY, Jeong L, Kang YO, Lee SJ, Park WH (2009) Electrospinning of polysaccharides for regenerative medicine. Adv Drug Deliv Rev 61(12):1020–1032

Lee E-J, Teng S-H, Jang T-S, Wang P, Yook S-W, Kim H-E, Koh Y-H (2010) Nanostructured poly (e-caprolactone)–silica xerogel fibrous membrane for guided bone regeneration. Acta Biomater 6:3557

Lee C, Shin J, Lee JS, Byun E, Ryu JH, Um SH, Kim DI, Lee H, Cho S-W (2013) Bioinspired, calcium-free alginate hydrogels with tunable physical and mechanical properties and improved biocompatibility. Biomacromolecules 14(6):2004–2013

Lemire JA, Harrison JJ, Turner RJ (2013) Antimicrobial activity of metals: mechanisms, molecular targets and applications. Nat Rev Microbiol 11(6):371–384

Levy SB, Marshall B (2004) Antibacterial resistance worldwide: causes, challenges and responses. Nat Med 10(12 Suppl):S122–S129

Li WR, Xie XB, Shi QS, Duan SS, Ouyang YS, Chen YB (2011) Antibacterial effect of silver nanoparticles on Staphylococcus aureus. Biometals 24(1):135–141

Liakos I, Rizzello L, Bayer IS, Pompa PP, Cingolani R, Athanassiou A (2013) Controlled antiseptic release by alginate polymer films and beads. Carbohydr Polym 92(1):176–183

Liakos I, Rizzello L, Scurr DJ, Pompa PP, Bayer IS, Athanassiou A (2014) All-natural composite wound dressing films of essential oils encapsulated in sodium alginate with antimicrobial properties. Int J Pharm 463(2):137–145

Liang HY, Wang WZ, Huang YZ, Zhang SP, Wei H, Xu HX (2010) Controlled Synthesis of Uniform Silver Nanospheres. J Phys Chem C 114(16):7427–7431

Liau SY, Read DC, Pugh WJ, Furr JR, Russell AD (1997) Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterial action of silver ions. Lett Appl Microbiol 25(4):279–283

Liu J, Hurt RH (2010) Ion release kinetics and particle persistence in aqueous nano-silver colloids. Environ Sci Technol 44(6):2169–2175

Liu X, Ma PX (2009) Phase separation, pore structure, and properties of nanofibrous gelatin scaffolds. Biomaterials 30:4094

Liu S-J, Kau Y-C, Chou C-Y, Chen J-K, Wu R-C, Yeh W-L (2010a) Electrospun PLGA/collagen nanofibrous membrane as early-stage wound dressing. J Membr Sci 355:53–59

Liu J, Sonshine DA, Shervani S, Hurt RH (2010b) Controlled release of biologically active silver from nanosilver surfaces. ACS Nano 4(11):6903–6913

Lo CM, Wang HB, Dembo M, Wang YL (2000) Cell movement is guided by the rigidity of the substrate. Biophys J 79(1):144

Lourenço BN, Marchioli G, Song W, Reis RL, van Blitterswijk CA, Karperien M, van Apeldoorn A, Mano JF (2012) Wettability influences cell behavior on superhydrophobic surfaces with different topographies. Biointerphases 7:46

Luciani A, Coccoli V, Orsi S, Ambrosio L, Netti PA (2008) PCL microspheres based functional scaffolds by bottom-up approach with predefined microstructural properties and release profiles. Biomaterials 29(36):4800–4807

Malmsten M (2011) Antimicrobial and antiviral hydrogel. Soft Matter 7:8725–8736. doi:10.1039/C1SM05809F

Mandoli C, Mecheri B, Forte G, Pagliari F, Pagliari S, Carotenuto F, Fiaccavento R, Rinaldi A, Di Nardo P, Licoccia S, Traversa E (2010) Thick soft tissue reconstruction on highly perfusive biodegradable scaffolds. Macromol Biosci 10:127

Mazzitelli S, Pagano C, Giusepponi D, Nastruzzi C, Perioli L (2013) Hydrogel blends with adjustable properties as patches for transdermal delivery. Int J Pharm 454(1):47–57

