Chapter 10
Gold Nanoparticles for Tissue Engineering

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Abstract Regenerative medicine is currently recognized as an emerging field of nano-medicine with promising opportunities to fully heal tissues damaged by disease, trauma or congenital issues. Within this field, tissue engineering aims at the combination of cells, new bio-materials, and biochemical factors to regenerate biological tissues. The societal impact of this research is significant due to the possibility of implanting natural, synthetic, or semi-synthetic tissues and organs that are fully functional from the start, or can grow into the required functionality. Recently advances of nanotechnology provided wide possibilities to fabricate

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N. Dasgupta et al. (eds.), *Environmental Nanotechnology*, Environmental Chemistry for a Sustainable World 14, https://doi.org/10.1007/978-3-319-76090-2_10

nanostructured scaffolds that mimic the tissue-specific microenvironment. The unique properties of a variety of nanomaterials allow to prepare scaffolds with improved biochemical, mechanical, and electrical properties and capable also to cell adhesion, proliferation, differentiation and to foster the cell growth. Within many types of nanoparticles, gold nanoparticles nowadays are widely used in biology and medicine due to the wide range of valuable chemical and physical properties. However, efficient application of gold nanoparticles for tissue engineering purposes is still at incipience stage.

Here we review the current advances of application of gold nanoparticles for tissue engineering. We will summarize (1) properties of gold nanoparticles relevant to tissue engineering, (2) interaction of gold nanoparticles with cell and toxicity, (3) the current advances in tissue engineering, focusing on cardiac, bone, neural and skin tissue engineering, recognized as the most significant fields of regenerative medicine. In addition, other fields of tissue engineering, where gold nanoparticles have been also applied, are also highlighted. The major point of this review is to highlight the relevance of gold nanoparticles as co-factor, that impart to the scaffolds properties valuable for tissue engineering. According to the reviewed publications the main function of gold nanoparticles in tissue engineering is aimed on enhancing scaffolds properties and delivery efficiency. Moreover, the examples of direct impact of gold nanoparticles on cells differentiation are also provided.

Abbreviation

LSPR Localized surface plasmon resonance

10.1 Introduction

Tissue Engineering has gained enormous interest as a means to restore, maintain and improve tissue function, capable to meet the increasing demand for replacement tissues and organs (Nerem et al. 2000). Such approach is a valuable alternative to replace a damaged tissue or organ instead of applying transplants. Tissue engineering lies at the interface of several disciplines and combines cell technology, materials development and fabrication, and creation of suitable biochemical factors to create artificial organs and tissues, or to regenerate damaged tissues (Liu et al. 2007; Langer and Vacanti 1993). One of the major strategy of tissue engineering encompasses the in vitro growing of cells onto a scaffold with subsequent implantation into the body (Liu et al. 2007). Therefore, the engineering of scaffolds with the proper architecture, high cyto-and tissue compatibility, bioactivity, and good mechanical properties is an important task in tissue engineering (Chan and Leong 2008). A number of scientific publications have already highlighted the advances in bone tissue engineering, cardiac tissue engineering, tissue engineering for the skin regeneration and wound

healing, and other areas, and many of corresponding publications have also discussed the future prospects of tissue engineering (Huu et al. 2013; Amini 2012a, b; Gong et al. 2016; Langer and Vacanti 2016; Dong and Lv 2016; Boccaccini and Harding 2012; Boccaccini and Ma 2014; Liverani et al. 2016; Skobot et al. 2015).

Nanotechnology is continuously being applied in the field of tissue engineering with increasing success since biomaterials to be engineered (extracellular fluids, bone marrow, cardiac tissue etc.) are of nanometer size. The application of nanotechnology to the tissue engineering field comprises the fabrication of nanofibers, 2D and 3D nano-structures and of nanoparticles for controlled-release approaches. These can be employed for building and functionalization of the scaffolds, aimed to enhance the repopulation of the scaffold by cells of the hosting organism (Kingsley et al. 2013; Chung et al. 2007). In addition, the optical and conductive properties of specific nanoparticles can significantly increase of cells growth (Tiwari and Siväjärvi 2016). Indeed, based on achieved results, nanotechnology showed the superiority in tissue engineering and in regenerative medicine in general, in comparison of conventional techniques (Kingsley et al. 2013; Kim et al. 2014). Beside the direct use in the fabrication of nanostructured scaffolds, other important properties of nanoparticles, such as surface chemistry and ability to deliver bioactive agents, play also an important role in enhancing the tissue engineering capabilities. Thus, tissue engineering strategies based on the combination of bioactive agent-loaded nanoparticles and scaffolds have significant increased in recent years, and biomaterial scaffolds can be combined with bioactive agents loaded into nanoparticles to improve tissue regeneration (Monteiro et al. 2015).

Various types of nanoparticles are applied nowadays in tissue engineering approaches. Nanocomposite scaffolds provide structural support for the cells. The nanoscale details of the scaffold may have significant effects on cell-scaffold adhesion, integrin-triggered signaling pathways and cellular function (Gong et al. 2015). For example, polymeric nanoscaffolds capable of continued release of bioactive growth factors, with different release profiles and surface hydrophilicity have been fabricated (Sahoo et al. 2010; Kim et al. 2014). "Functional" magnetite nanoparticles were developed for cell manipulation using magnetic force, and magnetic nanoparticles were applied to assemble more complex tissue structures than structures that are achieved by conventional scaffold-based tissue engineering strategies (Ito and Kamihira 2011; Lee et al. 2014; Zhang 2015a, b). Improved biocompatibility of carbon nanotubes, led to the possibility to apply carbon nanotubes as tissue scaffolding materials to enhance the organ regeneration (Veetil and Ye 2009; Haniu et al. 2012; Bosi et al. 2014). Mesoporous silica nanoparticles provide a flexible platform for controlled delivery of drugs and imaging agents in tissue engineering and stem cell therapy (Rosenholm et al. 2016; Li 2015b). Indeed, nowadays there are many reviews highlighting in details the application of different nanomaterials for tissue engineering approaches, for example nanomaterials in bone tissue engineering (Vieira et al. 2017); applications of magnetic nanoparticles for controlled tissue engineering (Lee et al. 2014); nanomaterials for tissue engineering in dentristy (Chieruzzi et al. 2016); nanomaterials for cardiac tissue engineering

(Zhang et al. 2011); application of carbon-based nanomaterials for tissue engineering (Ku et al. 2013). Summarized and detailed information regarding fabrication and application in tissue engineering of broad range of nanoparticles, for example polymeric nanomaterials, nanoporous biomaterials, carbon-based nanomaterials, nanofibrous scaffolds was published in 2013 (Gaharwar et al. 2013).

Nanoparticles of noble metals have been studied with growing interest, since they exhibit significantly distinct physical, chemical and biological properties from nanoparticles bulk counterparts (Armentano et al. 2010; Rosarin and Mirunalini 2011; Pandey and Pandey 2016). Within these types of nanoparticles, silver and gold nanoparticles are the most frequently used due to valuable optical, electronic, catalytic, biocompatible properties and potentially high surface reactivity (Rosarin and Mirunalini 2011). The nanoscale size of gold nanoparticles, wide range of easy preparation techniques, high surface area, and broad opportunities of surface functionalization make these nanoparticles attractive to fit the requirements of tissue engineering (Vial et al. 2016). Moreover, the unique optical properties of gold nanoparticles, e.g. Localized Surface Plasmon Resonances (LSPR), located in the Visible and Near-Infrared range, can be used to enhance scaffold properties and correspondingly improve cells adhesion, growth or differentiation.

However, despite the advances of gold nanoparticles application in cancer therapy, imaging and sensing agents, delivery platforms, the potential of gold nanoparticles in the tissue engineering field has not been sufficiently explored. Nevertheless, this interest is growing rapidly, and this is the main focus of current work. Therefore, firstly the properties of gold nanoparticles relevant to tissue engineering will be briefly highlighted. Then the recent advantages of gold nanoparticles application in most important fields of tissue engineering will be summarized and discussed. Moreover, current problems and restrictions to gold nanoparticles applications, together with possible solutions, will be also provided.

10.2 Properties of Gold Nanoparticles

Gold nanoparticles are among the most extensively studied nanoparticles, due to the high stability and facile synthetic preparation techniques (Chirico et al. 2015; Tateno et al. 2014). Moreover, gold nanoparticles have a range of unique properties (Yeh et al. 2011), that include tunable optical resonances, electronic properties and easy surface functionalization approaches. All these factors make gold nanoparticles versatile platforms for different nano-biological application (Yeh et al. 2011; Han et al. 2007; Chirico et al. 2015). Gold nanoparticles are also very attractive due to the well-controlled size and due to the possibility to fine tuning optical properties by shape and size (Herizchi et al. 2016). Based on these properties, nowadays gold nanoparticles are explored in diverse biomedical application e.g. genomics, biosensors, immunoassays, clinical chemistry, laser phototherapy of cancer cells and tumors, the targeted delivery of drugs, DNA and antigens, optical bioimaging and

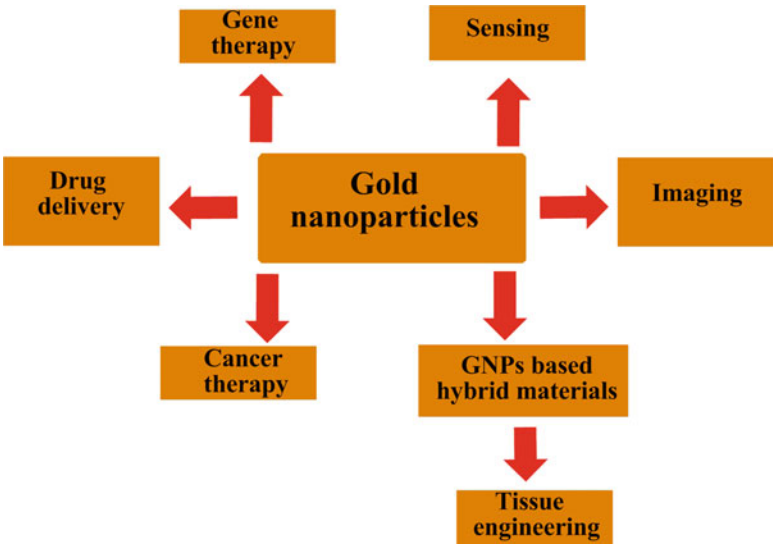

Fig. 10.1 Application of gold nanoparticles in biomedical fields

monitoring of cells and tissues (Dykman and Khlebstov 2011). The major possible applications fields of gold nanoparticles in biomedical field are shown in Fig. 10.1.

Therefore, it is useful to revise briefly the most essential properties of gold nanoparticles including the interaction of nanoparticles with cells before discussing the promising possibility of application in tissue engineering.

