Development of Nano-antimicrobial Biomaterials for Biomedical Applications

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Abstract Around the globe, there is a great concern about controlling growth of pathogenic microorganisms for the prevention of infectious diseases. Moreover, the greater incidences of cross contamination and overuse of drugs has contributed towards the development of drug resistant microbial strains making conditions even worse. Hospital acquired infections pose one of the leading complications associated with implantation of any biomaterial after surgery and critical care. In this regard, developing non-conventional antimicrobial agents which would prevent the aforementioned causes is under the quest. The rapid development in nanoscience and nanotechnology has shown promising potential for developing novel biocidal agents that would integrate with a biomaterial to prevent bacterial colonization and biofilm formation. Metals with inherent antimicrobial properties such as silver, copper, zinc at nano scale constitute a special class of antimicrobials which have broad spectrum antimicrobial nature and pose minimum toxicity to humans. Hence, novel biomaterials that inhibit microbial growth would be of great significance to eliminate medical device/instruments associated infections. This chapter comprises the state-of-art advancements in the development of nano-antimicrobial biomaterials for biomedical applications. Several strategies have been targeted to satisfy few important concern such as enhanced long term antimicrobial activity and stability, minimize leaching of antimicrobial material and promote reuse. The proposed strategies to develop new hybrid antimicrobial biomaterials would offer a potent antibacterial solution in healthcare sector such as wound healing applications, tissue scaffolds, medical implants, surgical devices and instruments.

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List of Abbreviations

2D	Two dimensional
3D	Three dimensional
A549	Human lung adenocarcinoma epithelial cell line
AB	Anti bacterial
AF	Antifungal
AgNP	Silver nanoparticles
AV	Antiviral
BAI	Biomaterial assisted infection
BEAS2B	Human normal bronchial epithelial cells
CACC	Calcium alginate-cotton cellulose
CFU	Colony forming units
CMC	Carboxymethyl chitosan
CNS	Carbon nanoscrolls
CNTs	Carbon nanotubes
CSNPs	Chitosan nanoparticles
CuNPs	Copper nanoparticles
CuO NPs	Copper oxide nanoparticles
DD	Degree of deacetylation
GNPs	Gold nanoparticles
GO	Graphene oxide
HA	Hydroxyapatite
HaCaT	Human keratinocyte
HAIs	Hospital acquired infections
HepG2	Human hepatoma cells
HNC	Hybrid nanocomposite
HNTs	Halloysite nanotubes
IPN	Inter-penetrating network
LBL	Layer-by-layer
MBC	Minimum bactericidal concentration
MDR	Multiple drug resistance
MIC	Minimum inhibitory concentration
MWCNTs	Multiple-walled carbon nanotubes
NCs	Nanocomposites
ND	Not Determined
NIR	Near-Infrared
NSP	Nanosilicate platelets
PTFE	Polytetrafluorethylene
QCS NPs	Quaternary ammonium chitosan derivative nanoparticles
rGO	Reduced grapheme oxide
ROS	Reactive oxygen species
SWCNTs	Single-walled carbon nanotubes
TEM	Transmission electron microscopy
USEPA	US environmental protection agency

VRE	Vancomycin resistant *Enterococci*
ZnO	Zinc oxide
ZoI	Zone of inhibition

1 Introduction

The recent progresses in health care sector have witnessed the use of biomaterials for improving life quality of critically ill patients. Biomaterials have revolutionized a few emerging areas such as biomedical engineering and tissue engineering by facilitating less-invasive techniques for continuous monitoring, improving drug administrating and enhanced mobility by either restoring or replacing organ functions (Hench and Polak 2002; Place et al. 2009; Hubbell 1995). As compared to the conventional technologies, biomaterials with improved functionality and durability have been utilized in form of vascular grafts, biocompatible coatings, medical implants, stents tissue scaffolds which might remain functional even for several months (Place et al. 2009; Ratner et al. 2004). Despite considerable success of biomaterials in ageing society, only a few biomaterials can prove their safety concerns under practically relevant conditions. Regardless of implant composition and applications, i.e., from prosthetic joints, artificial heart and dental implants to vascular valves and intraocular lenses, virtually all biomaterials behave as a "niche" to pathogenic microorganisms (Zaat et al. 2010; Busscher et al. 2012; Stewart and Costerton 2001). Under in vivo conditions, the microbes get attracted and subsequently lead to biofilm formation on biomaterial surface, leading to one of the major clinical complications often referred as biomaterial-associated infections (BAIs) (Percival et al. 2015; Campoccia et al. 2013a; Zaat et al. 2010; Busscher et al. 2012; Stewart and Costerton 2001). The greater incidences of biomaterial-associated infections thus compromise with the intended use of any implant or device and add risk to humans in terms of high morbidity and even mortality (Parsek and Singh 2003; Hall-Stoodley and Stoodley 2009). Moreover, it marks an adverse impact on economy since the existing treatment strategies to cure infection could cost even more than the initial biomaterial implantation. In a broad sense, a biomaterial faces two major challenges when implanted within the body i.e., to make suitable integration with native tissue while preventing colonization of microbes on its surface. In 1987, an orthopedic surgeon Anthony Gristina introduced a phrase "the race for the surface" referring that there exists a stern competition between tissue integration and bacterial attachment on biomaterial surface (Gristina 1987). A successful implantation of biomaterial without any infection would thus be a 'winning' situation for its intended use, though it is not the case with few of them.

Biomaterial-associated infections are most commonly caused by *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Staphylococcus capitis*, *Staphylococcus saprophyticus*, *Staphylococcus warneri*, *Staphylococcus*

cohnii, *Staphylococcus xylosus, Staphylococcus chromogenes, Staphylococcus schleiferi, Staphylococcus lugdunensis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Proteus vulgaris, Candida albicans, Propionibacterium acnes* including a few other bacterial strains having low virulence potential on healthy individuals but resides within skin and mucous membranes (Jukes et al. 2010; Mack et al. 2013). The cascade for pathogenesis of BAIs follows a series of common events (Costerton et al. 1999; Parsek and Singh 2003; Busscher et al. 2012; Stewart and Costerton 2001). First step involves an initial attachment of microbial cells to biomaterial surface while the attached cells start accumulating in multiple layers leading to the formation of biofilm as a second step. Subsequently, the maturation of microbial biofilm takes place and finally, microbial consortium is detached for spreading to other parts of biomaterial surface. The implants are thus susceptible to many infections, as direct contamination to biomaterial surface during surgery starts even after few hours of implantation i.e., preoperative contamination (Maathuis et al. 2005; Campoccia et al. 2013b). The site of implant can also be accessed to microbes during hospitalization, known as hospital-acquired infections. The spreading of microbes occur due to microbial contamination on several locations other than the implant site through blood stream i.e., post-operative infections and is inevitable (Siegel et al. 2007; Campoccia et al. 2013b). As a result, the durability and functionality of biomaterial implants is severely affected which is manifested by severe complications that arise during patient's recovery.

The accumulation of microbial biofilm leads to the secretion of extracellular polymeric substance (EPS), which tends to hide microbes within its polymeric mesh and make them inaccessible to host immune system and antimicrobial therapies (Costerton et al. 1999; Hall-Stoodley et al. 2004; Hall-Stoodley and Stoodley 2009; Stewart and Costerton 2001). Sessile and adherent bacteria are thus intrinsically more resistant towards host clearance and require nearly 500–5,000 times higher concentration of common clinical antibiotics than planktonic or non-biofilm forming pathogens (Boucher et al. 2009; Donlan and Costerton 2002; Subbiahdoss et al. 2013). The therapeutic and prophylactic use of antibiotics for curing post operative infections has even contributed towards the development of microbial strains with high resistance against those drugs making conditions unmanageable. Ultimately, the removal of an infected implant would be the only possible solution followed by weeks of antibiotic treatment to remove infection before implantation of new device (Maathuis et al. 2005; Campoccia et al. 2013b; Busscher et al. 2012; Ratner et al. 2004). For these reasons, local or topical administration of antibacterial agents is preferred over routine systemic approaches which would minimize an initial attachment of bacteria on implant surfaces. Accordingly, a promising strategy for reducing the occurrence of BAIs is to prevent an initial attachment of bacteria to implant and device surfaces (Bazaka et al. 2012; Salwiczek et al. 2014; Monteiro et al. 2009). This has spurred research efforts on developing antimicrobial surfaces and coatings that can be applied to biomedical devices so as to confer resistance against bacterial colonization. To achieve this, the antibacterial biomaterial surface should reflect non-cytotoxic characteristics against mammalian cells and it should not pose any adverse effects on healthier tissues and body fluids of patients

(Harding and Reynolds 2014; Norowski and Bumgardner 2009). Moreover, therapeutic approaches to inhibit bacterial colonization yet retaining the intended properties of biomaterials is always advisable, such as the visual clarity of contact lenses or the flexibility of vascular grafts would not be compromised.