Mehta M, Schmidt-Bleek K, Duda GN, Mooney DJ (2012) Biomaterial delivery of morphogens to mimic the natural healing cascade in bone. Adv Drug Deliv Rev 64:1257–1276

Merkel TC, Freeman BD, Spontak RJ, He Z, Pinnau I, Meakin P, Hill AJ (2002) Ultrapermeable, reverse-selective nanocomposite membranes. Science 296(5567):519–522

Milionis A, Martiradonna L, Anyfantis GC, Cozzoli PD, Bayer IS, Fragouli D, Athanassiou A (2013) Control of the water adhesion on hydrophobic micropillars by spray coating technique. Colloid Polym Sci 291:401

Milionis A, Fragouli D, Martiradonna L, Anyfantis GC, Cozzoli PD, Bayer IS, Athanassiou A (2014) Spatially controlled surface energy traps on superhydrophobic surfaces. ACS Appl Mater Interfaces 6:1036–1043

Mirzajani F, Ghassempour A, Aliahmadi A, Esmaeili MA (2011) Antibacterial effect of silver nanoparticles on Staphylococcus aureus. Res Microbiol 162(5):542–549

Moffa M, Polini A, Sciancalepore AG, Persano L, Mele E, Passione LG, Potente G, Pisignano D (2013) Microvascular endothelial cell spreading and proliferation on nanofibrous scaffolds by polymer blends with enhanced wettability. Soft Matter 9:5529

Monopoli MP, Walczyk D, Campbell A, Elia G, Lynch I, Bombelli FB, Dawson KA (2011a) Physical-chemical aspects of protein corona: relevance to in vitro and in vivo biological impacts of nanoparticles. J Am Chem Soc 133(8):2525–2534

Monopoli MP, Bombelli FB, Dawson KA (2011b) Nanobiotechnology: nanoparticle coronas take shape. Nat Nanotechnol 6(1):11–12

Morais DS, Rodrigues MA, Lopes MA, Coelho MJ, Maurício AC, Gomes R, Amorim I, Ferraz MP, Santos JD, Botelho CM (2013) Biological evaluation of alginate-based hydrogels, with antimicrobial features by Ce(III) incorporation, as vehicles for a bone substitute. J Mater Sci Mater Med 24(9):2145–2155

Morens DM, Folkers GK, Fauci AS (2010) The challenge of emerging and re-emerging infectious diseases (vol 430, pg 242, 2004). Nature 463(7277):122

Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramirez JT, Yacaman MJ (2005) The bactericidal effect of silver nanoparticles. Nanotechnology 16(10):2346–2353

Mou Z-L, Duan L-M, Qi X-N, Zhang Z-Q (2013) Preparation of silk fibroin/collagen/hydroxyapatite composite scaffold by particulate leaching method. Mater Lett 105:189

Musee N, Thwala M, Nota N (2011) The antibacterial effects of engineered nanomaterials: implications for wastewater treatment plants. J Environ Monit 13(5):1164–1183

Nandakumar A, Fernandes H, de Boer J, Moroni L, Habibovic P, van Blitterswijk CA (2010) Fabrication of bioactive composite scaffolds by electrospinning for bone regeneration. Macromol Biosci 10:1365

Navarro E, Piccapietra F, Wagner B, Marconi F, Kaegi R, Odzak N, Sigg L, Behra R (2008) Toxicity of silver nanoparticles to Chlamydomonas reinhardtii. Environ Sci Technol 42(23): 8959–8964

Neibert K, Gopishetty V, Grigoryev A, Tokarev I, Al-Hajaj N, Vorstenbosch J, Philip A, Minko S, Maysinger D (2012) Wound-healing with mechanically robust and biodegradable hydrogel fibers loaded with silver nanoparticles. Adv Healthc Mater 1(5):621–630

Ng R, Zhang X, Liu N, Yang ST (2009) Modifications of nonwoven polyethylene terephthalate fibrous matrices via NaOH hydrolysis: effects on pore size, fiber diameter, cell seeding and proliferation. Process Biochem 44:992