10.2.1 Synthesis of Gold Nanoparticles

Generally, gold nanoparticles are synthesized in a liquid phase by reduction of chloroauric acid ($HAuCl_4$) (Yeh et al. 2011; Chirico et al. 2015). This process usually contains two steps: (1) reduction using agents such as borohydrides, aminoboranes, hydrazine, formaldehyde, hydroxylamine, saturated and unsaturated alcohols, citric and oxalic acids, polyols, sugars, hydrogen peroxide, sulfites, carbon monoxide, hydrogen, acetylene, and monoelectronic reducing agents including electron-rich transition-metal sandwich complexes; (2) stabilization by agents such as trisodium citrate dihydrate, sulfur ligands (in particular thiolates), phosphorus ligands, nitrogen-based ligands (including heterocycles), oxygen-based ligands, dendrimers, polymers and surfactants (Zhao et al. 2013). Stable gold nanoparticles can be also synthesized by laser ablation without the addition of any external chemical reagent (Wender et al. 2011). The particle shape and size depend on a large numbers of parameters, for example, it can be controlled by the initial reagent concentrations (Zhou et al. 2009; Hostetler et al. 1998) and also on the nature of

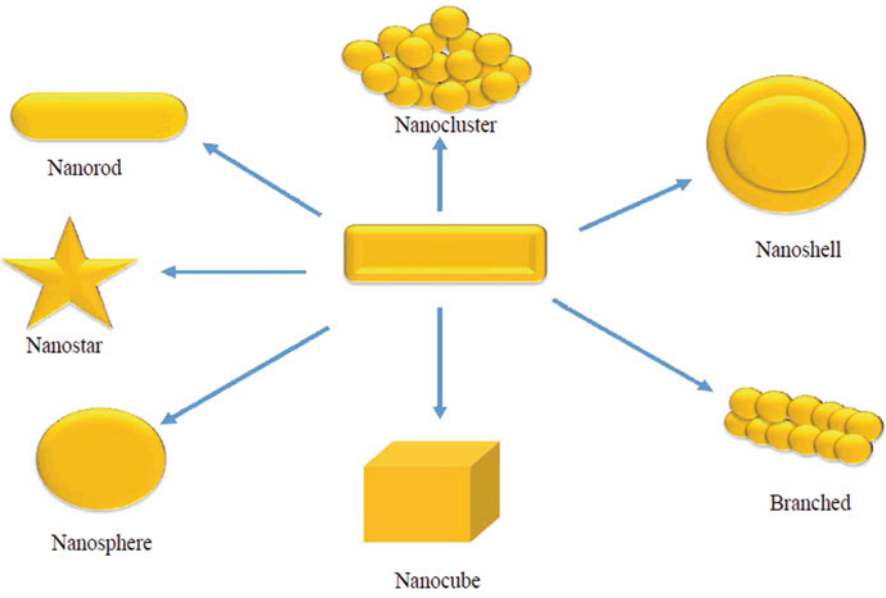

Fig. 10.2 Examples of different shapes of existing gold nanoparticles. (Modified after Alaqad and Saleh 2016)

surfactant (Pallavicini et al. 2013). Some of the various shapes of gold nanoparticles that can be prepared by the existing synthetic techniques are summarized in Fig. 10.2:

One of the simplest approach to produce gold nanoparticles is based on reduction of $HAuCl_4$ pioneered by *J. Turkevich* and refined by *G. Frens* in 1970s (Frens 1973). This method is used in general to obtain monodisperese spherical gold nanoparticles suspended in water with an average size 10–20 nm. Nowadays the *"in situ" Turkevich-Frens* method has been further improved for reproducible preparation of citrate-stabilized gold nanoparticles (Kimling et al. 2006; Polte et al. 2010). The two-phase *Brust-Schiffrin* method, published in 1994, was the first method for the *in-situ* synthesis of thiolate-stabilized gold nanoparticles, based *on* $NaBH_4$ as reduction agent (Brust et al. 1994). This method is performed in ambient conditions with relative high stability of the resulting gold nanoparticles with diameter located in the 2–5 nm range (Templeton et al. 2000; Sardar and Shumaker-Parry 2009).

Compared with the in situ synthesis, the recent widely used *seed-growth* technique enlarges the particles step by step, and it is much easier to control the sizes and shapes of resulted gold nanoparticles (Nikoobakht and El-Sayed 2003; Ziegler and Eychmüller 2011; Leng et al. 2015). This powerful strategy involves two main steps: small-size gold nanoparticles seeds preparation and adding of resulted seeds to a "growth" solution containing $HAuCl_4$ and the stabilizing and reducing agents

(Bastus et al. 2011; Casu et al. 2012; Pallavicini et al. 2013). Among other techniques *Martin method* discovered in 2010 allows to obtain stable "naked" gold nanoparticles in water by reducing $HAuCl_4$ with sodium borohidride (Martin et al. 2010). The resulted gold nanoparticles are easily functionalized with hydrophilic species or hydrophobic ligands in non-polar solvents.

The sonolysis synthetic approach based on ultrasound was introduced by Baigent and Müller (1980). Later, the ultrasound was applied to form gold nanoparticles during reaction of $HAuCl_4$ aqueous solution with glucose (Zhang et al. 2006). In this case, the reducing agents are hydroxyl radicals and sugar pyrolysis radicals that form at interface of collapsing cavities and the bulk water. The resulted gold nanoparticles have a shape of nanoribbons with width 30–50 nm and length of several micrometers.

10.2.1.1 "Green" Synthesis of Gold Nanoparticles

Common methods of gold nanoparticles synthesis usually lead to the adsorption of some toxic chemical species e.g. surfactants on the nanoparticles surface that can cause a problem for effective application in the medical field, such as tissue engineering (Singh and Scrivastava 2015). This drawback forced researchers to utilize biofriendly and eco-compatible natural compounds for the reduction of Au-containing salts needed for the synthesis of gold nanoparticles (Mandal et al. 2006). These new approaches also improve substantially the production efficiency, being based on a one-step process, with no need of supplementary surfactants or polymers, capping agents etc. In addition, these synthetic techniques can significantly improved the biocompatibility of gold nanoparticles. Recently, the following natural sources have been successfully utilized in gold nanoparticles "green" synthesis: *plant extracts* (Sujitha and Kannan 2013; Bhau et al. 2015; Sett et al. 2016; Patra and Baek 2015), *aglae* (Ramakrishna et al. 2016; Rajeshkumar et al. 2013; Parial et al. 2012), *bacteria and fungi* (Kitching et al. 2015; Mukherjee et al. 2002), *polysaccharides* (Huang and Yang 2004; Potara et al. 2009; Pandey et al. 2013), and *proteins* (Leng et al. 2016). Another one-step "green" approach is based on thermal evaporation of gold from surfaces to obtain gold nanoparticles (Anantha et al. 2012).

This subchapter provided very brief review of classical and recently developed techniques of gold nanoparticles preparation. The importance of "green approaches" of gold nanoparticles synthesis for tissue engineering should be highlighted. All previously mentioned "green" methods are being actively investigated since, beside the economical benefits that comes from the use of cheap compounds, absence of toxic compounds, the antibacterial, antifungal and antioxidative properties of gold nanoparticles prepared by these "green" techniques, could also bring additional valuable properties of gold nanoparticles based scaffolds.

10.2.2 Surface Decoration of Gold Nanoparticles

Surface functionalization of the nanoparticles with suitable ligands is essential to ensure the stability of nanoparticles against aggregation or to enhance the targeting efficiency for cells (Park et al. 2013; Tiwari et al. 2011). Indeed, finely tuned surface decoration of the nanoparticles, which determines the interaction of nanoparticles with the environment and provides biocompatibility, is strongly required for an efficient application of gold nanoparticles in nanomedicine in general and in particular for tissue engineering (Sperling and Parak 2010). Briefly, the proper surface decoration of gold nanoparticles brings following advantages: makes them biocompatible, stable in physiological media, provides the possibility to carry and deliver, drugs and target molecules, reduces the non-specific binding, and enhances accumulation of gold nanoparticles. Moreover, proper tuning of surface chemistry promotes effective cells adhesion proliferation, and growth. Therefore, surface functionalization of gold nanoparticles is an important condition for efficient application in tissue engineering. For this purpose, the most common strategies of functionalization of gold nanoparticles are reviewed.

Gold nanoparticles can be easily functionalized using different kind of ligands, synthetic and nature origin polymers, biomolecules, etc. depending on the final application (Spampinato et al. 2016). The most widely applied approaches are following:

(a) functionalization of gold nanoparticles with thiol and disulfide containing molecules;
(b) embedding of gold nanoparticles into inorganic/polymer shell;
(c) non-covalent functionalization of gold nanoparticles.

These methods are pictorially summarized in Fig. 10.3:

The direct interaction of the gold surface with thiols (-SH) and disulfides (-S-S-), forming self-assembled monolayers, is the most widely employed method for grafting a coating onto the gold nanoparticles (Borzenkov et al. 2015). This may be either a small molecule or a polymer or a combination of both (Daniel and Astruc 2004; Gronbeck et al. 2000). Biocompatible polyethylene glycols (PEGs), with terminal –SH groups, are very frequently used as coatings for gold nanoparticles, due to many advantages in bio-medical applications (Borzenkov et al. 2015; Chirico et al. 2015). PEG coating is also used for nanoparticle specific functionalization, as many commercial PEGs feature, in addition to the thiol function for grafting on gold, terminal functional groups (e.g. -OH, -COOH, $-NH_2$) suitable for further chemical modification (Manson et al. 2011; Shenoi et al. 2013). The corresponding bifunctional linkers allow also to conjugate gold nanoparticles with biomolecules e.g. DNA, peptides, antibodies and proteins (Ojea-Jimenez and Puntes 2009; Li et al. 2013; Jazayeri et al. 2016).

Embedding gold nanoparticles into inorganic/polymer shell allows to enhance several properties of the nanoconstructs. The shell structure usually bears functional groups for further modification or for recognition of and binding with bio-molecules,

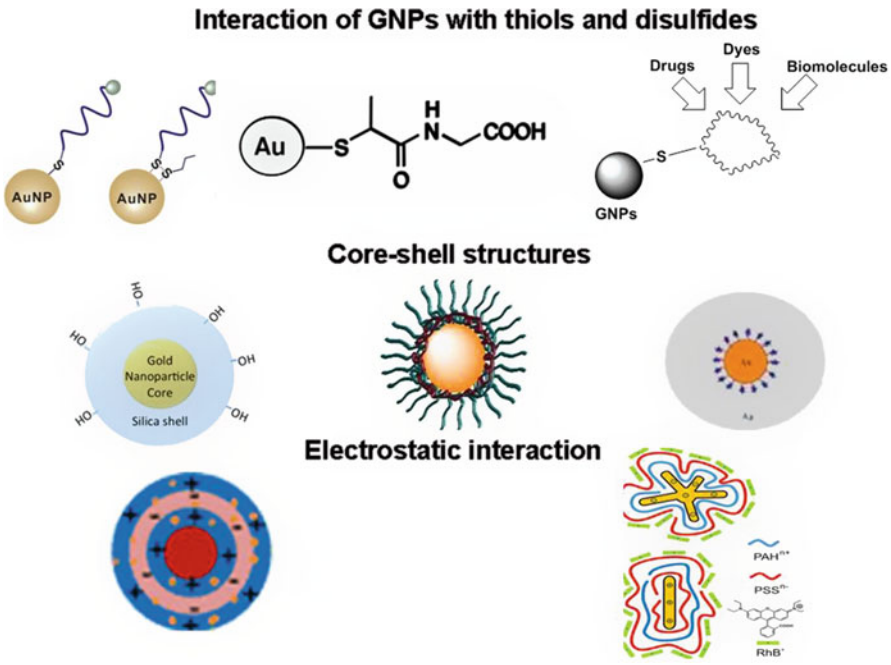

Fig. 10.3 Common functionalization methods of gold nanoparticles: covalent functionalization, preparation of core-shell structures, and electrostatic interaction

such as cellular receptors (Vial et al. 2016). For example, incorporating of gold nanoparticles into polymer nanospheres is attractive for developing biocompatible nanomaterials and plasmonic photonic crystals (Khan and MacLachlan 2015). Such structures exhibit high biocompatibility, biodegradability and high cell uptake (Vial et al. 2016; Jokerst et al. 2012). A broad range of gold nanoparticles core-shell structures were studied, with various shell nature such as iron oxide (Liang et al. 2009), silica (Kandpal et al. 2007; Liz-Marzan et al. 1996; Fales et al. 2011), silver (Lu et al. 2013; Pustovalov et al. 2012), polymers like poly(N-isopropylacrylamide), chitosan, amino-terminated polystyrene (Kanahara et al. 2014; Dong et al. 2014; Wu et al. 2016).

The non-covalent functionalization strategy is based on a combination of electrostatic and hydrophobic interactions of the molecules and the gold surface (Rayavarpu et al. 2007). One of the most frequently used technique of non-covalent coating is the so-called layer-by-layer method that provides multilayer structures (Vial et al. 2016). Charged target molecules can be electrostatically bound onto the outer layer, or better incorporated within alternate layers. The multiply layers were formed using different charged compounds such as polyelectrolytes (Mayya et al. 2003; Toccoli et al. 2012; Dorris et al. 2008), proteins (Brewer et al. 2005; Takahashi et al. 2008), oligonucleotides (Elbakry et al. 2009; Bishop et al. 2015), antibodies (Jazayeri et al. 2016).