In last few years, nanotechnology has provided immense opportunities to manipulate substances at nano scale altering their physicochemical properties and transform them into potential antimicrobial agents. Owing to its small size, nanomaterials have high surface area to volume ratio which makes them more effective even at relatively lower dose concentration than their bulk form (Mauter and Elimelech 2008; Sharma et al. 2009; Rai et al. 2009; Singh et al. 2008). Moreover, the mechanism of antimicrobial action of nanomaterials can be mediated through several pathways, i.e., disruption of bacterial membrane, formation of holes and pits on cell wall, generation of ROS, binding to sulfhydryl groups of metabolic enzymes of the bacterial electron transport chain to inhibit respiratory activity, and integration with DNA (Kumar et al. 2008; Morones et al. 2005; Rai et al. 2009; Hill 2009; Zhang et al. 2012a; Panáček et al. 2006; Sharma et al. 2009; Chopra 2007). This provides inability of microorganism to develop resistance against them. Recently few reports have elucidated a more efficient, direct contact killing action of silver nanoparticles to the bacterial cell wall, which do not even require the internalization of nanoparticles into the cells and thus would be more efficient than antibiotics to inhibit bacterial resistance. As a result, nanomaterials are particularly very effective to kill the multiple drug resistant microbial strains. In particular, the inherent antimicrobial properties of coinage metals i.e., silver, gold and copper were known to us from ancient times, these metallic nanoparticles have been utilized by researchers as potential disinfectants in biomedical and water purification applications (Atiyeh et al. 2007; Russell et al. 1994). They are being introduced as one of the important component in our daily life style. Imagine an odorless textiles (antibacterial T shirts) to public hygiene (deodorants, toothbrushes, washing machines) to water purifier to processed foods packing material, to antibacterial bandages, sunscreen lotions, and cosmetics that you would certainly feel the existence of nanomaterials. More recently, silver nanoparticles are used in the coating of medical equipments such as catheters, infusion systems and dental composites. In addition, there is increasing interest in utilization of 'nanoparticulate' forms of metal, metal oxides like copper/copper oxides, zinc oxides (ZnO) and biopolymers which exhibit remarkable antimicrobial properties.

Despite this, materials at nano scale pose certain challenges which limit their development as an efficient antimicrobial agent. In the absence of any support material, the nanoparticles tend to aggregate due to their high surface reactivity such that their actual antimicrobial efficacy is severely inhibited (Gupta and Silver 1998; Li and Lenhart 2012; Morones et al. 2005; Agnihotri et al. 2012, 2013, 2015). Moreover, colloidal nanomaterials cannot be used repeatedly and thus seem to be uneconomical under practically relevant conditions. Over past few years, tremendous research activities have been focused to minimize these limitation by either immobilizing or incorporating nanoparticles onto solid support with an aim to enhance their antibacterial activities and promote their reusability

(Agnihotri et al. 2015, 2012, 2013; Zhou et al. 2014; Bakare et al. 2016; Cao et al. 2010; Lin et al. 2013; Ifuku et al. 2015; Zheng et al. 2016; Chernousova and Epple 2013). In general, various immobilization approaches fall in one of three categories; (1) incorporation and entrapment of segregated nanoparticles inside a porous matrix, (2) simultaneous in situ generation and immobilization of nanoparticles on to a support matrix and (3) immobilization of nanoparticles on a surface functionalized solid support. Among all approaches, one common procedure that would facilitate a stable association between nanoparticles and the support matrix is the selection of appropriate surface modification methods. However, the choice of the method to be employed for immobilization would certainly depend on many other factors such as type of solid support used, size/shape, morphology, surface functionalization and stability of nanoparticles, and the kind of application for which it is used. For example, a low-moderate level of immobilization may give the desired signals for optical/biosensor applications (Johnsson et al. 1991) while, a relatively higher level of immobilization would always be desired for the long term antibacterial effects. Moreover, in order to develop a antimicrobial biomaterial, the physical behavior of a biomaterial during nanomaterials integration must be in compliance with clinical requirements (Stickler 2000). For example, the mechanical specifications of antimicrobial biomaterial would be desirable for a very high load as in case with hip and knee implants. On the other hand, a biomaterial should either have high transmittance for designing intraocular/contact lenses or should be highly elastic while fabricating artificial blood vessels. In many cases, haemo-compatibility and cytocompatibility of a biomaterial is compromised while introducing the nano-antimicrobial component, which ultimately would lead to immunological rejection of implant. A thorough understanding of the interaction of nano antimicrobial moiety with the biological environment like proteins, cells and tissue, is therefore crucial in order to be able to improve the functionality of nano-biomaterial interfaces.

The current chapter summarizes the recent progress and state-of-art facts on developing novel hybrid nanomaterials based systems as antimicrobial agents for various biomedical applications. The fabrication of nano-antimicrobial biomaterials would be explained on the basis of various mechanism through which a nanomaterial is bound to a biomaterial surface such as by (i) covalent immobilization (ii) impregnation (iii) sustained release of antimicrobial component (iv) synergistic action due to inherent antimicrobial action of support material. The aforementioned strategies aim for one common objective i.e., to provide an effective, stable and long term antibacterial efficacy to the biomaterial, promoting their reuse without causing any cytotoxicity responses against normal cells. The role of silver, gold, copper/copper oxide, zinc oxide, chitosan and their hybrid nanocomposites will be considered while designing new nano-antimicrobials for much needed applications in wound healing, tissue scaffolds, medical implants, coating surgical devices and instruments.

2 Unsupported Nano-Silver as Antimicrobial Agent

Silver and its compounds have been recognized for its antimicrobial efficacy since antiquity. Greeks and Romans used silver coins and vessels to disinfect potable water and keeping the milk fresh (Hill 2009). However, silver based antimicrobial therapy was first documented by Ravelin in 1889 which demonstrated that silver when used in ultra low concentration, proved to be highly germicidal (Zhang et al. 2012a). In late eighteenth century, eye drops constituting of 2% silver nitrate solution were also used to thwart the ocular infections in newborns which lead to blindness and against the treatment of typhoid and anthrax. In 1920s, the US Food and Drug Administration (FDA) agency has recognized colloidal silver as an effective agent to manage wound healing (Chopra 2007). Silver was then continued as a promising strategy for controlling infections during burns till World War II, which outraged its role with huge demands and development of new antibiotics (Dunn and Edwards-Jones 2004). However, such a heavy use of antibiotics lead to the development of drug resistant microbial strains which propelled the researchers to find novel remedies based on silver. In concurrence with growing interest in exploiting materials at nano level, nano silver has shown the highest level of commercialization (Agnihotri et al. 2014). Silver nanoparticles constitute an important component in nearly 57% (435 out of 762 products) of nano enabled consumer products (health care and fitness sector) available in market (Vance et al. 2015). As a result, AgNPs have emerged as the most exploited antimicrobial nanomaterial in diverse applications such as cosmetics, textiles and fabrics, dietary supplements, food packaging, surgical coatings, silver dressings, water sanitation and disinfection (Rai et al. 2009; Chen and Schluesener 2008; Dunn and Edwards-Jones 2004; Raghupathi et al. 2011; Dorobantu et al. 2015; Gajbhiye and Sakharwade 2016).

It is widely accepted that smaller the size of nanoparticles, higher would be its antimicrobial action. These results might be possible due to higher penetration rates of small sized nanoparticle owing to their high surface to volume ratios (Chen and Schluesener 2008; Rai et al. 2009). The antimicrobial property of AgNPs is governed by several other factors such as shape, aggregation state, stability, dispersion medium, types of capping agents, and methods of surface functionalization of nanoparticles. Even similar sized nanoparticles show variation in antimicrobial action against two different strains of same microbial species, known as strain-specific biocidal killing (Agnihotri et al. 2014; Mukherji et al. 2012; Ruparelia et al. 2008). Regarding shape, the truncated AgNPs appear to be more potent than spherical AgNPs in terms of their antimicrobial efficacies (Pal et al. 2007). However, spherical AgNPs are usually preferred over other shapes due to their ease in synthesis, control on particle size, handling and recovery for use either as colloidal state, or immobilized form (Agnihotri et al. 2013, 2014).

Regarding in vitro evaluation of the antibacterial activity of AgNPs, the potency is quantified either by using disk diffusion tests in solid media or by serially diluting the antibacterial material in liquid culture (Agnihotri et al. 2014; Ruparelia et al.
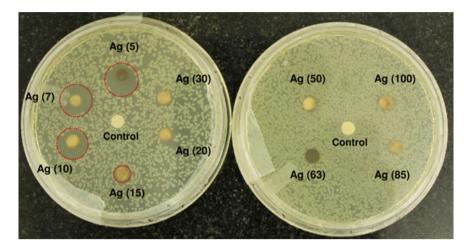
Fig. 1 Disk diffusion tests for different sized (5–100 nm) silver nanoparticles against the *E. coli*. The zone of inhibition (ZoI) is highlighted with a dashed circle indicating a noticeable antibacterial effect. The number in parentheses indicates the average size of silver nanoparticles in nanometer (Reproduced from Agnihotri et al. (2014), Royal Society of Chemistry)

2008). In disk diffusion studies, the sensitivity of a microbe is tested by calculating the diameter of zone of inhibition (ZoI) created by nanoparticles-laden disc by inhibiting the microbial growth surrounding that disc. Thus, a higher value of ZoI would indicate a more sensitive microbial strain and a more effective antimicrobial nanomaterial (Fig. 1). On the other hand, the liquid broth assay is used to quantify the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of nanoparticles as shown in Table 1. The minimum inhibitory concentration (MIC) is defined as the minimum concentration of antimicrobial

Table 1 (a) Minimum inhibitory concentration (MIC, µg ml^{-1}) and (b) minimum bactericidal concentration (MBC, µg ml^{-1}) values for silver nanoparticles of varying size[a]. (Reproduced (Agnihotri et al. 2014), Royal Society of Chemistry)

Bacterial strain	Different sized silver nanoparticles (nm)									
	(5)	(7)	(10)	(15)	(20)	(30)	(50)	(63)	(85)	(100)
(a)										
E. coli MTCC 443	20	20	30	30	40	50	80	90	90	110
E. coli MTCC 739	60	90	90	90	100	100	120	140	160	160
B. subtilis MTCC 441	30	40	40	50	50	60	80	90	110	120
S. aureus NCIM 5021	70	70	80	100	90	100	130	160	180	200
(b)										
E. coli MTCC 443	30	30	40	50	50	80	100	110	130	140
E. coli MTCC 739	90	100	100	110	120	120	140	170	170	180
B. subtilis MTCC 441	40	50	50	60	70	80	100	120	130	140
S. aureus NCIM 5021	80	90	100	110	100	120	160	200	>200	>200

[a]Studies were done at 10^5–10^6 CFU ml^{-1} initial bacterial concentrations

agent that inhibits the visible growth of microbes whereas, the lowest concentration of biocidal agent that kills 99.9% of microbial population is termed as minimum bactericidal concentration (Ruparelia et al. 2008).