Ng R, Zang R, Yang KK, Liu N, Yang S-T (2012) Three-dimensional fibrous scaffolds with microstructures and nanotextures for tissue engineering. RCS Adv 2:10110

Nitanan T, Akkaramongkolporn P, Rojanarata T, Ngawhirunpat T, Opanasopit P (2013) Neomycin-loaded poly(styrene sulfonic acid-co-maleic acid) (PSSA-MA)/polyvinyl alcohol (PVA) ion exchange nanofibers for wound dressing materials. Int J Pharm 448(1):71–78

Nomiya K, Takahashi S, Noguchi R, Nemoto S, Takayama T, Oda M (2000) Synthesis and characterization of water-soluble silver(I) complexes with L-histidine (H2his) and (S)-(-)-2-pyrrolidone-5-carboxylic acid (H2pyrrld) showing a wide spectrum of effective antibacterial and antifungal activities. Crystal structures of chiral helical polymers [Ag(Hhis)]n and ([Ag (Hpyrrld)]2)n in the solid state. Inorg Chem 39(15):3301–3311

Notin L, Viton C, Lucas J-M, Domard A (2006) Pseudo-dry-spinning of chitosan. Acta Biomater 2:297–311

Nowack B, Krug HF, Height M (2011) 120 years of nanosilver history: implications for policy makers. Environ Sci Technol 45(4):1177–1183

Nowack B, Ranville JF, Diamond S, Gallego-Urrea JA, Metcalfe C, Rose J, Horne N, Koelmans AA, Klaine SJ (2012) Potential scenarios for nanomaterial release and subsequent alteration in the environment. Environ Toxicol Chem 31(1):50–59

Oliveira SM, Song W, Alves NM, Mano JF (2011) Chemical modification of bioinspired superhydrophobic polystyrene surfaces to control cell attachment/proliferation. Soft Matter 7:8932

Pal S, Tak YK, Song JM (2007) Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the Gram-negative bacterium Escherichia coli. Appl Environ Microbiol 73(6):1712–1720

Palchesko RN, Zhang L, Sun Y, Feinberg AW (2012) Development of polydimethylsiloxane substrates with tunable elastic modulus to study cell mechanobiology in muscle and nerve. PLoS ONE 7:e51499

Pamula E, Bacakova L, Filova E, Buczynska J, Dobrzynski P, Noskova L, Grausova L (2008) The influence of pore size on colonization of poly(L-lactide-glycolide) scaffolds with human osteoblast-like MG 63 cells in vitro. J Mater Sci Mater Med 19:425

Pamula E, Filová E, Bacáková L, Lisá V, Adamczyk D (2009) Resorbable polymeric scaffolds for bone tissue engineering. J Biomed Mater Res A 89A:432

Panacek A, Kvitek L, Prucek R, Kolar M, Vecerova R, Pizurova N, Sharma VK, Nevecna T, Zboril R (2006) Silver colloid nanoparticles: synthesis, characterization, and their antibacterial activity. J Phys Chem B 110(33):16248–16253

Panacek A, Kolar M, Vecerova R, Prucek R, Soukupova J, Krystof V, Hamal P, Zboril R, Kvitek L (2009) Antifungal activity of silver nanoparticles against Candida spp. Biomaterials 30(31): 6333–6340

Park EJ, Yi J, Kim Y, Choi K, Park K (2010) Silver nanoparticles induce cytotoxicity by a Trojan-horse type mechanism. Toxicol In Vitro 24(3):872–878

Penchev H, Paneva D, Manolova N, Rashkov I (2009) Electrospun Hybrid nanofibers based on chitosan or N-carboxyethylchitosan and silver nanoparticles. Macromol Biosci 9:884–894

Pillai CKS, Paul W, Sharma CP (2009) Chitin and chitosan polymers: chemistry, solubility and fiber formation. Prog Polym Sci 34(7):641–678

Place ES, George JH, Williams CK, Stevens MM (2009a) Synthetic polymer scaffolds for tissue engineering. Chem Soc Rev 38:1139

Place ES, Evans ND, Stevens MM (2009b) Complexity in biomaterials for tissue engineering. Nat Mater 8(6):457–470