10.2.3 Conductive Properties of Gold Nanoparticles

Electrical stimulation was shown to enhance cardiac, muscle and nerve tissues growth therefore raising the interest of researchers for diverse conductive polymers that could be used for scaffolds fabrication (Ghasemi-Mobarakeh et al. 2011; Amezcua et al. 2016). In this line of research, the incorporation of conductive nanoparticles (e.g. metal, carbon nanotubes) in scaffolds was also applied (Martins et al. 2014; You et al. 2011). The electrical conduction properties of gold nanoparticles have been intensively exploited for the development of gold nanoparticles based devices and sensors including also nanoelectronic devices for biomedical applications (Tateno et al. 2014; Wuelfing et al. 2010; Zotti et al. 2008; Homberger and Simon 2010; Edwardson et al. 2016).

Therefore, the electrical properties of gold nanoparticles can be advantageously used to fulfill the needs of tissue engineering, particularly of cardiac and nerve tissues growth. For example, the intercellular electrical communications can be enhanced presence of gold nanoparticles in hybrid fibrous scaffolds (Shevach et al. 2013). In addition, engineering of gold nanoparticles based hybrid scaffolds can promote superior electrical signal conductivity (Fleischer et al. 2014).

10.2.4 Optical Properties of Gold Nanoparticles

Unique optical properties of gold nanoparticles provide additional advantages to be applied in tissue engineering. For example, they assist to monitor cells differentiation process, cells uptake and migration (Vial et al. 2016). Therefore, the whole tissue regeneration process can be easily studied. In addition, their photo-thermal properties can promote cells growth and differentiation as will be discussed below. For this reason the brief discussion of Localalized Surface Plasmon Resonance of gold nanoparticles as a key-factor of their photo-thermal properties is provided. Gold nanoparticles absorb and scatter light with extraordinary efficiency with respect to organic compounds. Such impressive interaction with lights is explained by the fact that the conduction electrons on the gold surface undergo a collective oscillation when they are excited by light at certain wavelengths (Huang and El-Sayed 2010; Freddi et al. 2013). This interaction produces coherent localized plasmon oscillations with a resonant frequency that strongly depends on the composition, size, geometry, dielectric environment and particle–particle distance of nanoparticles (Chirico et al. 2015).

The Localized Surface Plasmon Resonance (LSPR) response arises from the electric field of the incident light driving surface conduction electrons collectively away from the metal nanoparticle lattice (Messersmith et al. 2013). As a result of corresponding collective factors, the absorption and scattering cross-sections of gold nanoparticles are much higher than non-plasmonic nanoparticles. Apart of particle size and local surface refractive index, the shape of the LSPR spectra can be widely tuned also by varying of gold nanoparticles shape (Chirico et al. 2015; Nehl and

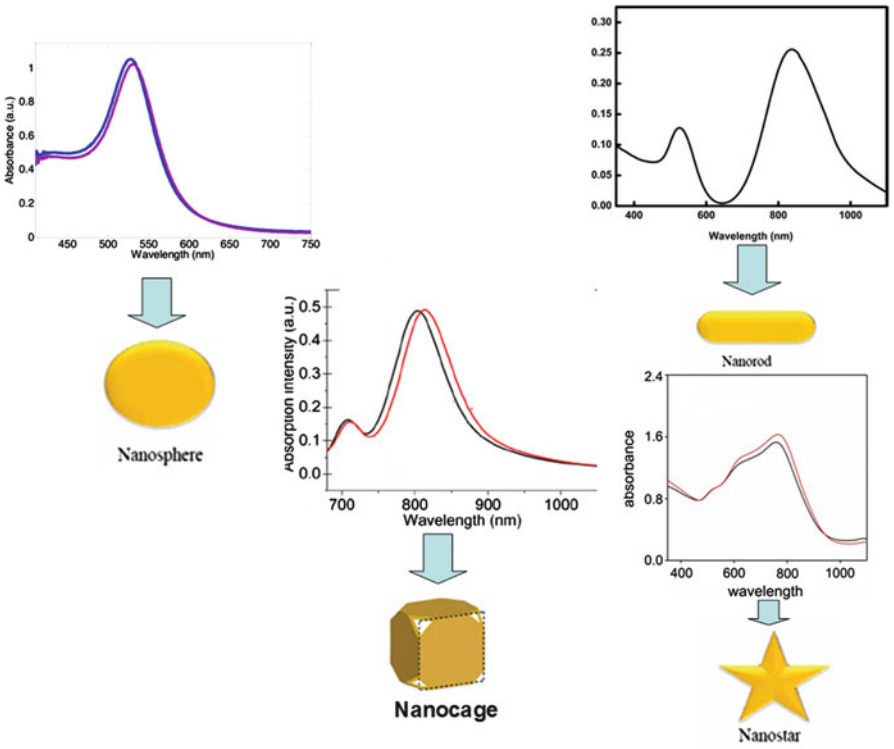

Fig. 10.4 Localized Surface Plasmon Resonance (LSPR) positions of different types of gold nanoparticles

Hafner 2008; Noguez 2007). The LSPR positions of spherical and some types of nonspherical gold nanoparticles are schematically shown in Fig. 10.4:

Precise tuning of LSPR position can be also achieved by regulating concentrations of reagents during preparation stage (Casu et al. 2012; Pallavicini et al. 2013). The sensitivity of LSPR to environment changes and surface modification of gold nanoparticles have led to development gold nanoparticles based biosensors (Messersmith et al. 2013). In addition to wavelength-selective photon absorption and scattering, an important consequence of LSPR excitation is the local electromagnetic field enhancement that lies at the heart of surface-enhanced spectroscopy (Butler et al. 2015; Vo-Dinh et al. 2010).

The capability of gold nanoparticles to locally release heat upon irradiation with wavelength that matches $LSPR_{max}$ of nanoparticles is promising and valuable property, that has been already applied in cancer hyperthemal treatments (Huff et al. 2007; Faheem and Banu 2014). In addition it provides a prospective opportunity also for tissue engineering, as suggested recently for optical stimulation of cells growth (Paviolo et al. 2013; Gentemann et al. 2017). Therefore, this phenomenon should be discussed more in details.

10.2.4.1 Photo-Thermal Effect of Gold Nanoparticles

The possibility to trigger by Near-Infrared irradiation a localized heat release from metal nanoparticles, is an attractive approach to provide spatial and temporal control of heat both in vivo and in vitro (Kabb et al. 2015; Avvakumova et al. 2016). The application of gold nanoparticles in local hyper-thermal treatment exploits high absorption cross-sections of gold nanoparticles in the Near-Infrared (700–1200 nm) which is the range of the so called biological transparent window (Borzenkov et al. 2016). The absorption cross-section has a large dependence on the gold nanoparticles shapes and the corresponding LSPR band can be tuned by changing the shape parameters (Richardson et al. 2006). Beside the direct hyperthermal effect, gold nanoparticles can be used also as smart drug delivery vehicles, in which the release of the bound compounds can be triggered by the localized heating obtained by Near-Infrared irradiation (Bakhtiari et al. 2009; Borzenkov et al. 2015). Closely related to the medical field, also the possibility to exploit the local photo-thermal effect for controlled deformations of macroscopic shape-memory polymeric nanocomposites was reported (Zhang et al. 2014).

The group of El-Sayed has pioneered the use of gold nanoparticles induced hyperthermia for cellular treatments. Spherical gold nanoparticles with a peak absorption at 530 nm have been initially exploited. They were irradiated at 514 nm to kill cancer cells in vitro (El-Sayed et al. 2006). However, spherical gold nanoparticles absorb only visible light, being generally poor prospects for tissue heating since the penetration of ultraviolet and visible light in tissues is limited. Non-spherical gold nanoparticles can instead be used to locally release heat in the tissues, when irradiated in Near-Infrared region where tissues are transparent (Huang et al. 2006). For example, gold nanostars with LSPR finely tuned by synthetic conditions in the range 700–1100 nm, displayed a pronounced photo-thermal effect under Near-Infrared irradiation in solutions, as monolayers grafted on the dry glass surface, and when printed on coated flexible paper substrates, showing higher temperature increase in the latter case (Casu et al. 2012; Borzenkov et al. 2015, 2016; Pallavicini et al. 2014, 2015). In addition, it was shown that the photo-thermal action of the gold nanostars monolayer on glass efficiently induces cell death in *S. aureus* biofilms upon Near-Infrared irradiation (Pallavicini et al. 2014).

The direct monitoring of the temperature around gold nanoparticles is also essential to test therapeutic efficiency of nanoparticles (Shao et al. 2013; Zharov et al. 2006). Freddi et al. proposed an all-optical method to measure the temperature of nonspherical gold (nanorods and nanostars) and magnetite nanoparticles under Near-Infrared and radiofrequency excitation, based on the temperature dependence of the excited state lifetime of Rhodamine B, bound at ≈20 nm from the nanoparticles surface (Freddi et al. 2013). It was shown that gold nanostars are ≈3 and ≈100 times more efficient that than gold nanorods and magnetite nanoparticles in inducing localized hyperthermia as it shown in Fig. 10.5:

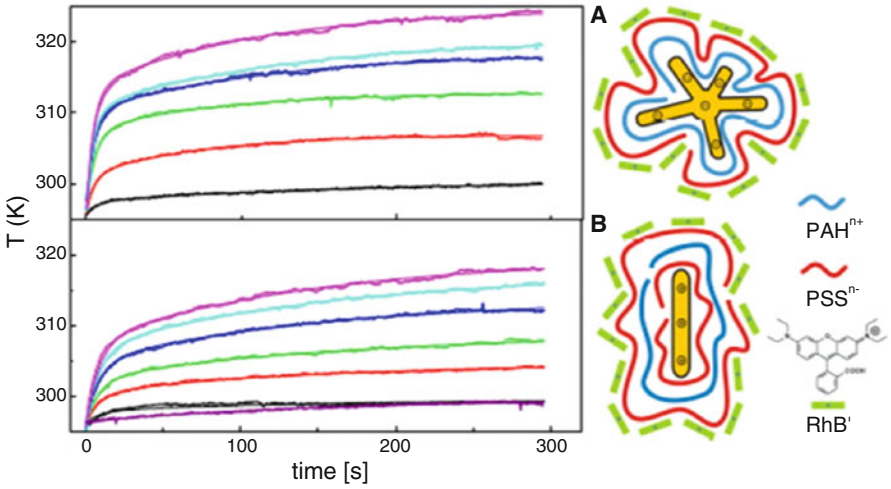

Fig. 10.5 The temperature increased profile of gold nanostars and gold nanorods coated with polyelectrolytes and decorated with Rhodamine B (Reprinted with permission from Freddi et al. 2013)

10.2.4.2 Gold Nanoparticles as Tracking and Contrast Agents

Nanoparticles are also widely used as contrast agents for molecular targeting imaging by exploiting various types of interactions. Single particle tracking is an important tool to investigate dynamic biological processes by following the movement of individual labeled molecules with high spatial and temporal resolution using various microscopic techniques (Rong et al. 2008). Regarding to tissue engineering field an accurate imaging of cells, differentiation process and subsequent tissue regeneration process is crucial for in vitro and in vivo studying of the scaffold performance (Vial et al. 2016; Appel et al. 2013). Four major imaging techniques employing gold nanoparticles as contrast agents have been developed: *optical fluorescence imaging*, *X-ray imaging*, *photoacoustic imaging*, and *dark field optical microscopy*.