The antimicrobial property of silver nanoparticles strongly depends on synthesis routes by which they were produced. Out of several methods (physical, chemical and biological) explored for producing silver nanoparticles, biological methods are gaining enormous interest due to its eco-friendly, non-toxic nature and often synthesize AgNPs with higher antimicrobial properties than that of produced by any other means (Panáček et al. 2006; Sharma et al. 2009). For instance Nanda and Saravanan (2009) investigated the antibacterial activity of biogenic AgNPs (160–180 nm) using S. *aureus* and found that these NPs showed excellent biocidal efficacy against clinically pathogenic multidrug resistant *Staphylococcus aureus* (*MRSA*), multidrug resistant *Staphylococcus epidermis (MRSE), and S. pyogenes.* Similarly, Ingle et al. (2008) reported the extracellular synthesis of AgNPs using *Fusarium accuminatum*, isolated from infected ginger and demonstrated nearly 2–3 times higher biocidal activity of AgNPs than bulk silver (Ag$^+$) against highly pathogenic bacterial strains i.e., MRSA, *Salmonella typhi*, *S. epidermidis*, and *Escherichia coli*. Results revealed that the antimicrobial activity of silver nanoparticles is 2.4–2.9 times that of silver ions. In a recent study, AgNPs (average size, 19–54 nm) synthesized using whole plant extract and callus extract of *Linum usitatissimum* demonstrated good efficacy against pathogenic strains *E. coli, Klebsiella pneumoniae and S. aureus* (Anjum and Abbasi 2016). Similarly, Shanthi et al. (2016) used cell free extract of *Bacillus licheniformis* to produce 18–64 nm AgNPs which exhibited strong antibacterial and antibiofilm properties against *Vibrio parahaemolyticus* Dav1. Silver nanoparticles synthesized through green route have also demonstrated good antimycotic activity against various fungal species viz, *Candida albicans, Dermatophyte Trichophyton* and *Mentagrophytes* (Panáček et al. 2009; Rodrigues et al. 2013). These strains are among few of the most common pathogens that cause hospital-acquired sepsis in immunocompromised patients with nearly 40% mortality rate (Panáček et al. 2009).

Silver nanoparticles can prove to be effective to prevent infectious diseases mediated through viruses. Rogers et al. (2008) demonstrated that AgNPs (10 nm) with biocompatible coating would significantly inhibit Monkey pox virus infection under laboratory conditions. Speshock et al. (2010) elucidated that AgNPs are capable of inhibiting viral infection by significantly reducing the production of viral RNA and release of progeny viruses. The authors however claimed that AgNPs treatment would be effective only if administered within initial 2–4 h of replication stage. A recent study showed antibacterial, antifungal and antiviral activity against variety of microorganisms *E. coli, K. pneumoniae, S. sonnei, P. aeruginosa, S. epidermidis, MRSA, S. bovis, A. flavus, C. albicans,* and Bean Yellow Mosaic Virus (BYMV) by AgNPs produced from micro organisms (Elbeshehy et al. 2015). Similarly, Lu et al. (2008) elucidated antiviral activity of AgNPs against Hepatitis B virus. Another study reported the potential antiviral activity of biogenic AgNPs (size range, 20–46 nm) against human parainfluenza virus type 3, Herpes Simplex Virus 1, and Herpes Simplex Virus 2 (Gaikwad et al. 2013).

3 Silver Based Hybrid Nanocomposites

Considering several limitations associated with using conventional antibiotics and rising demands for better hygiene has motivated researchers to develop effective yet affordable antimicrobial nanomaterials. Antimicrobial activities of metals like silver, gold, copper, zinc etc. can be enhanced by incorporating them into a material matrix thus obtaining a composite material. Nanocomposites are defined as composites in which at least one of the phases shows dimensions in the order of nanometre range. On the basis of types of matrix material, silver based nanocomposites fall into four major categories, silver-polymeric, silver-inorganic, silver-organic, and hybrid metal nanocomposites each with distinct properties that can be utilized in several biomedical applications.

3.1 Silver-Polymeric Nanocomposites

The use of polymers in medical sector continues to grow, thanks to some of its interesting properties like its resistance towards heat, irradiation and chemicals, inert nature, clarity, durability and flexibility to be molded into various sizes and/or shapes (Sastri 2013). Admittedly, the growing concerns for single usage disposable items that have succeeded in reducing the chances of infection in hospitals are made up of polymeric materials. However, the major drawback associated using polymers is that they also provide the necessary surface for microbial contamination, colonization, migration and somehow mimic the conditions require for their subsequent biofilm formation (Hall-Stoodley et al. 2004; Hall-Stoodley and Stoodley 2009). As a result, a large portion of hospital acquired infections (HAIs) are spread through surface contacts with disposable syringes, blood sachets, bottles, pipings, hospitals furniture/wardrobes, which are mostly based on polymeric (polypropylene and polyethylene) materials. It was estimated that roughly 80% of hospital-acquired urinary tract infections originate from urinary catheters, which are of polyvinyl chloride (PVC) origin (Curtis 2008).

Polymer-silver nanocomposites (NCs) are gaining importance as a new generation broad spectrum antimicrobial material in biomedical applications due to their ease in modifications, haemocompatibility, biodegradable nature, and enhanced activity of incorporated AgNPs within the polymeric network. Moreover, the presence of AgNPs would impart an additional biocidal feature to polymer without compromising its properties desired for a particular application. For the preparation of polymer/metal nanocomposites, metals can be incorporated via two approaches; (1) ex situ, in which pre synthesized nanoparticles are incorporated into the surface modified polymeric matrix and (2) in situ, in which polymeric matrix acts both as a nano reactor for synthesizing nanoparticles as well as a template for their subsequent immobilization. The immobilization is achieved through surface modification that allows favorable interaction between the nanoparticles and the support matrix.

Although the current discussion is limited to the incorporation of AgNPs on to polymeric template, similar methods can also be employed for immobilization copper, gold, ZnO nanoparticles in later sections.

AgNPs are quite commonly used as antimicrobial filler in polymeric nanocomposites (Muñoz-Bonilla and Fernández-García 2012) with diverse biomedical applications ranging from wound dressings, coating medical implants and devices, to tissue scaffolds. The polymeric support can be fabricated into various structures such as nanofibers, thin films, solid support and porous gel that act as a template for immobilizing silver nanoparticles (Mukherji et al. 2012). Among various structures, nanofibers have emerged as the most promising bio-material scaffolds owing to its nano scale architecture similar to natural human tissue along with microporous morphology which facilitates adhesion, proliferation, and differentiation of cells for tissue engineering application (Dahlin et al. 2011). Nanofibers posses a high surface area to volume ratio while its characteristics such as composition, biodegradation, and mechanical strength can be manipulated to the intended role, which is beneficial for other biomedical applications as well (Peng et al. 2016). For example, Almajhdi et al. (2014) incorporated AgNPs (1–7 wt%, 5–10 nm diameter) on polylactic-co-glycolic acid (PLGA) nanofibers through electrospinning process (Fig. 2) and the antibacterial activities were tested against
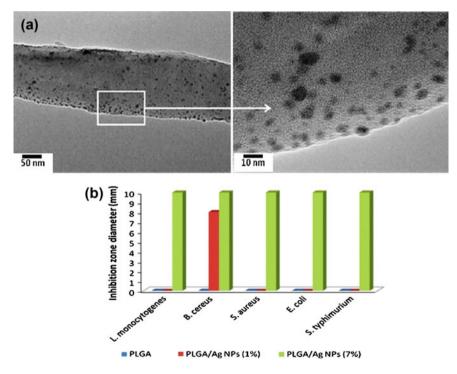

Fig. 2 **a** A highly dense incorporation of silver nanoparticles on the surface of PLGA nanofibers as shown through transmission electron microscopy (TEM). **b** Variation in the sensitivity of microbial pathogens against silver/polymer nanocomposite as marked by difference in their zone of inhibition. (Reproduce with permission from Almajhdi et al. (2014), Springer)

five pathogenic strains *E. coli* o157:H7, *S. aureus* ATCC 13565, *Bacillus cereus* EMCC 1080, *Listeria monocytogenes* EMCC 1875 and *S. typhimurium* ATCC25566 using disc diffusion method. PLGA nanofibers with 7 wt% AgNP demonstrated the best antimicrobial action displaying the highest ZoI (10 mm) against all tested strains. PLGA/Ag nanofibers were found to be suitable for therapeutic applications since they enhanced the anticancer activity along with the biocidal nature without posing any cytotoxicity effects to normal cells.

In another study, Raghavendra et al. (2013) synthesized polymer/Ag nanocomposite fibers based on cellulose for antibacterial skin scaffolds using gum acacia and gaur gum (0.3–0.7 wt%) as biogenic reductants. The incorporation of AgNPs improved mechanical strength and thermal stability of resulting nanocomposite than pristine cellulose fibers along with promising antibacterial activity against pathogenic strains of *E. coli*. Kim et al. (2009) successfully prepared a biodegradable electrospun poly(ethylene oxide)/AgNP NC which showed efficient biocidal control against *S. aureus* and *K. pneumoniae* pathogens. Similar to previous study, they also reported that incorporating AgNPs on to polymeric nanofibers enhanced their mechanical strength without any significant decline in antimicrobial efficacy of AgNPs. Regarding biocompatibility and biodegradability, electrospun nanofibers made from PLGA, polylactic acid (PLA), polycaprolactam (PCL) polymers have been used in many biomedical applications such as fabricating sutures, scaffolds, guided conduits for nerve tissue regeneration wherein the antimicrobial effect to fibers is bestowed due to the presence of AgNPs (Vargas-Villagran et al. 2014).