Polini A, Scaglione S, Quarto R, Pisignano D (2013) Composite electrospun nanofibers for influencing stem cell fate. Methods Mol Biol 1058:25–40

Powers JH (2004) Antimicrobial drug development—the past, the present, and the future. Clin Microbiol Infect 10:23–31

Qin Y (2008) The gel swelling properties of alginate fibers and their applications in wound management. Polym Adv Technol 19:6–14

Rapraeger AC (2000) Syndecan-regulated receptor signaling. J Cell Biol 149(5):995–997

Reddy PR, Varaprasad K, Sadiku R, Ramam K, Reddy GVS, Raju KM, Reddy N (2013) Development of gelatin based inorganic nanocomposite hydrogels for inactivation of bacteria. J Inorg Organomet Polym Mater 23(5):1054–1060

Reynolds NP, Styan KE, Easton CD, Li Y, Waddington L, Lara C, Forsythe JS, Mezzenga R, Hartley PG, Muir BW (2013) Nanotopographic surfaces with defined surface chemistries from amyloid fibril networks can control cell attachment. Biomacromolecules 14:2305

Richardson TP, Peters MC, Ennett AB, Mooney DJ (2001) Polymeric system for dual growth factor delivery. Nat Biotechnol 19(11):1029–1034

Rieger KA, Birch NP, Schiffman JD (2013) Designing electrospun nanofiber mats to promote wound healing—a review. J Mater Chem B 1:4531–4541

Roh JY, Sim SJ, Yi J, Park K, Chung KH, Ryu DY, Choi J (2009) Ecotoxicity of silver nanoparticles on the soil nematode Caenorhabditis elegans using functional ecotoxicogenomics. Environ Sci Technol 43(10):3933–3940

Rouet V, Hamma-Kourbali Y, Petit E, Panagopoulou P, Katsoris P, Barritault D, Caruelle JP, Courty J (2005) A synthetic glycosaminoglycan mimetic binds vascular endothelial growth factor and modulates angiogenesis. J Biol Chem 280(38):32792–32800

Rujitanaroj P-o, Pimpha N, Supaphol P (2008) Wound-dressing materials with antibacterial activity from electrospun gelatin fiber mats containing silver nanoparticles. Polymer 49: 4723–4732

Sachlos E, Czernuszka JT (2003) Making tissue engineering scaffolds work. Review: the application of solid freeform fabrication technology to the production of tissue engineering scaffolds. Eur Cell Mater 5:29–39, discussion 39–40

Saltzman WM, Olbricht WL (2002) Building drug delivery into tissue engineering. Nat Rev Drug Discov 1(3):177–186

Schmidt S, Madaboosi N, Uhlig K, Köhler D, Skirtach A, Duschl C, Möhwald H, Volodkin DV (2012) Control of cell adhesion by mechanical reinforcement of soft polyelectrolyte films with nanoparticles. Langmuir 28:7249

Schneider A, Wang XY, Kaplan DL, Garlick JA, Eglesa C (2009) Biofunctionalized electrospun silk mats as a topical bioactive dressing for accelerated wound healing. Acta Biomater 5(7): 2570–2578

Schwaber MJ, De-Medina T, Carmeli Y (2004) Epidemiological interpretation of antibiotic resistance studies - what are we missing? Nat Rev Microbiol 2(12):979–983

Schwarz US, Safran SA (2013) Physics of adherent cells. Rev Mod Phys 85:1327

Seyednejad H, Gawlitta D, Dhert WJA, van Nostrum CF, Vermonden T, Hennink WE (2011) Preparation and characterization of a three-dimensional printed scaffold based on a functionalized polyester for bone tissue engineering applications. Acta Biomater 7:1999

Seyednejad H, Gawlitta D, Kuiper RV, de Bruin A, van Nostrum CF, Vermonden T, Dhert WJA, Hennink WE (2012) In vivo biocompatibility and biodegradation of 3D-printed porous scaffolds based on a hydroxyl-functionalized poly(ε-caprolactone). Biomaterials 33:4309

Sintubin L, De Gusseme B, Van der Meeren P, Pycke BF, Verstraete W, Boon N (2011) The antibacterial activity of biogenic silver and its mode of action. Appl Microbiol Biotechnol 91(1):153–162