In addition to chemical stability and wide opportunities of surface functionalization, the strong two-photon luminescence of gold nanoparticles coupled to a specific targeting makes these nanoparticles ideal candidates as contrast agents for optical microscopy, which is the most adequate to investigate in vivo samples (Chirico et al. 2015). Major advantages of the use of gold nanoparticles, compared to the organic dyes, are the large absorption cross-section of the gold nanoparticles and extreme photophysical stability. Gold nanoparticles do not blink or bleach unlike many fluorescent dyes or quantum dots (Rong et al. 2008). Indeed, different types of gold nanoparticles have recently been successfully examined as contrast agents for biomedical imaging because of brightness of gold nanoparticles at near-infrared wavelengths, which can penetrate through tissue better than visible light (Chirico et al. 2015).

Gold induces also a strong X-ray attenuation making gold nanoparticles attractive for in vivo computer tomography molecular imaging (Popovtzer et al. 2008). Hybrid nanoparticles such as antibiofouling polymer-coated gold nanoparticles, gadolinium coated gold nanoparticles, PEG coated gold nanoparticles were developed as computer tomography contrast agents (Kattumuri et al. 2007; Alric et al. 2008; Cai et al. 2007). It was demonstrated that the assembly of gold nanoparticles that form exclusively on the targeted cancer cells yield a strong selective X-ray attenuation that is distinct from the attenuation obtained by identical but untargeted cancer cells or by normal cells (Popovtzer et al. 2008).

Gold nanoparticles as exogenous contrast agents have also great potential for photoacoustic imaging due to inherent and geometrically induced optical absorption (Li and Chen 2015). In addition, at the LSPR wavelengths gold nanoparticles have higher extinction coefficients than conventional organic dyes (Song et al. 2016). The most widely utilized gold nanoparticles for photoacoustic imaging are spheres, rods, shells, prisms, cages, stars and vesicles (Li 2015). Dark field optical microscopy is another commonly applied technique to monitor cellular uptake, cell migration and molecular affinity (Nenasheva et al. 2012; Ahijado-Guzman et al. 2014). It should be noted that upon light excitation only frequencies matching the LSPR are strongly scattered resulting to visualization of gold nanoparticles as bright spots (Vial et al. 2016). Recently super-resolution imaging of fluorescence-free plasmonic gold nanoparticles was achieved using enhanced dark-field illumination based on wavelength-modulation (Zhang 2015).

10.2.5 Interaction of Gold Nanoparticles with Cells and Toxicity of Gold Nanoparticles

The information about long-term and short-term biological effects of gold nanoparticles nanotoxicity is crucial for safe application in tissue engineering (Yen et al. 2009). Potential risks and possible biomedical effects of gold nanoparticles on human organism must be assessed firstly, as utilization of non-toxic or biodegradble components is important requirement in the tissue engineering field (Wang 2015a, b; Vial et al. 2016). In physiological conditions, gold nanoparticles can be internalized, trafficked, stored, or secreted by cells (Wang 2015; Wang et al. 2011). However, the interaction of gold nanoparticles with cells is rather complicated interfacial process in space and time (Wang 2015).

Gold nanoparticles can be internalized by cells along at least two major pathways: receptor-mediated endocytosis and phagocytosis (Wang 2015). The impact of the gold nanoparticles size and surface effects on cell interactions has been widely investigated (Chirico et al. 2015; Liu et al. 2013). For example, it was demonstrated that, for spherical gold nanoparticles stabilized by citric acid ligands, 50-nm diameter is an optimal size to maximize the rate of uptake and intracellular concentration in mammalian HeLa cells (Chithrani et al. 2006). Once internalized, nanoparticles

may affect the cellular activity at different levels, by interacting with vital cell components such as the membrane, mitochondria, or nucleus (Alkilany and Murphy 2010). Star-shaped gold nanoparticles with average size 180 nm increased firing rate of hippocampal neurons modifying excitability of neurons (Salinas et al. 2014). Gold nanoparticles can also induce cell differentiation as it was demonstrated in case of mesenchymal stem cells and NG108-15 neuronal cells (Yi et al. 2010; Paviolo et al. 2013).

However, the nanotoxicological screening studies done in vitro could not predict accurately in vivo toxicity (Griffith and Swartz 2006). Gold nanoparticles are found to be non toxic due to numerous reports (Connor et al. 2005; Alkilany 2010; Villiers et al. 2009). On the other hand, other reports claimed some toxicity of gold nanoparticles (Goodman et al. 2004; Pan et al. 2009). Such contradictory results may rise from the variability of the used toxicity assays, cell lines, and nanoparticles chemical/physical properties. The strong need to systematize all collected data suggests employing an appropriate metric, e.g. the particle concentration, that could help in comparing and organizing the available data in a more accurate way (Fratoddi et al. 2015). In fact, it was demonstrated that at constant numerical particle concentration (number of particles per unit volume of cell culture), at least in the case of HeLa cells, no effect of diverse functionalization could be observed (Fratoddi et al. 2015). At least for this cell line, the particle concentration was the single parameter that triggered toxicity. Therefore, it is mandatory to define the therapeutic window together with synthetic and coating protocols where gold nanoparticles can be employed without side effects (Fratoddi et al. 2015). In addition, the important issue is also to understand the long term effect of gold nanoparticles on organism, clearly defining the difference between toxicity and cell damage. From this short discussion is evident that there is a strong need or reliable models for detailed in vivo investigations (Rushton et al. 2010; Fratoddi et al. 2015).

10.3 Gold Nanoparticles in Cardiac Tissue Engineering

The main goal of cardiac tissue engineering is the development of functional tissue constructs that can reestablish the structure and function of injured myocardium (Vunjak-Novakovic et al. 2010). In cardiac tissue engineering contracting cells are seeded within supporting biomaterial scaffolds that provide cells with the microenvironment which is essential for the assembly of functional cardiac tissue (Shapira et al. 2016). A wide range of approaches and methods for cardiac tissue engineering scaffolds creation have been proposed and investigated up to now. In addition to bulk synthetic and natural materials, nanomaterials are being actively investigated. They are considered particularly promising as they are able to significantly improve mechanical, electrical, optical and magnetic properties due to large surface-area-to-volume ratio and surface roughness of nanomaterials (Amezcua et al. 2016). The limiting factor of most engineered scaffolds for cardiac tissue engineering is that they are electrically insulating (Suuronen and Ruel 2015). Cardiac muscle is a type

of tissue that would benefit from an electrically conductive scaffold to regenerate lost or lower functional areas (Mckeon-Fischer and Freeman 2012). The native myocardium displays a direct current conductivity of 0.1 S/m and the conduction is facilitated by electrically conducting Purkinje fibers (Shin et al. 2013; Suuronen and Ruel 2015). Therefore, one of the main conditions of successful application of scaffolds for cardiac tissue engineering is the ability to reduce electrical impedance of the cellular environment.

Incorporation of gold nanoparticles into scaffold structure can improve the cardiac excitability and the cellular attachment. Microporous thiol-hydroxyethyl methacrylate scaffolds with immobilized gold nanoparticles for cardiac tissue engineering were fabricated and tested (You et al. 2011). Cardiac muscle tissues cultured on such scaffolds demonstrated improved levels of connexin 43. However, the sophisticated preparation technique and absence of cell adhesive functions limited the application. The application and limitation of conductive polyaniline and gold nanoparticles for fabrication conductive scaffolds for cardiac and muscle tissues growth was discussed by Mckeon-Fischer and Freeman (2012). Gold nanoparticles incorporated into PET polymer structure enhanced the cellularity of the reconstituted tissue, reduced ROS, and reduced bacteria adhesion to PET (Whelove et al. 2011). Significantly enhanced biocompatibility of the polyurethane – gold nanoparticles composites over the original polyurethane was also achieved by extensive modification of the surface morphology and by increasing the free radical scavenging ability (Hsu et al. 2008).

With particular regard to cardiac tissue engineering, electrospinning is considered to be most effective fabrication technique. These structures mimic extracellular matrix fibrous structure and enhance the cell adhesion (Tallawi et al. 2015). For example, peptide-functionalized gold nanoparticles were also successfully incorporated within polymethylglutarimide nanofibers through electrospinning (Jung et al. 2012). Grafting functionalized gold nanoparticles led to high-density localization of the cell-adhesive peptides on the nanofiber and to the enhancement of HeLa cell adhesion that potentiated cardio-myocyte differentiation of human pluripotent stem cells.

Cohen-Karni et al. combined controlled cell-biomaterial interactions and improved cell proliferation and differentiation by fabricating silk nanofibers containing gold nanoparticles that were subsequently decorated with peptides (Cohen-Karni et al. 2012). Silk fibroin mixed with gold seed nanoparticles was electrospun to form nanofibers doped with gold seed nanoparticles. Following gold reduction, there was a twofold increase in particle diameter confirmed by the appearance of a strong absorption peak at 525 nm. The resulted scaffolds displayed improved Young's modulus and were then modified with RGD peptide due to the interaction with gold nanoparticles. It was shown that Human mesenchymal stem cells cultured on such scaffold had a more than twofold larger cell area compared to the cells cultured on bare silk fibroin scaffold.

With a similar approach the research group led by *Tal Dvir* demonstrated that gold nanoclusters can be incorporated into macroporous scaffolds to increase the matrix conductivity and enhance the electrical signal transfer between cardiac cells

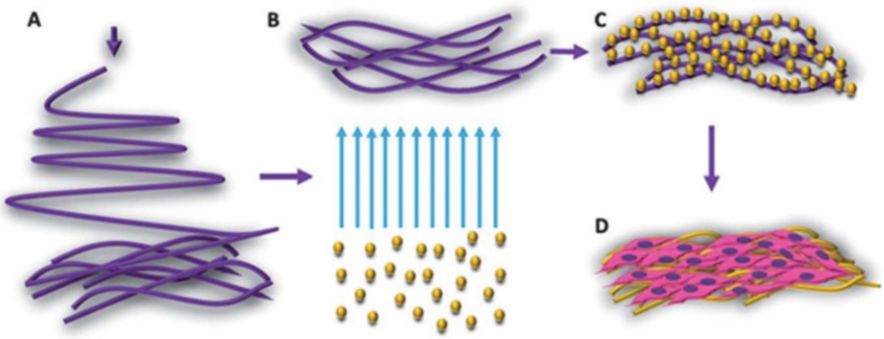

Fig. 10.6 Fabrication of gold nanoparticles based hybrid scaffold for cardiac tissue regeneration. (**a**) Fabrication of polycaprolactone–gelatin fiber scaffolds by electrospinning. (**b**) Evaporation of gold nanoparticles onto the fibers to create nanocomposite scaffolds. (**c**) Cardiac cells are seeded in the nanocomposite scaffolds for engineering a functional cardiac tissue. (Reprinted with permission from Shevach et al. 2013)

(Shevach et al. 2013). For this purposes gold nanoparticles were evaporated on the surface of polycaprolactone–gelatin fibrous scaffolds fabricated by means of electrospinning creating nanocomposites with a nominal gold thickness between 2 and 14 nm. The scaffold fabrication strategy is shown in Fig. 10.6.

The authors showed that cardiac cells seeded on the nano-gold scaffolds assembled into more elongated and aligned tissues. Also, the presence of gold nanoparticles in scaffold structure promoted the growth of cardio-myocytes with significantly higher aspect ratio and promoted massive cardiac sarcomeric actinin expression. In general, cardiac cells grown on gold nanoparticles decorated scaffolds exhibited significantly higher contraction amplitudes and rates, as compared to those grown on scaffolds without gold.

It is worth mentioning that in a previous work Tal Dvir et al. showed that incorporation of gold nanowires within alginate scaffolds can bridge the electrically resistant pore walls of alginate and improve electrical communication between adjacent cardiac cells (Dvir et al. 2011). Gold nanowires were grown by anisotropic elongation of the seeds (Kim et al. 2008). Tissues grown on these composite matrices were thicker and better aligned than those grown on pristine alginate and it was expected that the integration of conducting gold nanowires improve the therapeutic value due to enhanced electrical properties. These works laid the basis for the more recent development of composite nanofibrous structures incorporating gold nanoparticles. As briefly outlined below, two major approaches are related to the use of fibrous synthetic compounds or hydrogels, or to the use of decellularized matrices.