Currently, the use of natural polymers in tissue scaffolds, drug delivery systems, layer by layer (LBL) assembled films, and as a cargo for bioactive compounds has increased the demand for investigation in biomedical fields. Polymers of natural origin like cellulose, chitosan, dextran, hyaluronan, collagen, alginate have been traditionally used as sources of wound dressings, suture threads, vaccines, and as bioactive compounds (anti-ageing, anti-coagulants and antibacterial agents) in their natural or modified forms (Travan et al. 2009; Ahamed et al. 2015; Anna et al. 2013; Azizi et al. 2014; Lavorgna et al. 2014; Pinto et al. 2009; Raghavendra et al. 2013; Zahran et al. 2014). Interestingly, polymers from natural sources offer many advantages over synthetic ones which make them suitable as biomaterials in regenerative medicine and therapeutics (Allen et al. 2015). Being natural, they are inherently biocompatible, biodegradable, renewable, nontoxic and are relatively cheaper (Dang and Leong 2006). Moreover, natural polymers are easy for chemical modifications thereby improving the structural and functional properties required for the biomaterials (Allen et al. 2015). With this approach, several biocidal agents including AgNPs have been incorporated on natural polymers after surface functionalization in order to enhance their utility as nano-antimicrobials in biomedical applications.

In order to design an efficient and greener polymeric nanocomposites, Pinto et al. (2009) reported in situ synthesis of AgNPs on the surface of cellulosic fibers for biomedical applications. Authors demonstrated that positively charged Ag^+ ions can form stable electrostatic interactions with functional moieties available at the surface
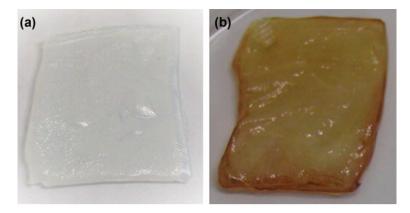
Fig. 3 Photographic images of **a** pristine cellulose membrane and **b** Ag-cellulose nanocomposite membrane fabricated by in-situ synthesis of AgNPs. (Reproduced with permission from Pinto et al. (2009), Elsevier)

of cellulose, and subsequently can be reduced to nanoscale under UV irradiation. The successful immobilization of AgNPs was evidenced by observing a visual change in the color of nanocomposites after immobilization (Fig. 3). Electron microscopy analyses confirmed a highly dense and homogenous distribution of AgNPs over the nanocomposite. The nanocomposite with high Ag content (0.57–4.4 wt%) exhibited strong antibacterial activity toward *S. aureus, K. pneumoniae and B. subtilis* strains. A modified form of cellulose i.e., carboxymethylcellulose has also been utilized as a template for incorporating copper, silver and even iron oxide nanoparticles with an aim to fabricate antimicrobial and antifungal coatings as novel therapeutics (Nadagouda and Varma 2007).

Azizi et al. (2014) synthesized poly(vinyl alcohol)/chitosan (PVA/CS) based nanocomposites using different proportions of zinc oxide and silver nanoparticles as multifunctional nano fillers. As compared to pristine PVA/Cs, presence of nano ZnO and AgNPs increased the mechanical strength (from 0.055 to 0.205 GPa), demonstrated good visibility and UV-shielding effects along with excellent antimicrobial properties against *Salmonella choleraesuis* and *S. aureus* strains. Recently, a cellulose-chitosan hybrid nanocomposites containing a unique blend of AgNPs and antibiotic gentamicin was prepared for wound dressing applications (Ahamed et al. 2015). For preliminary experiments performed on rats, the physicochemical and biochemical studies revealed faster healing pattern in wounds while the presence of AgNPs ruled out the chances for contamination. The authors claim this nanocomposite as an eco-friendly wound dressing material for humans after being successfully implemented on other animals. Zahran et al. (2014) described an eco-friendly approach for synthesizing Ag/alginate nanocomposite using a one step in situ reduction of Ag^+ ions in alginate solution, which acted as both reducing and stabilizing agent. The resulting nanocomposite was applied on cotton fabrics so as to testify its antimicrobial potential on clinical isolates. The

modified cotton fabrics showed excellent antibacterial activity towards *E. coli, S. aureus and P. aeruginosa* strains. A slight reduction in the antibacterial efficacy of modified fabrics was observed when used repeatedly for 20 washing cycles, however 90% bacterial killing was still achievable with such high number of washing steps. Authors claimed this NC as a promising approach for fabricating antibacterial finishing for wound healing purposes. Similarly, Ag/collagen based hybrid nanocomposites have been exploited as tissue scaffold for promoting bio-cidal response against *E. coli, P. mirabilis, B. cereus, and S. aureus* pathogens (Mandal et al. 2012). Due to their good mechanical strength, biological function-alities and potential to immobilize metallic nanoparticles, collagen based scaffolds can successfully be utilized in fabricating prosthetic heart valves. Polymers isolated from non-primate sources such as crustaceans (chitosan, chitin), *Bombyx mori* (silk fibroin) has also been employed as a bio-template for in situ production of AgNPs besides acting both as a reducing agent and stabilizer to prevent their aggregation (Fei et al. 2013; Lavorgna et al. 2014). The resulting nanocomposites have demonstrated efficient killing of MRSA, *S. epidermidis*, and *K. pneumoniae* with disruption of biofilm formation afterwards. Nevertheless, the scientific advance-ments prompted towards establishing polymers-silver nanocomposites as an ideal antimicrobial biomaterial are still poised with several challenges such as broadening their applicability while combating against virulent pathogens.

3.2 *Silver-Inorganic Nanocomposites*

In past two decades, the application of inorganic nanomaterials in biomedical fields has drawn attention among the researchers. Inorganic nanocomposites consisting of micro and mesoporous silica, glass (silicon dioxide), silicates and zeolites have particularly been exploited for potential antimicrobial actions. Other than being inert, inorganic nanomaterials can easily be engineered into desired morphology while its porous architecture contributes toward dense immobilization of AgNPs (Agnihotri et al. 2013). Moreover, inorganic nanocomposite are inherently hydro-philic due to the presence of several functional groups such as hydroxyl, carboxyl,–SH and act as cargo vehicle for delivering drugs, bioactive molecules and even antimicrobial agent owing to its high surface area.

For instance, Song et al. (2013) reported synthesis of silver/polyrhodanine nanocomposites on silica nanoparticles as potential antimicrobial therapeutics. In this study, metal binding affinity of thiol-functionalized silica NPs was exploited for loading Ag^+ ions on its surface followed by treatment with rhodanine monomer solution. The polymerization of rhodanine was then carried at the silica surface, where silver ions were subsequently reduced to silver nanoparticles (average size, 7 nm) forming stable silver-polyrhodanine complex (Fig. 4). The antibacterial potential of Ag/PRh–SiO$_2$ nanocomposite was evaluated toward *E. coli* and *S. aureus* strains which showed MIC values of 1.5 and 2.5 mg ml^{-1} at an initial bacterial concentration of $10^5 - 10^6$ CFU ml^{-1}. The enhanced antimicrobial
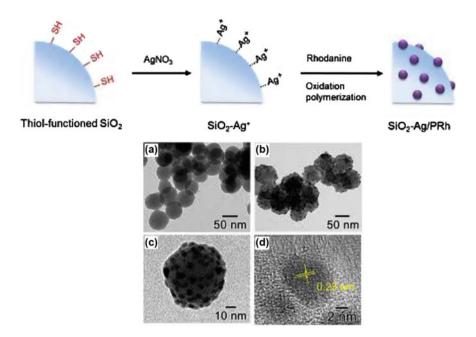
Fig. 4 Schematic representation for the synthetic protocol of Ag/PRh-SiO$_2$ nanocomposite. TEM images of **a** thiol-modified silica nanoparticles and **b, c** Ag/PRh-SiO$_2$ nanoparticles at lower and higher magnifications, respectively. **d** High resolution TEM image of Ag/PRh-SiO$_2$ nanocomposite. (Reproduced with permission from Song et al. (2013), American Chemical Society)

activity of silver/polyrhodanine-silica nanocomposite was attributed to the dual role of microbial killing through release of silver ions as well as direct contact with polyrhodamine.

In another study, fully exfoliated clay, i.e., nanosilicate platelets (NSP) were used as a dispersing agent and immobilizing matrix for depositing AgNPs and the Ag/NSP/Poly(ether)urethane (PEU) hybrid nanocomposites were evaluated for its biocompatibility, immunological response, and antimicrobial activities against few clinical isolates (Lin et al. 2013). Owing to its immobilization, AgNPs could not enter inside cells thereby lowering the risk associated with cellular uptake of AgNPs. The Ag/NSP composite having 20 ppm AgNP concentration were translated into an effective biocompatible material by further mixing with PEU which showed no cytotoxic responses to mouse skin fibroblasts (L929 cells) and human hepatoma cells (HepG2), yet exhibiting complete bacterial killing (99.9%) of *E. coli* cells (Fig. 5). The amount of leachable silver in form of either free Ag$^+$ or AgNPs was found to be 170 and 270 ppb, respectively, whereas the supernatant of silver nanohybrids did not show antibacterial activity after aging for 6 months. Authors thus claimed that this antimicrobial biomaterial can effectively be employed in biomedical application considering the biosafety associated with minimizing the excessive discharge of silver.
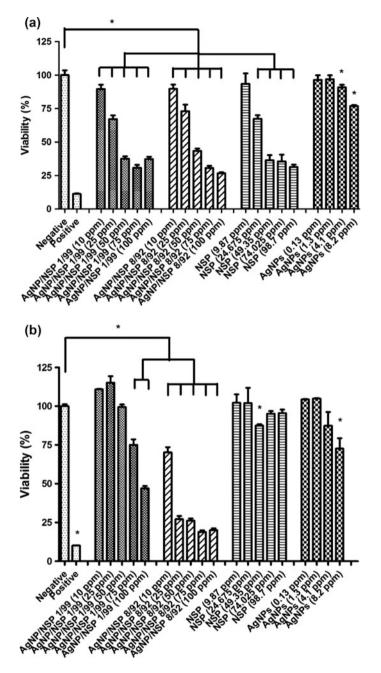
Fig. 5 Cytotoxic effects of AgNP/NSP (silver-nanosilicate platelets), NSP, and AgNPs on **a** L929 cells and **b** HepG2 cells. The concentration of NSP or AgNPs corresponded to the content of each component within the AgNP/NSP hybrid. The concentration was based on the total weight, e.g., AgNP/NSP 1/99 10 ppm contains 9.87 ppm NSP and 0.13 ppm AgNPs. * indicates a statistical difference from the control, $p < 0.05$. (Reproduced with permission from Lin et al. (2013), American Chemical Society)