Siow KS, Britcher L, Kumar S, Griesser HJ (2006) Plasma methods for the generation of chemically reactive surfaces for biomolecule immobilization and cell colonization—a review. Plasma Process Polym 3:392

Smetana AB, Klabunde KJ, Marchin GR, Sorensen CM (2008) Biocidal activity of nanocrystalline silver powders and particles. Langmuir 24(14):7457–7464

Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci 275(1):177–182

Song B, Wu C, Chang J (2012a) Controllable delivery of hydrophilic and hydrophobic drugs from electrospun poly(lactic-co-glycolic acid)/mesoporous silica nanoparticles composite mats. J Biomed Mater Res B Appl Biomater 100(8):2178–2186

Song B, Wu C, Chang J (2012b) Dual drug release from electrospun poly(lactic-co-glycolic acid)/mesoporous silica nanoparticles composite mats with distinct release profiles. Acta Biomater 8 (5):1901–1907

Spellberg B (2008) Dr. William H. Stewart: mistaken or maligned? Clin Infect Dis 47(2):294

Spellberg B, Guidos R, Gilbert D, Bradley J, Boucher HW, Scheld WM, Bartlett JG, Edwards J, Amer IDS (2008) 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

Stupack DG, Cheresh DA (2002) ECM remodeling regulates angiogenesis: endothelial integrins look for new ligands. Sci STKE 2002(119):e7

Sue YM, Lee JY, Wang MC, Lin TK, Sung JM, Huang JJ (2001) Generalized argyria in two chronic hemodialysis patients. Am J Kidney Dis 37(5):1048–1051

Suganya S, Senthil Ram T, Lakshmi BS, Giridev VR (2011) Herbal drug incorporated antibacterial nanofibrous mat fabricated by electrospinning: an excellent matrix for wound dressings. J Appl Polym Sci 121(5):2893–2899

Sun T, Qing G (2011) Biomimetic smart interface materials for biological applications. Adv Mater 23:H57

Sun W, Darling A, Starly B, Nam J (2004) Computer-aided tissue engineering: overview, scope and challenges. Biotechnol Appl Biochem 39:29–47

Sun T, Qing G, Su B, Jiang L (2011) Functional biointerface materials inspired from nature. Chem Soc Rev 40:2909

Sušec M, Ligon SC, Stampfl J, Liska R, Krajnc P (2013) Hierarchically porous materials from layer-by-layer photopolymerization of high internal phase emulsions. Macromol Rapid Commun 34:938

Suwantong O, Ruktanonchai U, Supaphol P (2010) In vitro biological evaluation of electrospun cellulose acetate fiber mats containing asiaticoside or curcumin. J Biomed Mater Res A 94(4): 1216–1225

Taipale J, KeskiOja J (1997) Growth factors in the extracellular matrix. FASEB J 11(1):51–59

Takenaka S, Karg E, Roth C, Schulz H, Ziesenis A, Heinzmann U, Schramel P, Heyder J (2001) Pulmonary and systemic distribution of inhaled ultrafine silver particles in rats. Environ Health Perspect 109(Suppl 4):547–551

Tanabe Y, Jessell TM (1997) Diversity and pattern in the developing spinal cord (vol 274, pg 1115, 1996). Science 276(5309):21

Teo W-E, He W, Ramakrishna S (2006) Electrospun scaffold tailored for tissue-specific extracellular matrix. Biotechnol J 1(9):918–929

Torres-Giner S, Martinez-Abad A, Gimeno-Alcañiz JV, Ocio MJ, Lagaron JM (2012) Controlled delivery of gentamicin antibiotic from bioactive electrospun polylactide-based ultrathin fibers. Adv Eng Mater 14:B112–B122

Travan A, Pelillo C, Donati I, Marsich E, Benincasa M, Scarpa T, Semeraro S, Turco G, Gennaro R, Paoletti S (2009) Non-cytotoxic silver nanoparticle-polysaccharide nanocomposites with antimicrobial activity. Biomacromolecules 10(6):1429–1435