Regarding the employment of synthetic matrices, most of the applications appeared on polymeric nanofibers. Coiled electrospun fibers with gold nanoparticles were presented as a new promising nanocomposite scaffold for cardiac tissue engineering (Fleischer et al. 2014) since the natural heart matrix contains a unique subpopulation of coiled perimysial fibers that provide the mechanical properties

crucial for efficient and continuous contractions (Robinson et al. 1988; Fleischer et al. 2014). Gold nanoparticles were obtained by evaporation of gold on polycaprolactone fibers. It was shown that integrated gold nanoparticles provided anisotropic transfer of the electrical signals throughout engineered cardiac tissues. Cultivation of cardiac cells within the fabricated hybrid scaffolds led to cell organization into elongated and aligned tissues generating a strong contraction force, high contraction rate and low excitation threshold.

Nanocomposite scaffold based on gold nanotubes/nanowires incorporated into biodegradable castor oil based polyurethane was fabricated to be applied in cardiac tissue engineering (Ganji et al. 2016). H9C2 cardiomyocyte cells were cultured on the scaffolds for one day, and electrical stimulation was applied to improve cell communication and interaction in neighboring pores. Gold nanotubes/nanowires were fabricated by using template-assisted electrodeposition and gold in the form of nanowires allowed the formation of conductive bridges between pores and enhanced cell communication. Also, the addition of gold nanoparticles caused the formation of hydrogen bonding with the polyurethane matrix and improved the thermomechanical properties of nanocomposites. It was demonstrated that gene expression level was up-regulated by the incorporation of gold nanotubes/nanowires into the polyurethane scaffolds, in particular after electrical stimulation. Moreover, hybrid scaffolds displayed more native morphology and enhanced proliferation compared to gold-free scaffolds.

Gold nanoparticles with the size of 16 nm were embedded into nanofibrous polycaprolactone together with vitamin B12, *aloe vera* and silk fibroin to fabricate novel scaffolds to differentiate mesenchymal stem cells into cardiac lineage (Sridhar et al. 2015). Nanoparticle loaded nanofibrous scaffold displayed a mechanical strength of 2.56 MPa matching that of the native myocardium. The most important conclusion is that phenotype and cardiac marker expression in differentiated cells were highly resonated in gold nanoparticle loaded nanofibrous scaffolds.

Regarding the use of hydrogels, the use of chitosan based thermosensitive conductive hydrogel with a highly porous network of interconnected pores, as scaffold for cardiac tissue engineering was reported (Baei et al. 2016). Chitosan stabilized gold nanoparticles were evenly dispersed throughout the polymer matrix in order to provide electrical cues. The fabricated scaffolds supported viability, metabolism, migration and proliferation of mesenchymal stem cells. Therefore, it was confirmed that incorporation of electro-conductive gold nanoparticles into hydrogel structure enhances the properties of myocardial constructs. Hybrid material based on a natural collagen scaffold with incorporated gold nanoparticles was also reported recently (Li et al. 2016). Gold nanoparticles, namely gold nanorods were immersed in collagen matrix uniformly providing easier adhesion and better matching of the cell on substrate. The presence of gold nanoparticles improved mechanical properties of scaffolds, increased protein expression and Ca^{2+} fluctuation. The authors demonstrated that such hybrid scaffolds with suitable stiffness distribution properties could efficiently regulate ID assembly and formation in

cultured cardiac myocytes. Previously it was shown, that nanocomposites based on I type collagen containing a small amount (17.4, 43.5, and 174 ppm) of gold nanoparticles (approximately 5 nm) displayed improved biocompatibility and promoted proliferation and migration of mesenchymal stem cells (Hung et al. 2014). Gold-coated collagen nanofibers produced by a single-step reduction process were found to be biocompatible and to improve the myocardial and neuronal differentiation process of the mesenchymal stem cells (Orza et al. 2011).

Ultraviolet-crosslinkable gold nanorod-incorporated gelatin methacrylate hybrid hydrogels with enhanced material and biological properties for cardiac tissue engineering were fabricated (Navaei et al. 2016). Embedded nanorods promoted electrical conductivity and mechanical stiffness of the hydrogel matrix. Later, gelatin methacrylate hydrogel constructs comprised of surface micro-topographies incorporated with electrically conductive gold nanorods were developed to provide simultaneous electrical and topographical cues that mimic physiological relevant myocardium function (Navaei et al. 2017). Notably, that only electrically conductive cardiac tissues showed a consistent response in changing beat rate as a result of external stimulation.

Along similar research lines, a novel therapeutic hybrid scaffold that can couple electrical, mechanical, and biological properties suitable for cardiac tissue engineering was developed (Ravichandran et al. 2013). The electrospun scaffold was based on BSA/PVA and embedded gold nanoparticles in the ratios BSA/PVA/Au of 2:1:0.1. The structure of fabricated gold nanoparticles based scaffold is shown in Fig. 10.7. Results indicated that obtained gold nanoparticles based scaffolds could

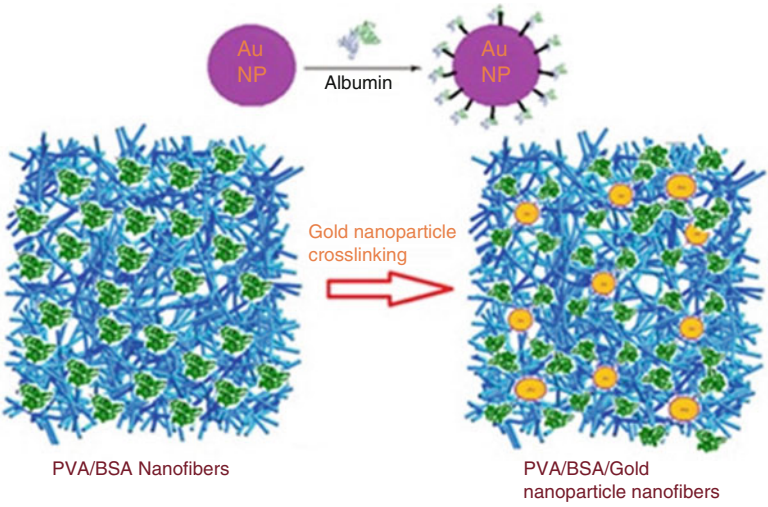

Fig. 10.7 Fabrication of PVA/BSA/Gold nanoparticles scaffolds for cardiac tissue engineering. (Reprinted with permission from Ravichandran et al. 2013)

lead to enhanced cardiomyogenic differentiation and result in superior biological and functional effects on infarcted myocardium regeneration.

Decellularized matrices have been considered since long times as promising and valuable scaffolds for engineering functional cardiac patches but the lack of quick and efficient electrical coupling between adjacent cells may jeopardize the success of the treatment (Shevach et al. 2014). To overcome this drawback gold nanoparticles were deposited on fibrous decellularized matrices and morphology, conductivity, and degradation of scaffolds was studied (Shevach et al. 2014). Gold nanoparticles were deposited on the scaffold's fibers using an e-beam evaporator. The authors showed that cardiac cells engineered within the hybrid scaffolds exhibited elongated and aligned morphology, massive striation, and organized connexin 43 electrical coupling proteins. In addition it was demonstrated that the hybrid patches display superior function as compared to pristine patches, including a stronger contraction force, lower excitation threshold, and faster calcium transients.

Interesting results have been reported very recently by utilizing photothermal properties of gold nanoparticles for cells simulation. Gentemann et al. applied a 532 nm picosecond laser to heat gold nanoparticles on cardiomyocytes leading to calcium oscillations in the HL-1 cardiomyocyte cell line (Gentemann et al. 2017). The authors observed a contraction rate increase in calcium containing buffer with neonatal rat cardiomyocytes. Notably, that in all tested cells these reactions were observed only in presence of gold nanoparticles. Therefore, this finding can provide novel approaches for a light based, nanoparticle mediated stimulation systems.

Indeed, incorporation of gold nanoparticles into scaffold structure can significantly improve mechanical, electrical properties, cell attachment on surface due to decoration of gold nanoparticles surface with proper ligands – all factors essential for successful application of designed scaffolds in cardiac tissue engineering. However, the important aspect is related to gold nanoparticles toxicity and impact on cardiac tissues in living organism. For example, the treatment of rat's heart tissue with 100 μl of 10 and 20 nm gold particles for 3 days or 7 days induced congestion in the heart muscle with prominent dilated blood vessels and extravasations of red blood cells. On the other hand, the treatment with 100 μl of 50 nm particles for 3 or 7 days demonstrated normal looking heart muscle with normal muscle direction and fascicles (Abdelhalim 2011). To have a comprehensive evaluation of the chronic cardiac toxicity of gold nanoparticles to the heart, PEGylated gold nanoparticles at three different sizes (10, 30 and 50 nm) were administrated to mice via tail vein for 14 consecutive days (Yang et al. 2016). Gold nanoparticles with smaller size displayed higher accumulation in mouse heart and faster elimination. It was shown, that none of the three sizes of gold nanoparticles affected cardiac systolic function. However, results indicated that the accumulation of small size gold nanoparticles can induce reversible cardiac hypertrophy. Therefore, the apart of numerous benefits of gold nanoparticles based scaffolds for cardiac tissue engineering, the toxicity of employed gold nanoparticles and impact on heart should be studied more precisely.

10.4 Gold Nanoparticles in Neural Tissue Engineering

Nerve regeneration process is a complex biological activity. In the peripheral nervous system, nerves can regenerate if injuries are tenuous (Schmidt and Leach 2003). However, larger injuries and spinal cord injuries are complicated processes, where self nerve regeneration is inhibited by numerous factors and neural tissue engineering can be a valuable help in the treatment of these injuries. For the peripheral nervous system the traditional treatment is based on nerve grafting. However, for spinal cord injury not much can be done without exogenous implants. Therefore, neural tissue engineering is also focused on the development of suitable environments for regeneration of neural tissue (Schmidt and Leach 2003). One of the first examples of the use of nanotechnology for neural tissue engineering was the effect of the surface nano-structure on the neuronal pattern of growth (Baranes et al. 2012, 2016). Indeed, nanotechnology can provide microstructured scaffolds to promote regeneration and direct repair by reconnecting axons (Limongi et al. 2017). Moreover, metal nanoparticles that act as anchoring sites for the small filopodial projections on 2D surfaces, allowed significant improvement of neurite–substrate interactions, leading to controlled growth of the neuronal processes (Baranes et al. 2016; Alon et al. 2014).

Park et al. evaluated the potential of gold nanoparticles (20 nm) to deliver electrical stimulation to nerve cell cultures in vitro to induce nerve regeneration (Park et al. 2008). For this puprpose authors introduced a novel method for the fabrication of a nanostructured 2D substrate in which gold nanoparticles were attached to the surface of cover glass via an adsorption system. By the electrical stimulation (250 mV for 1 h) through the network the authors observed an increase of the PC12 cells showing outgrowth length of neutrite, with a mean length of 98.5 μm. For comparison, the neutrite outgrowth length without electrical stimulation was approximately 10–20 μm. Interesting, that the alternating current stimulation showed good viability (<90%) of cells, while a high percentage of dead cells (more than 30%) was observed under constant current stimulation.

2D surfaces were therefore largely developed for neural tissue engineering purposes. On the other hand, 3D neuronal networks seem to give promising opportunities for repairing damaged spinal cord (Baranes et al. 2016). 3D composite scaffolds based on polycaptolactone/gelatin electrospun nonofibers decorated with gold nanoparticles (≈10 nm) encouraged a longer outgrowth of the neurites, as judged by the total length of the branching trees and the length and total distance of neurites (Baranes et al. 2016). The authors demonstrated that decorated nonofibers with gold nanoparticles provided additional topographical and anchoring sites for superior morphogenesis leading to more complex neuronal networks as shown in Fig. 10.8.