It is a matter of immense discussion whether the mode of antibacterial action of AgNPs is mediated solely on the basis of either release of silver ions or nanoparticles-specific, or may be both (Li et al. 2006; Hoop et al. 2015; Wang et al. 2013). Another study hypothesizing the dual role of antibacterial action of Ag/SiO_2 based nanocomposites was given by (Agnihotri et al. 2013) where a high localized immobilization of AgNPs (8.6 nm, average size) was achieved on an amine-functionalized silica substrate using 3-(2-aminoethylaminopropyl) trimethoxysilane as a cross linker molecule. The bactericidal potential of AgNP–glass nanocomposite was tested against two *E. coli* strains, MTCC 443 and MTCC 739, and one *Bacillus subtilis* strain, MTCC 441, in both deionized water and phosphate buffer medium. The antibacterial tests were performed independently at two different initial bacterial concentrations i.e., 10^3 and 10^5 CFU ml^{-1}, where bacterial counts were reduced to zero within 120 min under all the test conditions. It was concluded that contact killing is the predominant bactericidal mechanism and surface immobilized nanoparticles showed greater efficacy than other sources of silver (free AgNPs, bulk Ag and bulk AgCl) and released even less than 25 ppb of silver in solution (Fig. 6). Interestingly, AgNP–SiO$_2$ substrate was reused 11 times
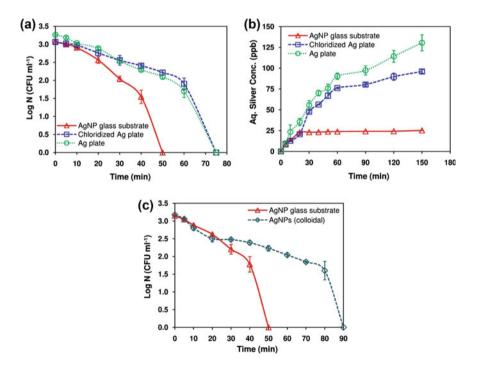

Fig. 6 Comparative effect of various Ag sources, i.e., pure silver, AgCl and AgNP–glass substrate, all with same dimensions on **a** disinfection and **b** silver release profile is presented. **c** Comparative bactericidal potential of AgNP–glass substrate and AgNP colloidal suspension (average size 8.6 nm) against the *E. coli* MTCC 443 strain having a similar content of silver. (Reproduced with permission from Agnihotri et al. (2013), Royal Society of Chemistry)

without losing its bactericidal efficacy. This indicates that the proposed immobilization protocol could prove to be effective while minimizing the toxicity issues associated with excess release of AgNPs into solution as required in case with antimicrobial coatings, especially for surgical devices and synthetic implants.

A research group lead by Prof. Alexander Seifalian at University College London (UK) has developed a novel nanocomposite biomaterial based on polyhedral oligomeric silsesquioxane-poly(carbonate-urea)urethane (POSS-PCU) having required mechanical properties and histo-compatibility for cardiovascular applications (Ghanbari et al. 2016; Kannan et al. 2006; Raghunath et al. 2009). This polymer has been successfully implanted in humans in form of vascular bypass graft, a lachrymal duct, and tracheal implants. However, the biomaterial suffers from graft infection involving MRSA, *S. epidermidis* which prevails with serious consequences including bacteremia, systemic sepsis, higher incidences of amputation, and even death. With an aim to impart biocidal component to this polymer, pre synthesized silver nanoparticles (average diameter, 15 nm) were mixed with POSS-PCU at different concentrations (16, 32, 64, 128 mg) and its effect on the platelets was evaluated (de Mel et al. 2012).

Platelet adhesion on test surfaces was quantified using the Alamar blue assay, which is a direct measurement of metabolic activity of platelets and is proportionate to the color intensity. Authors demonstrated that POSS-PCU up to 64 mg AgNPs marked no significant variation in platelet adhesion as compared to POSS-PCU without AgNPs. However, for higher conc. of AgNPs (i.e., 128 mg), POSS-PCU-demonstrated a 50% reduction in platelet adhesion as with pristine POSS-PCU. While comparing the morphology of the platelets, the positive controls (Collagen and PTFE) showed the existence of platelets in a highly aggregated state with extended pseudopodia. In contrast to this, the platelets treated with Ag incorporated POSS-PCU showed a very few platelets with a rounded appearance at their initial state of adhesion. Comparing other results, it was evidenced that the incorporation of AgNP not only enhanced the anti-thrombogenic properties of POSS-PCU, it also provided an additional benefits in terms of its biocidal nature, and thus potentially can be used in fabrication of cardiovascular implants.

Shameli et al. (2011) developed a new method for in situ synthesis and immobilization of AgNPs within interlayer space of montmorillonite (MMT), a modified silicate clay. To this composite, chitosan polymer was intercalated through cationic exchange and hydrogen bonding processes so as to convey some important properties like biocompatibility, biodegradability, non toxicity, and bioactivity in the resulting nanocomposites for potential biomedical applications. The modified clay (MMT/chitosan) not only acted as a stabilizing agent preventing the AgNPs from being aggregated, it also assisted in reducing silver ions to AgNPs under room temperature conditions. The antibacterial activity of Ag/MMT/chitosan bio-nanocomposite was examined against *S. aureus*, MRSA *E. coli* O157:H7, and *P. aeruginosa* by disc diffusion method having different sizes (6.2–9.8 nm) of AgNPs. Results indicated that bio-nanocomposite was found to be highly bactericidal against all pathogenic strains where the range of ZoI varied from 7.6 to 11.9 mm showing its strain specific sensitivity. Authors claimed that the

Ag/MMT/chitosan nanocomposites can successfully be applied as biocompatible antimicrobial coating in surgical devices and as drug delivery vehicles. In a different study, Ag/MMT/chitosan nanocomposites have also been evaluated for topical treatment of chronic skin lesions during the treatment of skin ulcers (Sandri et al. 2014). The antimicrobial properties were examined against four bacterial strains, *S. aureus, S. pyogenes, E. coli,* and *P. aeruginosa* which often complicate skin lesions during wound healing.

3.3 Silver-Carbon Nanocomposites

Several attempts have been made to incorporate silver on various carbon based nanostructures like single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), graphene, graphene oxide and carbon aerogels for antibacterial applications. The size (diameter) of nanotubes is considered to be an important factor for assessing the bactericidal potential of CNTs since SWCNTs are more lethal to microbes than MWCNTs (Kang et al. 2008). Through gene expression data, it was evidenced that *E. coli* expressed a higher levels of stress-related gene products when treated with SWCNTs as compared to MWCNTs. The enhanced toxicity of SWCNTs is attributed to their sharp edges which acted as nanosyringes for inducing a direct contact to microbes thereby causing damage to cell membrane (Afzal et al. 2013). In addition to this, the presence of carbon nanotubes in Ag-CNTs nanocomposite serves many purposes. First, CNTs act as an immobilizing template for dense localization of silver nanoparticles owing to its high surface area. Secondly, CNTs may undergo simple surface functionalization procedures and the modified CNTs can offer the required nucleation sites for in situ synthesis of AgNP via forming stable silver-CNTs complexes (Wildgoose et al. 2006). Most importantly, Ag-CNTs often mediate synergistic antibacterial effect due to the inherent bactericidal action of CNTs and thus strengthen their antimicrobial performance in addition to being acted as an immobilizing substrate material (Akhavan et al. 2011; Yu et al. 2014; Seo et al. 2014; Rangari et al. 2010).

Exploring the above possibilities, Mohan et al. (2011) described a wet chemical approach to immobilize AgNPs onto carbon nanotubes following surface functionalization procedure. In this study, silver ions were initially grafted over acid functionalized surface (–COOH) forming stable silver-MWCNTs complexes, which acted as the template for AgNP growth. After exposing it to reducing agent, AgNPs were decorated onto MWCNTs in a highly ordered fashion with dense packing. The antibacterial experiments performed against *E. coli* strain had shown that while Ag-MWCNT contributed toward 97% bacterial killing, the acid functionalized MWCNTs (without AgNPs) killed only 20% of bacterial population. Authors suggested their role as antibacterial coatings in biomedical devices and antibacterial controlling system.