Ueda E, Levkin PA (2013) Emerging applications of superhydrophilic-superhydrophobic micropatterns. Adv Mater 25:1234

Upert G, Bouillere F, Wennemers H (2012) Oligoprolines as scaffolds for the formation of silver nanoparticles in defined sizes: correlating molecular and nanoscopic dimensions. Angew Chem Int Ed Engl 51(17):4231–4234

Uppal R, Ramaswamy GN, Arnold C, Goodband R, Wang Y (2011) Hyaluronic acid nanofiber wound dressing-production, characterization, and in vivo behavior. J Biomed Mater Res B Appl Biomater 97(1):20–29

Üstündağ GC, Karaca E, Özbek S, ÇavuşoĞlu I (2010) In vivo evaluation of electrospun poly (vinyl alcohol)/sodium alginate nanofibrous mat as wound dressing. Tekstil ve Konfeksiyon 20:290–298

Uttayarat P, Jetawattana S, Suwanmala P, Eamsiri J, Tangthong T, Pongpat S (2012) Antimicrobial electrospun silk fibroin mats with silver nanoparticles for wound dressing application. Fiber Polym 13:999–1006

van der Pol U, Mathieu L, Zeiter S, Bourban P-E, Zambelli P-Y, Pearce SG, Bouré LP, Pioletti DP (2010) Augmentation of bone defect healing using a new biocomposite scaffold: an in vivo study in sheep. Acta Biomater 6:3755

Van Vlierberghe S, Schacht E, Dubruel E (2011) Reversible gelatin-based hydrogels: finetuning of material properties. Eur Polym J 47(5):1039–1047

Ventre M, Causa F, Netti PA (2012) Determinants of cell-material crosstalk at the interface: towards engineering of cell instructive materials. J R Soc Interface 9(74):2017–2032

Venugopal JR, Zhang Y, Ramakrishna S (2006) In vitro culture of human dermal fibroblasts on electrospun polycaprolactone collagen nanofibrous membrane. Artif Organs 30(6):440–446

Vertelov GK, Krutyakov YA, Efremenkova OV, Olenin AY, Lisichkin GV (2008) A versatile synthesis of highly bactericidal Myramistin(R) stabilized silver nanoparticles. Nanotechnology 19(35):355707

Villafiorita Monteleone F, Caputo G, Canale C, Cozzoli PD, Cingolani R, Fragouli D, Athanassiou A (2010) Light-controlled directional liquid drop movement on TiO2 nanorods-based nanocomposite photopatterns. Langmuir 26:18557

Voytik-Harbin SL, Brightman AO, Waisner BZ, Robinson JP, Lamar CH (1998) Small intestinal submucosa: a tissue-derived extracellular matrix that promotes tissue-specific growth and differentiation of cells in vitro. Tissue Eng 4:157

Wadhera A, Fung M (2005) Systemic argyria associated with ingestion of colloidal silver. Dermatol Online J 11(1):12

Walczyk D, Bombelli FB, Monopoli MP, Lynch I, Dawson KA (2010) What the cell "sees" in bionanoscience. J Am Chem Soc 132(16):5761–5768

Wan LQ, Jiang J, Arnold DE, Guo XE, Lu HH, Mow VC (2008) Mow, Calcium concentration effects on the mechanical and biochemical properties of Chondrocyte-Alginate constructs. Cell Mol Bioeng 1(1):93–102

Wang DA, Varghese S, Sharma B, Strehin I, Fermanian S, Gorham J, Fairbrother DH, Cascio B, Elisseeff JH (2007) Multifunctional chondroitin sulphate for cartilage tissue-biomaterial integration. Nat Mater 6(5):385–392

Wang C, Yan K-W, Lin Y-D, Hsieh PCH (2010) Biodegradable core/shell fibers by coaxial electrospinning: processing, fiber characterization, and its application in sustained drug release. Macromolecules 43(15):6389–6397

Wang S, Zhang Y, Wang H, Dong Z (2011a) Preparation, characterization and biocompatibility of electrospinning heparin-modified silk fibroin nanofibers. Int J Biol Macromol 48:345