The authors suggested that future works must focus on determining the exact role of the gold nanoparticles in neuronal tissue engineering, e.g. investigating whether these nanoparticles promote axon elongation and higher expression of neuronal markers because of topographical cues or due to the conductivity of

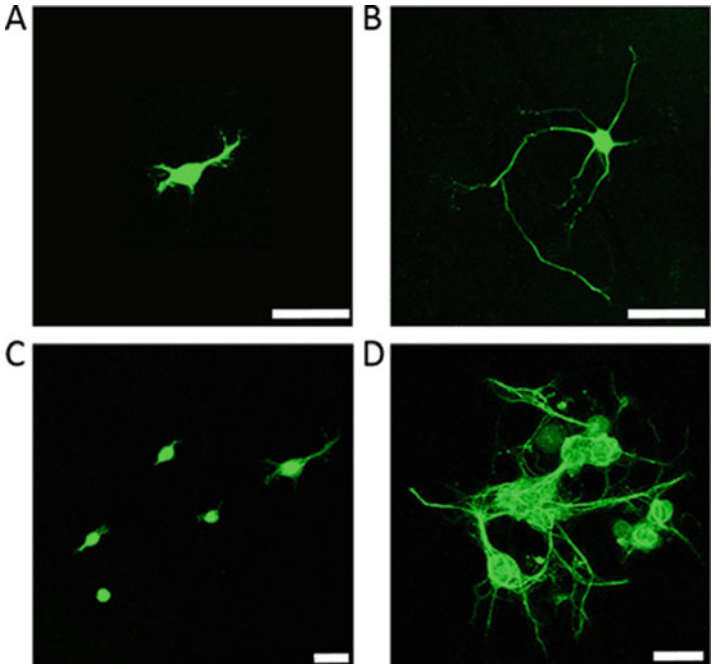

Fig. 10.8 Confocal microscopy images of single neurons cultivated on pristine (**a**) and gold nanoparticles (**b**) decorated 3D electrospun scaffolds. Neural network resulted on pristine (**c**) and gold nanoparticles based (**d**) scaffolds. (Reprinted with permission from Baranes et al. 2016)

corresponding nanoparticles. In addition, the effect of nanoparticle size and shape on neuronal tissue assembly must be also taken into account.

As demonstrated in opto-genetics, light absorption can modulate or trigger neural stimulation due to the action of light-activated membrane channels. Recently, the interest in extrinsic absorbers to assist neural stimulation in an aspecific way (typically through temperature) has grown. Gold nanoparticles have attracted some interest in this field due to the ability to efficiently absorb laser light at the plasmonic peak and to dissipate it into surrounding environment in form of heat (Chirico et al. 2015; Borzenkov et al. 2016; Freddi et al. 2013). Indeed, neuronal guiding and stimulation of neurons treated with gold nanoparticles can be achieved by applying Near-Infrared light at corresponding plasmonic resonance. In one of the first examples NG108-15 neuronal cells were treated with gold nanorods and then treated with low laser power (Paviolo et al. 2013). Bare, PSS and SiO_2 coated nanorods with LSPRs in range 750–810 nm were synthesized for this purpose. When the cells were irradiated with a 780 nm laser, the average number of neurons with neurites increased. Furthermore, when the NG108-15 cells were cultured with both bare and coated gold nanorods and then irradiated with 1.2–7.5 W/cm^2, they showed a neurite length increase up to 25 μm with respect to the control. Although the detailed mechanism of such

stimulation was not clear, the author connected this effect to the absorption of light by gold nanorods. This behavior was not specific to the gold nanoparticles surface chemistry.

The same authors demonstrated that intracellular calcium transients (Ca^{2+}) can be induced after Near-Infrared optical exposure of NG108-15 cells cultured with gold nanorods (Paviolo et al. 2014). The underlying mechanism was partially clarified in a subsequent detailed study and it is based on the transient heating associated with the optical absorption of gold nanoparticles, which can trigger neuronal cell differentiation and increase the level of intracellular calcium activity (Paviolo et al. 2015). The results indicated that nanoparticle absorbers can enhance and/or replace the process of infrared neural stimulation based on water absorption, with high potential for future applications in neural prostheses and cell therapies.

This phenomenon was confirmed also in other publications, though no definitive consensus has been found. Thus, the thermal transduction mediated by gold nanoparticles is particularly promising and can be a versatile tool to affect neuronal growth and to trigger the membrane depolarization from 0.025 to 25 ms illumination pulses (Colombo et al. 2016; Yong et al. 2014). Eom et al. reported that the neuronal stimulation could be activated by triggering temperature-sensitive channels in the rat sciatic nerve in vivo upon Near-Infrared illumination of gold nanoparticles (Eom et al. 2014). The authors demonstrated that temperature increase of 6 °C resulted in 5.7 times higher neuronal responsivity. From the other hand, PEGylated gold nanorods were shown to be effective in driving photothermal inhibition of the electrical activity of cultured neurons exposed to prolonged 785 nm laser pulses (Yoo et al. 2014).

Neurons can be directly stimulated with light to produce action potentials, particularly through the action of the opsins. In such approaches one does not rely on the localization of the absorbed light energy as it can be done by exploiting the high gold nanoparticles absorption. Recently it has been shown that gold nanoparticles can be conjugated to high-avidity ligands for a variety of cellular targets (Carvalho-de-Souza et al. 2015). Once bound to a neural membrane proteins, these particles transduce millisecond pulses of light into heat, which changes membrane capacitance, depolarizing the cell and eliciting the action potentials (Carvalho-de-Souza et al. 2015). The authors conjugated gold nanorods with LSPR ≈800 nm to molecules that bind neural membrane proteins with high avidity without damaging the cells. These findings indicate a possible promising alternative to optogenetics and potential applications for a variety of therapies involving neuronal photostimulation.

The light-assisted manipulation of cells can be exploited to control membrane activity or intracellular signaling (Lavoie-Cardinal et al. 2016). Lavoie-Cardinal et al. tested an optical method for the stimulation and the monitoring of localized Ca^{2+} signaling in neurons that exploits the plasmonic excitation of gold nanoparticles. They showed by means of confocal microscopy that the application of 800 nm laser pulses to neurons decorated with a few functionalized gold nanoparticles, triggers a transient increase in free Ca^{2+}. It is believed, that such gold nanoparticles-Assisted

Localized Optical Stimulation (NALOS) may bring a new complement to light-dependent methods for controlling neuronal activity and cell signaling.

The use of gold nanoparticles for neural tissue engineering has been tightly related also to development of hybrid polymeric matrices. In fact, nerve conduits derived from chitosan have favorable mechanical properties and slow biodegradability. Chitosan based scaffolds for neural tissue engineering, modified with other biodegradable polymers, were reported in publications (Shirosaki et al. 2014). Chitosan-gold nanocomposite materials were also investigated for similar purposes (Lin et al. 2008). The authors showed that gold nanoparticles improved the mechanical strength of the chitosan and also affected the behavior of neural stem cells in vitro. It was found that that 50 ppm of gold nanoparticles stimulated cell proliferation and gene expression. The analyses revealed that the number of myelinated axons in the regenerated nerve fibers was higher in animals where the nerve was reconstructed with the chitosan-gold nanocomposite (Lin et al. 2008).

A novel silk-gold nanocomposite based nerve conduit was tested on a model for a neurotmesis grade sciatic nerve injury in rats over a period of 18 months (Das et al. 2015). This conduit was fabricated by adsorbing gold nanoparticles onto silk fibers and transforming fibers into a nanocomposite sheet by electrospinning. The authors demonstrated that these implants are safe, stable and remain functional in vivo for a long duration. The use of gold nanoparticles synthesized by a "green technique" in the conduits was probably the key factor that inhibited the inflammatory response both in the host and in situ at the site of implantation. In addition, the strong interaction between the nanoparticles and nanofibers kept the gold nanoparticles embedded in the nanofibers, therefore limiting the migration of nanoparticles into the surrounding tissue. Based on these results it is likely that fabricated nanocomposites would enable chemical modifications of biomaterial and can potentially act as platforms for delivery of nerve growth factors providing chemical cues for cellular proliferation and directing neurite outgrowth.

Nevertheless, the effect of gold nanoparticles on central nervous system has still to be investigated accurately, since contradictory results are reported. For example, Söderstjerna et al. studied the size effect on the growth of human embryonic neural precursor cells with well-characterized commercial spherical 20 and 80 nm gold nanoparticles (Söderstjerna et al. 2013). It was demonstrated that only the highest dose of 20 nm gold nanoparticles significantly affected proliferation, whereas no effect was seen on apoptotic cell death. In another multi-parametric study, the exposure to gold nanoparticles affected cellular proliferation and differentiation in an immortalized neural cell line (Soenen et al. 2012). The effect of Gold/Fe_3O_4 nanoparticles on biocompatibility and differentiated properties of rat olfactory bulb stem cells was studied (Wang et al. 2013). The obtained results indicated that Gold/Fe_3O_4 nanoparticles at the concentrations of 40 μg/10^4 cells enhanced cell viability and decreased the cell death rate. The authors suggested the application of such nanoparticles as new nanotechnologies in stem-cell-based transplantation therapies for the treatment of central nervous system diseases. In terms of electrical activity, it was observed that intracellular nanoparticles might alter neuronal functions and

cause hyper-excitability in pathological conditions (Jung et al. 2014; Paviolo and Stoddart 2015). Indeed, for a successful application of gold nanoparticles as stimuli for neural cells growth, detailed studies on the adverse effects of gold nanoparticles on the developing central nervous system are mandatory.

10.5 Gold Nanoparticles in Bone Tissue Engineering

Bone defect repair using the tissue engineering approach is becoming a promising and highly effective method because the repair process may proceed with the patient's own tissue by the time the regeneration is complete (Laurencin et al. 1999; Amini 2012). Bone tissue engineering is considered as a complex process that starts with migration and recruitment of osteoprogenitor cells followed by cells proliferation and differentiation and by the matrix formation along with remodeling of the bone (Bose et al. 2012). Nowadays nanotechnology is integrated in the bone tissue engineering field to overcome some of the current limitations associated with bone regeneration methods including insufficient mechanical strength of scaffold materials, ineffective cell growth and osteogenic differentiation at the defect site, and stimulate bone cell growth (Kim and Fisher 2007). Nanomaterials can also act as carriers for bioactive molecules necessary for bone tissue formation. They stabilize the bioactive molecules through encapsulation or surface attachment and provide controlled release at the designated target (Walmsley et al. 2015). Based on a range of scientific reports, the following functions of gold nanoparticles applied to bone tissue engineering can be highlighted:

(a) act as osteogenetic agents;
(b) have antibacterial effect;
(c) enhance grafting of bone implants.

Gold nanoparticles are promising osteogenic agents for bone tissue regeneration, promoting osteogenic differentiation of mesenchymal stem cells (Liu et al. 2010; Yi et al. 2010). Gold nanoparticles with sizes 20 and 40 nm induce the increase of the osteogenic differentiation rate of MC3T3-E1 osteoblast-like cells (Liu et al. 2010). Furthermore, gold nanoparticles synthesized by citrate reduction of $HAuCl_4$, can affect osteoclast formation (Sul et al. 2010). In this direction a biodegradable hydrogel loaded with gold nanoparticles has been more recently developed as a new approach for bone tissue regeneration (Heo et al. 2014). The authors used photo-curable gelatin hydrogels in order to provide a proof of principle of gold nanoparticles loaded hydrogels in regeneration strategies for bone tissue engineering as shown in Fig. 10.9. The in vitro results revealed that the hydrogels loaded with gold nanoparticles promote proliferation, differentiation, and alkaline phosphate activities of human adipose-derived stem cells as they differentiate towards osteoblast cells in a dose-dependent manner. The in vivo results demonstrated that these hydrogels loaded with high concentrations of gold nanoparticles had a significant influence on new bone formation.