In order to construct an orthopedic implant biomaterial, Afzal et al. (2013) described Ag-reinforced composite material containing hydroxyapatite (HA) and

MWCNTs, where the presence of silver landed an antimicrobial character to the biomaterial without compromising their inherent mechanical, physiochemical and biological properties. In this study, HA-CNT composites were mixed with 5% Ag powder (particle size < 100 nm) while the samples were sintered in vacuum under uniaxial pressure of 30 MPa at 950 °C for 5 min. The antibacterial tests performed using *E. coli* and *S. epidermidis* showed a significant decrease (65–86%) in the number of bacteria adhered to Ag/HA/MWCNT composites. The density of *E. coli* on HA, Ag/HA, only CNT, and Ag/CNT was found as 330, 70, 1320, and 430 cells/mm^2, respectively. Similarly, the density of *S. epidermidis* on the corresponding substrates was estimated to be 370, 130, 350, and 50 cells/mm^2, respectively. This indicates that bacteria were proliferating more over the surface of pure HA and CNTs whereas the addition of Ag particles resulted in the inhibition of bacterial growth and bacterial proliferation was retarded. Later, the same group reported synthesis of ceramic biomaterial based on Ag/HA/CNTs for minimizing bacterial infections for bone replacement prosthesis (Herkendell et al. 2014). Introducing small amounts of silver (2–5 wt%) demonstrated a profound antibacterial effects as the bacterial adhesion was reduced to 60% in contrast to pure-CNTs and pure-HA who promoted bacterial growth on their surface by 8.5%. Several other approaches have also been applied to synthesize Ag/CNTs nanocomposites employing biocompatible, environmental benign molecules such as dendrimers (Murugan and Vimala 2011), PMMA (Rusen et al. 2014), and liposomes (Barbinta-Patrascu et al. 2014) which demonstrated excellent antibacterial activity against various pathogens strains *B. subtilis*, *S. aureus*, *E. coli*, and *E. faecalis*.

The development of toxic free biomaterials has become a great challenge in recent times. The chemical procedure for synthesizing Ag-carbon nano hybrids mainly involves the use of either sodium borohydride or hydrazine hydrate as reducing agents. These chemicals are inflammable, toxic and potentially hazardous, and their left over residues persists in the system despite several washing steps. As a result, they elicit cytotoxic effects both under in vitro and in vivo conditions and limit the applicability of synthesized biomaterial for long term use. Synthesis of nanocomposites by green method using some biocompatible reducing agents can potentially eliminate this problem. Recently, Yallappa et al. (2015) proposed a green method for synthesizing Ag-MWCNTs composite using *Terminalia arjuna* bark extracts under microwave irradiation. In this study, AgNO$_3$ precursor was introduced in aqueous dispersion containing MWCNTs and the biological extract. The phytochemicals present in the extract acted as reducing and stabilizing agent such that AgNPs were synthesized in situ and subsequently grown on the surface of MWCNTs. The hybrid nanocomposite was found to be very effective against bacterial and fungal strains, which are the causative agents for hospital-acquired infections. The antimicrobial activity was evaluated on the basis of disc diffusion studies, where the zone of inhibition (ZoI) was calculated in the range from 10–16 mm for bacterial strains and 7–8 mm for fungal strains. While comparing the antibacterial results with pristine MWCNTs, the order of bactericidal and antifungal potential of Ag/MWCNTs was observed as *E. coli* > *S. typhi* > *S. aureus* > *P. aeruginosa* and *C. albicans* > *T.*

rubrum ≈ *C. indicum*, respectively. Authors explained this enhanced antimicrobial activity of nanobiohybrids as a combined effect of CNTs, AgNPs, and the presence of phytochemicals from plant extract in the dispersing media. These results suggest that the biohybrid nanomaterials can compete with commercial antimicrobial agents.

Graphene i.e., a monolayer array of carbon atoms linked together in a 2D hexagonal lattice constitutes another class of nanomaterials that exhibits broad spectrum antimicrobial activity. The potential for using graphene based nanocomposites in films and coatings applications has been rapidly expanded over the past decade. Graphene is considered as a biocompatible material towards mammalian cells and osteoblasts while graphene oxide has been utilized as a carrier matrix to deliver bioactive agents and drugs into the cells. Hu et al. (2010) described a novel route for synthesizing graphene-based antibacterial paper via introducing several functional groups (hydroxyl, epoxy, and carboxyl) over graphene sheets enabling it to be water dispersible. The antibacterial activity of graphene paper was validated by observing a significant reduction (up to 70%) in metabolic activity of *E. coli* DH5α cells and suppressing bacterial growth up to 98.5% in presence of GO nanosheets. In another study, Wang et al. (2015) investigated the antibacterial potential of reduced graphene oxide/magnetic NPs/polyethylenimine nanocomposite onto which AgNPs were grown through in situ approach. The resulting biomaterial exhibited excellent antibacterial performance against model strain, *E. coli* O157:H7 with 99.9% killing rate (initial bacterial count, 10^7 CFU ml^{-1}) using a dosage of 0.1 μg ml^{-1} followed by a photothermal treatment (5 min) under a near-Infrared (NIR) laser irradiation. Moreover, a MBC value of 0.1 μg ml^{-1} could be achieved under near infrared (NIR) laser irradiation for 10 min, while no colony of *E. coli* O157:H7 was found in solid agar plate. The strong absorbance characteristics of graphene in NIR has been exploited in another study (Tian et al. 2014), where AgNP/GO/iron oxide nanocomposite showed synergistic antibacterial effect against *E. coli* and *S. aureus* strain. Moreover, due to the presence of iron oxide nanoparticles, the antibacterial composite were recoverable and hence can be used repeatedly.

Recently, a sandwich-like antibacterial nanomaterial was constructed by introducing halloysite nanotubes (HNTs) to Ag/graphene nanosheets combining the adhesive potential of 3, 4-dihydroxyphenylalanine (DOPA) after self polymerization (Yu et al. 2014). It was a single-step synthesis protocol and was performed under mild atmosphere without involving any hazardous chemicals or specific process conditions. Electron microscopy studies indicated that the presence of DOPA not only facilitated the intercalation of HNTs within GO sheets, it also caused reduction of silver ions to AgNPs and their subsequent attachment to both HNTs and graphene oxide (GO) nanosheets (Fig. 7). Synthesized AgNPs were found to be in a range between 5–15 nm through TEM micrographs. The antibacterial experiments indicated a very high bactericidal potential of Ag/HNTs/rGO (reduced graphene oxide) towards *E. coli* and *S. aureus* having MIC value of 2 μg/ml as compared to their control groups, i.e., Ag/GO (16 μg ml^{-1}), Ag/HNTs (32 μg ml^{-1}), colloidal Ag (64 μg ml^{-1}) and GO nanosheets (1064 μg ml^{-1}). Authors also demonstrated that Ag/HNTs/rGO nanocomposite can be fabricated into a paper-like antibacterial film
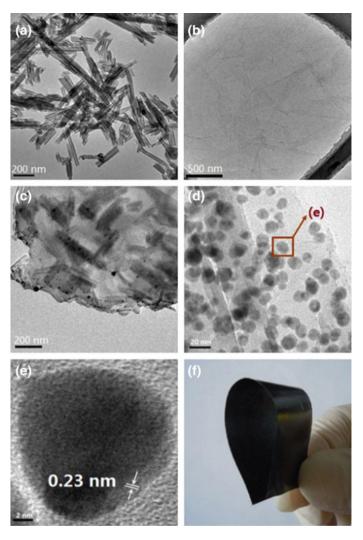
Fig. 7 TEM images of **a** HNTs, **b** GO nanosheets and **c**, **d**, sandwich-like nanomaterials at different magnifications. **e** HRTEM image of single entity silver nanoparticles with fringe spacing. **f** Photograph of antibacterial film prepared from Ag/HNTs/reduced GO. (Reproduced with permission from Yu et al. (2014), Nature publishing group)

by introducing a small proportion of polyethersulfone which showed excellent flexibility and can be used for biomedical purposes as antimicrobial coatings.

As evident from literature review, most of the studies involving use of Ag-graphene nanocomposites as antimicrobial biomaterial have targeted to exploit their efficacy either in terms of their ability to preventing bacterial colonization and/or inhibiting bacterial growth on the biomaterial surface. However, the clinical

relevance of these nanocomposites is not limited to their antibacterial nature since a few studies have specifically evaluated their antifungal effects as well. For examples, Li et al. (2013) explained a method for synthesizing carbon nanoscrolls (CNS) filled with AgNPs and was tested against *Candida albicans* (ATCC 90029) and *Candida tropical* fungal strains, isolated from a patient suffering from urethritis at a local hospital. The synthetic process involved in situ reduction of silver ions followed by their anchoring on the surface functionalized GO resulting in the formation of Ag–GO nanocomposite. Moreover, the composite was sonicated for next 6 h so that most of the exfoliated GO could be curled into scroll while wrapping most of AgNPs into it, called as carbon nanoscrolls.

At first, the antifungal activity of GO, Ag–GO and CNS–AgNPs were evaluated by modified agar disk diffusion method. Results indicate while no inhibition zone was observed for pure GO samples during 24 h of incubation, both GO–AgNPs and CNS–AgNPs showed a clear zone of inhibition (ZoI) even after an incubation period of 8 h. However, as the incubation time was increased to 12, 20, and 24 h, the no. of viable colonies were much lesser in CNS–AgNPs treated samples as compared to GO–AgNPs for same strains under similar test conditions. The antifungal activity of GO, GO-AgNPs and CNS–AgNPs also was evaluated by liquid culture assay broth micro dilution method. The MIC values of CNS–AgNPs against *C. albicans* and *C. tropical* strains were calculated as 0.25 and 0.125 mg mL^{-1}, respectively. On the contrary, GO–AgNPs demonstrated a higher MIC value of 0.5 mg mL^{-1} against both fungal strains. In order to elucidate the enhanced
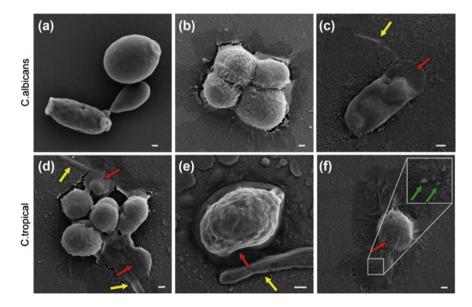