Wang Y, Zhang CL, Zhang Q, Li P (2011b) Composite electrospun nanomembranes of fish scale collagen peptides/chito-oligosaccharides: antibacterial properties and potential for wound dressing. Int J Nanomedicine 6:667–676

Wei G, Ma PX (2008) Nanostructured biomaterials for regeneration. Adv Funct Mater 18:3568

Wei G, Ma PX (2009) Partially nanofibrous architecture of 3D tissue engineering scaffolds. Biomaterials 30:6426

Wennemers H (2012) Peptides as asymmetric catalysts and templates for the controlled formation of Ag nanoparticles. J Pept Sci 18(7):437–441

Wharram SE, Zhang X, Kaplan DL, McCarthy SP (2010) Electrospun silk material systems for wound healing. Macromol Biosci 10(3):246–257

Whitesides GM, Ostuni E, Takayama S, Jiang X, Ingber DE (2001) Soft lithography in biology and biochemistry. Annu Rev Biomed Eng 3:335–373

Wijekoon A, Fountas-Davis N, Leipzig ND (2013) Fluorinated methacrylamide chitosan hydrogel systems as adaptable oxygen carriers for wound healing. Acta Biomater 9(3):5653–5664

Wijelath ES, Murray J, Rahman S, Patel Y, Ishida A, Strand K, Aziz S, Cardona C, Hammond WP, Savidge GF, Rafii S, Sobel M (2002) Novel vascular endothelial growth factor binding domains of fibronectin enhance vascular endothelial growth factor biological activity. Circ Res 91(1):25–31

Wnek GE, Carr ME, Simpson DG, Bowlin GL (2003) Electrospinning of nanofiber fibrinogen structures. Nano Lett 3(2):213–216

Xiaoling F, Wang H (2012) Spatial arrangement of polycaprolactone/collagen nanofiber scaffolds regulates the wound healing related behaviors of human adipose stromal cells. Tissue Eng Part A 18(5–6):631–642

Xiu ZM, Ma J, Alvarez PJ (2011) Differential effect of common ligands and molecular oxygen on antimicrobial activity of silver nanoparticles versus silver ions. Environ Sci Technol 45(20): 9003–9008

Xiu ZM, Zhang QB, Puppala HL, Colvin VL, Alvarez PJ (2012) Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Lett 12(8):4271–4275

Xu X, Zhong W, Zhou S, Trajtman A, Alfa M (2010) Electrospun PEG-PLA nanofibrous membrane for sustained release of hydrophilic antibiotics. J Appl Polym Sci 118(1):588–595

Yan L, Si S, Chen Y, Yuan T, Fan H, Yao Y, Zhang Q (2011) Electrospun in-situ hybrid polyurethane/nano-TiO2 as wound dressings. Fiber Polym 12(2):207–213

Yang C, Wu X, Zhao Y, Xu L, Wei S (2011) Nanofibrous scaffold prepared by electrospinning of poly(vinyl alcohol)/gelatin aqueous solutions. J Appl Polym Sci 121(5):3047–3055

Yang Y, Xia T, Chen F, Wei W, Liu C, He S, Li X (2012) Electrospun fibers with plasmid bFGF polyplex loadings promote skin wound healing in diabetic rats. Mol Pharm 9(1):48–58

Zelzer M, Majani R, Bradley JW, Rose FRAJ, Davies MC, Alexander MR (2008) Investigation of cell–surface interactions using chemical gradients formed from plasma polymers. Biomaterials 29:172

Zhang Y, Lim CT, Ramakrishna S, Huang Z-M (2005) Recent development of polymer nanofibers for biomedical and biotechnological applications. J Mater Sci Mater Med 16:933–946

Zook JM, Long SE, Cleveland D, Geronimo CL, MacCuspie RI (2011) Measuring silver nanoparticle dissolution in complex biological and environmental matrices using UV-visible absorbance. Anal Bioanal Chem 401(6):1993–2002

Zouani OF, Chollet C, Guillotin B, Durrieu MC (2010) Differentiation of pre-osteoblast cells on poly(ethylene terephthalate) grafted with RGD and/or BMPs mimetic peptides. Biomaterials 31(32):8245–8253

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