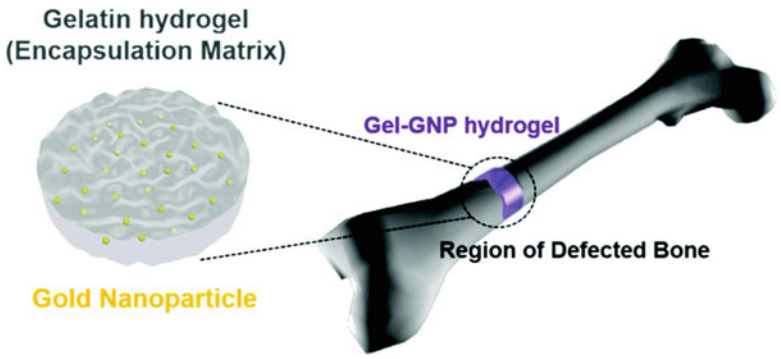

Fig. 10.9 Photo-cured gelatin hydrogel-gold nanoparticles network developed for bone tissue repair. (Reprinted with permission from Heo et al. 2014)

More recently, Choi et al. studied the effect of chitosan conjugated gold nanoparticles on the osteogenic differentiation (Choi et al. 2015). Positively charged gold nanoparticles with hydrodynamic diameter ≈40 nm were prepared using the chitosan reduction method. The results indicated that chitosan-conjugated gold nanoparticles increase the deposition of calcium content and the expression of marker genes related to osteogenic differentiation in human adipose-derived mesenchymal stem cells at nontoxic concentrations. The authors showed that such nanoconstructs can promote osteogenesis through the Wnt/β-catenin signaling pathway and can be applied as agents for bone formation.

The osteoinductive nature and the good bio-compatibility of hydroxyapatite makes it an optimal candidate as a graft material for bone repair, augmentation, and substitution (Chang et al. 2001). Moreover, gold nanoparticles with collagen form an efficient matrix for the growth of hydroxyapatite (Aryal et al. 2006). Such growth on gelatin–chitosan composite capped gold nanoparticles by means of wet precipitation was studied (Sobhana et al. 2009). These authors proved that gelatin–chitosan capped gold nanoparticles can act as a matrix for the growth of crystals, thus introducing low cost and effective approach.

Interesting results, appeared in *Nano Letters* in 2015, describing the fabrication of substrates with precisely spaced and tunable gold nanoparticles arrays (Schwab et al. 2015). Moreover these gold nanoparticles carried single bone morphogenetic proteins belonging to the transforming growth factor-β. In this work, glass cover slips were decorated with a regular arrangement of gold nanoparticles by using diblock copolymer micellar nanolithography, and gold nanoparticles 5–8 nm in size were functionalized with the morphogenetic proteins via bifunctional linker (11-mercaptoundecanoyl-*N*-hydroxysuccinimide ester). With such nanoconstructs, one can achieve controlled and sustained local delivery of different growth factors. Employing this set up it would be possible to assess, at unprecedented level, the minimum concentration (ranging from <0.5 to >3 ng/cm^2) of surface-bound growth factor needed to initiate individual signal pathways.

Too many orthopaedic implants undergo undesired bacterial infection. The subsequent inflammation leads to implant failure and second surgery. To overcome this drawback nanoparticle treatment of the prostheses has been applied. Beside silver, with known antimicrobial activity, also gold nanoparticles have been more recently used to load ceramic scaffolds and induce endogenous antimicrobial and anti-inflammatory activity that increase the success of bone implantation and tissue regeneration (Farag et al. 2012). The authors evaluated the effect of gold nanoparticles on the scaffold's mechanical properties, porosity and cell growth. Resulted scaffolds with incorporated gold nanoparticles displayed enhanced porosity, degradability and mechanical properties compared with the ceramic scaffolds. In a very recent publication the problem of rapid emergence of antibiotic resistance of bone tissue implants is also discussed (Ribeiro et al. 2017). The authors claimed that biomaterials must be modified to promote the tissue integration before bacterial adhesion. For this purpose, they fabricated silk fibroin/nanohydroxyapatite hydrogel modified with in situ synthesized silver and gold nanoparticles. In vitro antimicrobial studies revealed that hydrogels with both types of nanoparticles exhibited significant inhibition ability against both gram-positive and gram-negative bacteria. Cytocompatibility studies performed by using osteoblastic cells indicated that up to 0.5 wt% of silver nanoparticles, and for all concentrations of gold nanoparticles, the hydrogels can be effectively used as antimicrobial materials, without compromising cell behavior.

Gold nanoparticles can enhance grafting of bone implants. Ross and Roeder showed that bisphosphonate (alendronate)-functionalized gold nanoparticles exhibited more rapid binding kinetics and greater binding affinity to hydroxyapatite compared to carboxylate (L-glutamic acid) and phosphonate (2-aminoethylphosphonic acid) functional groups (Ross and Roeder 2011). However, it must be considered that the results for binding to synthetic crystals may not reflect real binding to tissue. Therefore, in following publication, the authors suggested that damaged bone tissue can be targeted by functionalizing gold nanoparticles with molecules exhibiting affinity for calcium (Ross et al. 2012). They studied the binding affinity of gold nanoparticles surface functionalized with carboxylate (L-glutamic acid), phosphonate (2-aminoethylphosphonic acid), or bisphosphonate (alendronate) in vitro (Ross et al. 2012). Based on obtained results, the authors suggested that bisphosphonate-functionalized gold nanoparticles have potential for targeted delivery to damaged bone tissue as shown in Fig. 10.10.

The effect of gold nanoparticles functionalization on grafting was also reported in paper published in *Biomaterials* in 2015 (Li 2015). In this study human bone marrow-derived mesenchymal stem cells were treated with amine (-NH2), carboxyl (-COOH) and hydroxyl (-OH) functionalized gold nanoparticles possessing different surface charges. These functionalized nanoparticles showed no acute toxicity and positively charged nanoparticles exhibited higher cellular uptake. All types of gold nanoparticles did not inhibit osteogenesis, though calcium deposition was markedly

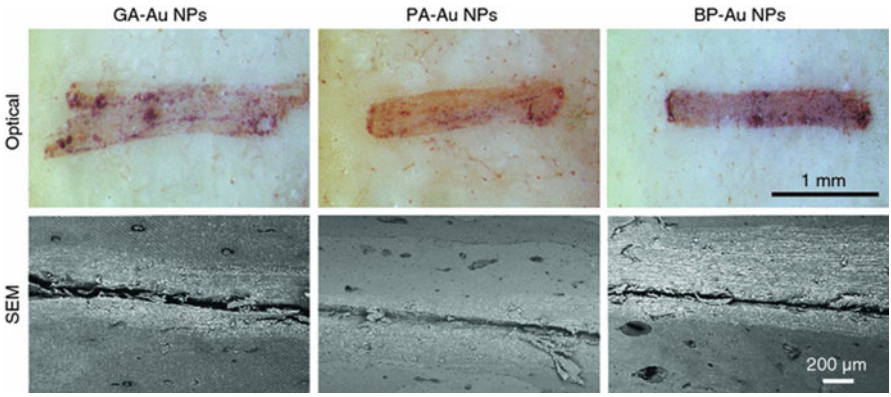

Fig. 10.10 Optical and backscattered electron micrographs showing surface damage (scratch) on bovine cortical bone specimens labeled with glutamic acid (GA)-, phosphonic acid (PA)-, and bisphosphonate (BP)-functionalized gold nanoparticles. The relative depth of the color or contrast observed in optical and backscattered electron micrographs, respectively, for damaged bone tissue inside the scratch versus undamaged tissue outside the scratch qualitatively suggested that BP-gold nanoparticles exhibited the greatest binding affinity for damaged bone tissue. (Reprinted with permission from Ross et al. 2012)

reduced during gold nanoparticles-COOH treatment. These results provided additional confirmation that functionalization of gold nanoparticles can play an important role during materials engineering.

Small non-coding RNAs, microRNAs (miRNAs) play an important role in stem cell differentiation through regulating target-mRNA expression. They are therefore important effectors in tissue engineering. In a very recent paper novel, surface-engineered, ultra-small gold nanoparticles (<10 nm) have been used as highly efficient miR-5106-delivery systems that enable the regulation of bone mesenchymal stromal cells differentiation (Yu et al. 2017). The authors exploited the effect of gold nanoparticles coated layer-by-layer with polyethylenimine and liposomes to enhance miR-5106-delivery activity and subsequent cells differentiation capacity. Coated gold nanoparticles showed negligible cytotoxicity, good miRNA-5106-binding affinity, highly efficient delivery of miRNAs to cells, and long-term miRNA expression. These finding can open a promising strategy for the rational design of ultra-small inorganic nanoparticles as highly efficient miRNA-delivery platforms for tissue regeneration. Another interesting delivery approach was developed on the basis of alginate made capsule systems capable of rapidly releasing multiple polyloads in response to ultrasonic signals (Kennedy et al. 2016). As a proof of concept, gold nanoparticles were decorated with bone morphogenetic protein-2 to demonstrate the potential bioactivity of the nanoparticle payloads. These nanoparticles were not cytotoxic and induced an osteogenic response in mouse mesenchymal stem cells.

Due to increasing interest of researchers to apply gold nanoparticles for bone tissue engineering, a few recent works have appeared on the effect of various sizes of

gold nanoparticles on the differentiation of human adipose-derived stem cells (ADSCs) into osteoblasts (Ko et al. 2015). Spherical gold nanoparticles with different sizes (15, 30, 50, 75 and 100 nm), at a single concentration of 1 μM, were used for this purpose. The results showed that independently of the size of the gold nanoparticles, no significant toxicity on cells was observed over 1 week of incubation. The authors found by means of dark field assays and optical microscope of the treated cells, that 30 nm and 50 nm gold nanoparticles were preferentially up taken. Moreover, it was also shown that all sized of gold nanoparticles promoted the differentiation of cells more than control. Nevertheless, 30 nm and 50 nm gold nanoparticles displayed the highest differentiation rates providing the most effective osteogenic differentiation.

Despite of promising results achieved recently by incorporation gold nanoparticles in bone tissue engineering, there are a few issues that still remain unclear and have to be solved in future. Indeed, the employment of gold nanoparticles brought relevant improvement of scaffolds properties. The ability to use gold nanoparticles as delivery vectors of biomolecules has been also explored. The study of the tissue growth and the scaffolds behavior in-vivo can be easily done exploiting the gold nanoparticles scattering or luminescence. However, the precise answer how gold nanoparticles promote bone formation still remains unclear (Vial et al. 2016). Primary goals for future studies are the development of protocols to reveal these mechanisms together with more detailed studies regarding the impact of nanoparticles sizes, shapes and surface properties on the bone tissue engineering. Further efforts should also be done towards the application of localized photothermal effect of gold nanoparticles in bone tissue engineering, similarly to what was done in the case of neural cells.

10.6 Gold Nanoparticles in Skin Tissue Engineering and Wound Healing

The skin is the largest organ of the body and engineered skin substitutes have a critical medical application to patients with a variety of injuries, for example with burn wounds (Wong and Chang 2009). Tissue-engineered skin includes endogenous cells implants, cells implanted within two- or three-dimensional biomaterials, biomaterials for replacement of the skin's dermal layer (both with and without cells), and biomaterials to support the replacement of both the epidermis and dermis (MacNeil 2008).

The ideal and primary goal of skin tissue engineering is to enable the rapid formation of a construct that will support and enable the complete regeneration of functional skin with all the skin appendages, the various layers (epidermis, dermit, fatty subcutis), and a fully functioning and scar free integration of the vascular and nerve network within the host tissue (Mohamed and Xing 2012). Skin regeneration and wound healing approaches that exploit the achievement of nanotechnology is the

topic treated in studies and investigations worldwide (Chaudhury et al. 2014; Parani et al. 2016). The current approaches of nanotechnology based methods for skin tissue engineering are:

1. Antimicrobial effect;
2. Anti-oxidative stress effect;
3. Growth factors in scaffolds for skin regeneration;
4. Nanofibrous scaffolds and nanoparticles loaded hydrogels with improved properties

Metal nanoparticles exhibit a range of useful properties for skin tissue engineering, such as enhancing mechanical strength, controlled release, and antibacterial activity against both bacteria and fungi, which make such nanoparticles excellent candidates for topical use in wound healing (Parani et al. 2016). For example, gold nanoparticles functionalized with antibiotics, antioxidants, and ROS scavengers can improve wound healing process. In addition, they can directly be applied in tissue welding and also act as vehicles for gene delivery (Parani et al. 2016).