Fig. 8 Scanning electron micrographs (SEM) of **a** native *C. albicans* cells, **b** *C. albicans* cells treated with pure GO and **c** CNS–AgNPs for 24 h. **d** and **e** *C. tropical* cells treated with CNS–AgNPs. **f** *C. tropical* cells treated with GO–AgNPs for 24 h. *Red*, *green* and *yellow arrows* indicate breakage of yeast cells, CNS–AgNPs and AgNPs on GO surface, respectively. (Reproduced with permission from Li et al. (2013), Elsevier)

Table 2 Silver nanocomposites based antimicrobial biomaterials and their biomedical applications

Silver based nanocomposites (NCs)	Size of AgNP	Activity	Microbes tested	Evaluation parameters	Biomedical applications	References
Ag/Polyurethane (PU)	5 nm	AB	B. subtilis, E. coli	ND	Antibacterial catheter	Hsu et al. (2010)
Ag/Polystyrene NCs	8 nm	AB	P. fluorescens, E. coli, B. circulans, S. aureus	ZoI: 2–27 mm	Antibacterial coatings	Kamrupi et al. (2011)
Ag/TiO$_2$ nanocomposite films	10–30 nm	AB	E. coli	ZoI: 7 mm	Antibacterial coatings, Antibiofilm material	Yu et al. (2011)
Ag/Calcium phosphate		AB	S. aureus, S. epidermidis	85–98% inhibition	Antibacterial scaffold	Ewald et al. (2011)
Ag/PLA thin films	3–4 nm	AB	E. coli, S. aureus, V. parahaemolyticus	ZoI: 9–15 mm	Antibacterial scaffold, Biomedical coatings	Shameli et al. (2010)
Ag/Carbon/platinum NC	3–5 nm	AB	Staphylococcus, P. aeruginosa	ND	Antibiofilm coatings	Narayan et al. (2005)
Ag/Calcium phosphate NC	2.7 nm	AB	S. mutan, S. sobrinus	75% inhibition	Antibiofilm plaques	Cheng et al. (2012)
Ag/PU/PCL/PMMA	20–27 nm	AB	E. coli	10^6 fold reduction	Antibiofilm implants	Sawant et al. (2013)
Ag/Bioactive glass/chitosan	<50 nm	AB	S. aureus	ZoI:16 mm	Antimicrobial coatings	Pishbin et al. (2013)
Ag/pCBMA NC	ND	AB	E. coli	99.8% inhibition	Antimicrobial coatings, Anti adhesive biomaterial	Hu et al. (2013)
Ag/Nylon-6/CNT hybrid NCs	5–10 nm	AB	S. aureus, S. pyogenes, E. coli, Salmonella enterica	ZoI: 19–28 mm	Antimicrobial coatings, Disinfectant filters	Rangari et al. (2010)
Ag/Polyamide-6 NC	<100 nm	AB	E. coli	100% inhibition	Antimicrobial material	Damm et al. (2007)

(continued)

Table 2 (continued)

Silver based nanocomposites (NCs)	Size of AgNP	Activity	Microbes tested	Evaluation parameters	Biomedical applications	References
Ag/hydroxyapatite NC	20–30 nm	AB	E. coli	100% inhibition	Bone substitute material, Implant coatings	Liu et al. (2013)
Ag/Graphene hydrogel	10 nm	AB	E. coli, S. aureus	ZoI: 9.7–11.8 nm	Burns wound healing	Fan et al. (2014)
Ag/Montmorillonite/chitosan NC	2–3 nm	AB	E. coli, S. aureus, MRSA	ZoI: 8–9.5 mm	Coating surgical devices, Delivery system	Ahmad et al. (2009)
Ag/PES/SPES film	40–50 nm	AB	S. aureus, S. albus, E. coli	ZoI: 1–2.5 mm	Coatings medical devices	Cao et al. (2010)
Ag/collagen scaffold	30–60 nm	AB	E. coli, P. mirabilis, B. cereus, S. aureus	ND	Tissue scaffold	Mandal et al. (2012)
Ag/collagen/PHBV film	ND	AB	E. coli, S. aureus, P. aeruginosa	100% inhibition	Tissue scaffold	Bakare et al. (2016)
Ag/PLGA electrospun nanofibers	5–10 nm	AB	ND	ND	Tissue scaffolds Wound dressing	Khalil et al. (2013)
Ag/chitosan/PEG film		AB	E. coli	88% inhibition	Wound dressing	Rao et al. (2012)
Ag/Cellulose	5–11 nm	AB	E. coli, S. aureus	ZoI: 2–3.5 nm	Wound dressing	Maneerung et al. (2008)
Ag/gelatin electrospun pads	11–20 nm	AB	P. aeroginosa, E. coli, MRSA, S. aureus	ZoI: 1.9–2.4 mm	Wound dressing	Rujitanaroj et al. (2008)
AgNP/chitosan	–	AB	S. aureus, B. subtilis, E. coli, S. choleraesuis	MIC: 0.03–0.06 mg/ml	Wound dressing	Chen et al. (2014)
Ag/Cellulose acetate nanofibres	21 nm	AB	S. aureus, E. coli, K. pneumoniae, P. aeruginosa	99.9% inhibition	Wound dressing Tissue scaffolds	Son et al. (2006)
AgNP/Cu-loaded mullite NC		AB	E. coli, S. aureus	MIC: 1.6 mg/ml	Wound dressings, Antimicrobial coatings	Kar et al. (2014)

(continued)

Table 2 (continued)

Silver based nanocomposites (NCs)	Size of AgNP	Activity	Microbes tested	Evaluation parameters	Biomedical applications	References
Ag/silica polystyrene	1–10 nm	AB, AF	*S. aureus, C. albicans, K. pneumonia, E. coli; P. fluorescens, A. niger Salmonella enterica*	MIC: 62.5 µg/ml	Antimicrobial coatings	Egger et al. (2009)
AgNPs/rice-paper plant	<100 nm	AB, AF	*E. coli, C. albicans*	MIC: 14–28 mg/l	Wound dressing	Zeng et al. (2007)
AgNPs/CMC/PEO nanofibers	12–18 nm	AB, AF	*S. aureus, E. coli, P. aeruginosa, C. albicans*	ZoI: 12–20 mm	Wound dressing	Fouda et al. (2013)
AgNP/Chitosan nanofibers	10 nm	AF	*Alternaria sps., Bipolaris oryzae, B. cinerea, P. digitatum, C. higginsianum, Fusarium oxysporum*	40–90% reduction in spore germination	Antifungal therapies	Ifuku et al. (2015)
Ag/Graphene oxide NC	30–50 nm	AF	*C. albicans,C. tropical*	ZoI: 15 nm MIC: 0.125 µg/ml	Nosocomial infections, Local antifungal therapy	Li et al. (2013)
Ag/Carbon supported matrix	20 nm	AB, AV	*E. coli, B. subtilis,* Bacteriophage M13	60–80% inhibition in bacterial and fungal counts,	Antibacterial material Antiviral infection control	Vijayakumar and Prasad (2009)
AgNP/Chitosan NCs	3.5–13 nm	AV	H1N1 influenza A	60–85% inhibition in viral replication	Antiviral infection control	Mori et al. (2013)
AgNP/Graphene NC film	5–25 nm	AV	feline coronavirus, infectious bursal disease virus	25% inhibition in viral replication	Antiviral infection control	Chen et al. (2016)

AB Antibacterial; *AF* Antifungal, *AV* Antiviral, *ND* Not Determined

antifungal activity of CNS-AgNPs over GO, scanning electron microscopy was employed (Fig. 8). The SEM micrographs indicated that no significant morphological change was observed in *C. albicans* and *C. tropical* cells treated with pure GO. However, both fungal strains treated with CNS-AgNPs demonstrated a distinct damage to cytoplasmic membrane such that their intracellular contents were leaked completely. Moreover, release of silver ions from CNS-AgNPs also caused a more severe effect such that a clear concave zone was observed in the cell membrane. Similarly, a hydrogel based contact lens has recently been tested as antimicrobial biomaterial comprising of quaternized chitosan, AgNPs and graphene oxide (Huang et al. 2016). Contact lenses loaded with AgNP and GO demonstrated good mechanical properties and excellent antifungal efficacy under both in vitro and in vivo conditions. Authors indicated its therapeutic use as drug delivery vehicle for the treatment of fungal keratitis, a severe ocular disease in developing countries which often leads to blindness and ocular morbidity. Analogous antimicrobial performance of various silver based nanocomposites and their biomedical applications has been summarized in Table 2.

3.4 Nano Silver Based Antimicrobial Hydrogels

Hydrogel is a porous 3D semi interpenetrating polymeric network which has high water holding capacity than its own weight without getting dissolved into it (Mukherji et al. 2012; Agnihotri et al. 2012). Due to their soft architectures and ability to mimic the microenvironment of native tissue, they can be engineered to a myriad of applications such as in drug delivery, tissue engineering, stem cell engineering, immunomodulation, molecular therapies and even in cancer research (Lee and Mooney 2001; Drury and Mooney 2003). Another expanding area where hydrogels have gained enormous attention is in wound dressings and coating surgical devices to prevent nosocomial infections. Other than being non-toxic, hydrophilic, biocompatible and biodegradable, hydrogel exhibits several other remarkable properties such as oxygen permeability, good adhesion and easy handling, which make them an ideal candidate for biomedical applications (Peppas et al. 2006; Hoffman 2012; Zhu and Marchant 2011; Jones and Milton 2000). Especially for wound healing purposes, the water holding ability of hydrogel keeps the wound hydrated and prevents scar formation, which is inevitable several times. Moreover, their low abrasion characteristics and ability to supply nutrients in a controlled manner accelerates healing process and alleviate pain. Some commercial hydrogel based products like Hydrofiber® and Aquagel® are already in the market which allow to keep a moist environment around the wound site and promotes wound healing (Jones et al. 2006).