Bacterial infections are severe problems in skin treatments. The impact of surface chemistry of gold nanoparticles antimicrobic activity was then studied in detail by means of gold nanoparticles carrying different cationic functionalities varying in chain length and nonaromatic and aromatic characteristics (Li et al. 2014). The authors demonstrated that gold nanoparticles with cationic and hydrophobic functional groups were the most effective against Gram-negative and Gram-positive bacteria. The light-absorbing capability of gold nanoparticles for tissue welding to facilitate healing of cut wounds was also studied (Gobin et al. 2005). In this work synthesized gold nanoshells with Near-Infrared LSPR were used as exogenous chromophores to absorb the near-infrared energy while the histological examination revealed good wound-healing response. It was also shown that antibiotics display enhanced activity when conjugated to gold nanoparticles (Parani et al. 2016). As an example, it was demonstrated that vancomycin capped gold nanoparticles acted as a rigid polyvalent inhibitor of vancomycin resistant enterococci (Gu et al. 2003). Moreover, it was shown that gold nanoparticles had unexpected direct activity against E coli strain. A prospective application for gold nanoparticles in wound healing was also demonstrated by a study on the fibroblast cell detachment, patterning, and regrowth on artificially engineered gold nanoparticle-based surfaces that were triggered by laser irradiation (Kolesnikova et al. 2012). The antimicrobial activity can be obtained also by decorating the gold nanoparticles with short peptides. Chen et al. reported the use of gold nanorods functionalized with the antimicrobial peptide surfactin (Chen et al. 2015). These decorated nanorods showed high antimicrobial activity likely related to bacterial membrane disruption. In addition, in vivo wound healing studies on rats demonstrated good biocompatibility, faster healing and better epithelialization.

Wound healing is also largely hindered by the inflammation due to oxidative stress (Parani et al. 2016; Chen et al. 2012). Also in this case, it has been shown that

the combination of antioxidants, such as epigallocatechin gallate and α-lipoic acid, with gold nanoparticles displayed significant antioxidation effect and enhanced the action of the growth factor of endothelial cells (Chen et al. 2012; Leu et al. 2012). Despite the fact that such combination accelerates the wound healing process, the combined effect of all components requires more accurate studies. Gold nanoparticles can also be used in gene therapy for wound healing. Anti-miR-378a RNA fragments were conjugated to methoxy PEG thiol and coated on the surface of gold nanoparticles (Gaharwar et al. 2014). The RNA decorated gold nanoparticles showed better wound closure compared to the wounds treated with gold nanoparticles carrying blank vector.

Regarding the use of gold nanoparticles in scaffolds for skin regenerations, there are a few studies on the growth of epithelial cells. Rosman et al. first studied the growth of these cells on substrates decorated with gold nanorods (Rosman et al. 2014). These nanoparticles were coated either with a positively charged (cytotoxic) surfactant or with a biocompatible polymer exhibiting one of two different terminal groups, resulting in a neutral or negative surface charge. It was found that all particles supported cell adhesion with no evidence of directed cell migration or particle internalization. The authors found an impaired cell growth correlated to the cytotoxicity of the surface bound surfactant. However, in the case of the presence of a biocompatible polymer on the nanoparticles, they observed no effect on cell growth for the functional terminated –COOH group, whereas the $–NH_2$ group reduced adherence and proliferation compared to cells growing on a bare glass substrate. It was concluded that the impact of basolateral exposure of gold nanorods on epithelial cells depends critically on the exposed chemical moiety in contact with the cell membrane. Along this line of studies, negatively charged and positively charged gold nanoparticles were incorporated into a decellularized porcine diaphragm to produce a biocompatible scaffold suitable for wound healing (Cozad et al. 2011). The incorporation of gold nanoparticles led in this case to enhanced cell proliferation and free radical generation, suggesting that also the shape and concentration of the gold nanoparticles may affect the growth of epithelial cells.

Volkova et al. have recently explored the possibility of using cryopreserved human of fibroblasts cultured with gold nanoparticles to treat experimental burns (Volkova et al. 2016). The immunofluorescent analysis emphasized that the use of these fibroblasts accelerated the skin synthetic processes and was helpful in recovering type I and III collagen content on day 21 after therapy. The authors ascribed the observed phenomenon to the unique structure and antimicrobial properties of gold nanoparticles. Interestingly, in an earlier work, gold nanoparticles immobilized on a silica substrate forming 500 nm SiO_2/gold nanoparticles core-shell structure promoted proliferation of mouse embryonic fibroblast cells (Li 2015). This observation was explained by the fact that the silica substrate kept gold nanoparticles outside the cells and the nano-size concavo-convex gold shell facilitated to cell adhesion, resulting in the proliferation. It was also shown that resulted core-shell nanomaterials can promote wound healing also due to anti-inflammatory and antioxidation properties of gold nanoparticles.

Incorporation of gold nanoparticles into polymeric network can bring advances in wound dressing fabrication. Gold nanoparticles-chitosan composites showed enhanced proliferation of human fibroblasts in vitro in comparison with pure chitosan (Hsu et al. 2011). In recent study nanocomposite collagen scaffolds incorporating gold nanoparticles were fabricated for wound healing applications (Akturk 2016a, b). It was shown that intercalation of gold nanoparticles into cross-linked scaffolds enhanced the stability against enzymatic degradation and increased the tensile strength. These nanocomposites displayed also inhibition of the inflammatory response and had a pronounced effect on skin tissue formation. Nevertheless, the authors noted that further studies are needed to investigate if and how higher loading of gold nanoparticles affects positively these results in a statistically meaningful manner (Akturk 2016). Citrate-capped gold nanoparticles were also incorporated in different concentrations into collagen/poly(ethylene oxide) nanofibrous matrixes for novel skin tissue engineering approaches (Aktürk and Keskin 2016). Nanocomposites with 14.27 ppm of gold nanoparticles showed the best morphology. The cell attachement and proliferation onto these scaffolds were similar to commercially available Matriderm substrate. In another paper published by the same authors 3D silk fibroin matrices loaded with citrate-capped gold nanoparticles (d = 24 nm) were used for skin tissue engineering applications (Akturk 2016). Also in this case, gold nanoparticles incorporation improved degradation profiles and mechanical properties significantly. Though, bare and gold nanoparticles based scaffolds showed similar cells attachment and layer by layer proliferation, more flattened and spread cells morphology was observed on gold nanoparticles based nanocomposite. Therefore, incorporation of gold nanoparticles into fibroin matrix at 14.27 ppm led to enhancement of matrix properties and did not cause toxicity both in vitro and vivo conditions.

In a paper published in 2015 Kim et al. studied the therapeutic effect of phytochemically stabilized gold nanoparticles grafted on hydrocolloid membrane, as shown in Fig. 10.11, for curing cutaneous wounds (Kim et al. 2015). Phytochemically stabilized gold nanoparticles are synthesized under non-toxic conditions suitable for medical applications.

The authors demonstrated that topical application of gold nanoparticle coated membranes for 15 days induced the acceleration of wound healing including tissue regeneration, connective tissue formation, and angiogenesis. It was also shown that such membranes did not induce toxicity.

10.7 Other Examples of Gold Nanoparticles Application in Tissue Engineering

Having discussed the application of gold nanoparticles in the most emerging fields of tissue engineering, it is also worth to mention very briefly other fields of tissue engineering, where corresponding nanoparticles have been already applied. An

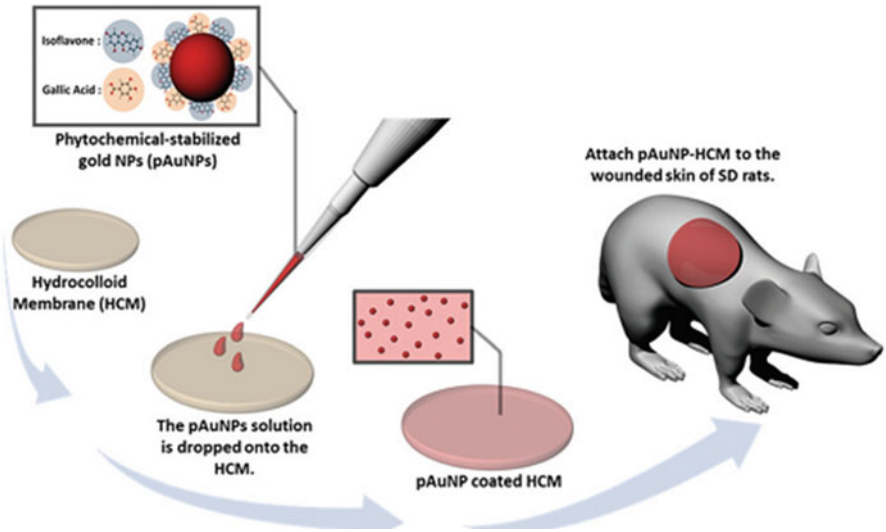

Fig. 10.11 Preparation of phyto-chemically stabilized gold nanoparticle coated membranes for skin damage treatment. (Reprinted with permissions from Kim et al. 2015)

active patch composed from decellularized tissue conjugated to 100 nm gold nanoparticles was shown to be efficient biomaterial for vascular repair (Ostdiek et al. 2015). Recently, it was demonstrated that systemically administered collagen-targeted gold nanoparticles were capable to bind to arterial injury following vascular interventions, therefore providing new opportunities in vascular injury treatments (Meyers et al. 2017). Gold nanoparticles were applied also to improve materials for hernia repair. Thus, it was demonstrated, that gold nanoparticles covalently linked to polypropylene mesh displayed improved biocompatibility as compared to pristine mesh (Grant et al. 2011). Also, a couple of examples regarding the application of gold nanoparticles for muscle tissue engineering are provided. Poly(L-lactic acid) and gold nanoparticles were used to prepare electrospun scaffolds with improved biodegradable, biocompatible and conductive scaffolds for skeletal muscle tissue regeneration (Mckeon-Fischer and Freeman 2011). The photo-thermal properties of gold nanoparticles can be also successfully applied in muscle tissue engineering. In the recent paper published in 2017 mild heat stimulation of muscle cells induced by Near-Infrared irradiation of gold nanoshels efficiently induced myotube contraction (Marino et al. 2017). The reported "wireless" activation can bring advances in tissue engineering and bionics, where large cell population can be simultaneously activated.

10.8 Conclusions

Without much doubts recent advances of nanotechnology brought significant advances in tissue engineering field. Among different types of nanomaterials, gold nanoparticles allowed significantly improvement the scaffold properties together with direct effect on the growth of different types of cells. The origin of gold nanoparticles efficacy lies likely in their multifunctional nature. Therefore, as it has been shown in this review, gold nanoparticles can be successfully applied for cardiac, neural, bone and skin tissues regeneration. The future trends are detailed studies of all observed challenging results in all mentioned fields providing summarized protocols. However, beside these promising results, many questions still remain unsolved, e.g. the formulation of clear models of their effect on cells growth. Long-term studies of the prolonged treatment of cells with gold nanoparticles, while growing on scaffolds, are required. Therefore, these factors have to be investigated in future opening an opportunity for further clinical trials.

Again, most studies still need more details on protocols to achieve best results in tissue engineering assisted by gold nanoparticles. Moreover, many studies still remain at in vitro level. Therefore, more investigations performed in vivo conditions are required. Finally, detailed protocols regarding the toxicity and side effects of gold nanoparticles in tissue engineering are important topics to be addressed for any efficient clinical application.

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