Despite several advantages associated with hydrogels, their utilization had been limited for two main reasons. First, hydrogels suffer with poor mechanical durability which restrict their applications in several domains such as tissue engineering and corneal implants where tough and flexible properties are specifically needed

(Zhu and Marchant 2011). Secondly, with few exceptions, hydrogels are generally more susceptible to get infected and their applications at the infected site may elevate the risk of spreading infection to surrounding tissues (Jones and Milton 2000). Integration of nanotechnological advances to hydrogel thus have succeeded to minimize these limitations and extended its accepted applications beyond treating sloughy and necrotic wounds. Reinforcing hydrogel with nanomaterials such as silica nanoparticles, graphene oxide, carbon nanotubes, clay nanosheets as nano-fillers have tremendously improved their mechanical strength. On the other side, introduction of nano-antimicrobials to hydrogel is becoming an utmost concern for its clinical relevance in much needed areas such as prostheses, ocular surgery, biodegradable sutures, and coating surgical implants and devices.

There exists a plethora of techniques for making nanocomposites hydrogels for diverse applications (Fig. 9). However, the development of nano antimicrobial hydrogels involves two classical approaches. In one approach, a wide range of nanomaterials with antimicrobial characteristics such as metallic nanoparticles (Ag, Au, Cu), carbon based (nanotubes, graphene, graphene oxide), polymeric, and inorganic materials (SiO_2, TiO_2) can be incorporated within the hydrogel structure so as to obtain nanocomposites with tailored functionalities. Secondly, polymeric materials which can form hydrogels and also contain innate antibacterial properties can be used with nanomaterials to demonstrate their synergistic effects. As compared to synthetic polymers, polymers having natural origin (cellulose, starch, chitosan, alginate, etc.) are increasingly utilized for biomedical applications due to their biocompatible, biodegradable and low cost attributes.

For example, Sacco et al. (2015) demonstrated the use of AgNP impregnated tripolyphosphate-chitosan hydrogels for the treatment of non-healing wounds. They investigated that there exist a synergism between AgNPs and chitosan, responsible for the enhanced antibacterial action of hydrogel against *S. aureus*, *E. coli*, *S. epidermidis*, and *P. aeruginosa* strains. Moreover, the hybrid gel contributed toward inhibiting the maturation of their biofilms whereas no harmful effects on the viability of keratinocytes and fibroblasts cells were observed through the biocompatibility tests. Another natural polymer isolated from microbial strain *Acetobacter xylinum* TISTR 975 i.e., bacterial cellulose was tested as an immobilizing template for AgNPs (Maneerung et al. 2008). Silver nanoparticles were synthesized in situ within bacterial cellulose by introducing polymeric material into $AgNO_3$ solution followed by the borohydride mediated reduction. The resulting hydrogel exhibited good physicochemical properties and a strong bactericidal performance against *E. coli* and *S. aureus* strains. Panacek et al. (2014) synthesized sodium polyacrylate stabilized AgNPs (size 10 nm) and incorporated into methylcellulose to form a hydrogel based nanocomposite which could be used as a potential topical antimicrobial formulation for treatment of burns and wounds. The hybrid nanocomposite showed excellent antibacterial and antifungal efficacy against infective pathogens *S. aureus, C. albicans, E. coli, P. auregenosa, S. epidermis* with MIC values of 25 mg L^{-1}. As claimed by the authors, this material could act as a barrier preventing the attack of microorganisms causing infections at wound site.
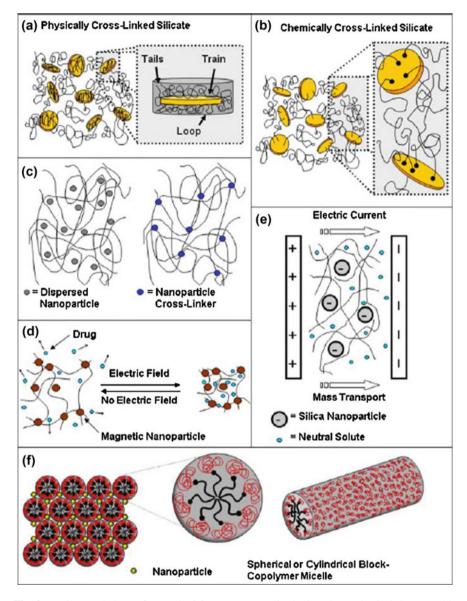
Fig. 9 Various techniques for synthesizing nanocomposite hydrogels. **a** Physical, but unstable interactions between polymeric chains and nanoparticles. **b** Nanoparticles are chemically bonded to polymers, often during radical polymerization processes. **c** Nanoparticles are chemically crosslinked within semi interpenetrating polymeric chains so as to enhance its antimicrobial properties. **d** Polymer–magnetic nanocomposites, with NPs dispersed within and/or crosslinking polymer chains for drug delivery applications. **e** Electro-osmotic flow of NPs embedded within polymeric matrix for the mass transport of drugs, proteins, and bioactive molecules. **f** Template block-copolymer gel with nanoparticles residing in the interstitial space between neighboring micelles. (Reproduced with permission from Schexnailder and Schmidt (2009), Springer)

Similarly, Vimala et al. (2011) incorporated an additional agent, curcumin obtained from *Curcuma longa* which has intrinsic wound healing, antibacterial, anti-inflammatory and anti-cancer properties into chitosan-PVA/Ag nanocomposite hydrogel. Antimicrobial assays yielded a noteworthy antibacterial and antifungal activity against *E. coli, Staphylococcus, Micrococcus, C. albicans, P. aeruginosa* with the diameter of ZoI ranging from 1 to 2.1 mm whereas, pristine hydrogel could not contributed towards forming a distinct zone of inhibition. In addition to that, the hydrogel nanocomposites exhibited satisfactory mechanical properties in order to employ them for wound dressings in treating/preventing infections. Agnihotri et al. (2012) also exploited chitosan–PVA-based hydrogel with dual functionalities
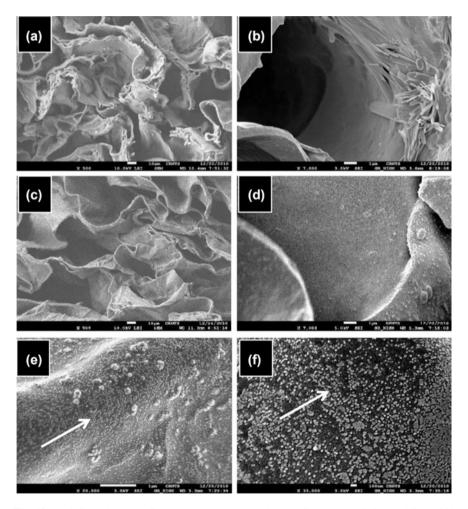

Fig. 10 FEG-SEM images of pure chitosan–PVA hydrogel (CP-50) at **a** 9500, and **b** 97,000 magnification. **c–f** demonstrated Ag-loaded chitosan–PVA hydrogel at different magnifications: **c** 9500, **d** 97,000, **e** 920,000, **f** 933,000. (Reproduced with permission from Agnihotri et al. (2012), Springer)

serving as a nano reactor in addition to its role for AgNP immobilization. SEM analyses indicated that semi-interpenetrating network of hydrogel not only facilitated a controlled and uniform distribution of AgNPs (Average size, 13 nm), it also precluded the requirement of adding any stabilizer to keep AgNPs in segregated state (Fig. 10). Swelling studies confirmed that the incorporation of silver incorporation enhanced the porosity and chain entanglement of the polymeric species of the hydrogel. The AgNP-hydrogel exhibited good antibacterial activity and was found to cause significant reduction in growth of *E. coli* in 12 h while such activity was not observed for the hydrogel without AgNPs.

A series of antibacterial superabsorbent hydrogels have been successfully prepared using polyacrylamide for biomedical applications. For example, Varaprasad et al. (2010) synthesized a semi-IPN Ag/poyacryalamide nanocomposite through free radical polymerization of acrylamide monomer in aqueous suspension containing desired amount of ammonia persulfate (as a cross linker), polyvinyl acetate, and ionic silver. The polymerization process was continued up to 8 h at 35 °C where silver ions were anchored at the surface of hydrogel through electrostatic interactions. To this polymeric mixture, sodium borohydride was introduced as to convert silver ions into AgNPs yielding hydrogel–silver nanocomposites. The antibacterial tests performed in solid agar demonstrated a significant and distinct ZoI while good antibacterial activity was observed against *E. coli* strains using liquid broth assays. Murthy et al. (2008) demonstrated the use of polyacrylamide/polyvinyl pyrrolidone semi-IPN hydrogel with AgNPs (size 3–5 nm) as excellent antibacterial biomaterials with 100% reduction in growth rate of *E. coli* under in vitro conditions. On a similar concept, Aggor et al. (2010) incorporated AgNPs (average size range, 1–12 nm) into polyacrylamide-co-acrylic acid hydrogel network and found that the hydrogel nanocomposite exhibits strong antibacterial and antifungal activity against *E. coli, S. aureus, B. subtilis,* and *C. albicans* strains. While increasing the dose of silver in hydrogel from 0.01 to 0.04 g/g of monomer mixture, there was a significant rise in antimicrobial study as manifested through a rise in the zone of inhibition (ZoI) from 10 to 20 mm. Moreover, the extraordinary swellable characteristics of hydrogel nanocomposite established its suitability as antimicrobial coatings for diverse biomedical applications.

A novel approach combining the antimicrobial therapy with nano-dressings has recently been cited for controlling foot infection in diabetic patients (El-Naggar et al. 2016). The nano-formula describes the fabrication of starch-chitosan/AgNP based dressing membranes through the conventional in situ synthesis of AgNPs within porous chitosan networks. The antibacterial experiments were tested against clinical pathogens isolated from the patients suffering from diabetic ulcers which mainly include *S. aureus, P. aeruginosa, K. pneumoniae, Proteus mirabilis* and *S. pyogenes*. Results indicated that chitosan-AgNP hydrogels always mediated higher antibacterial performance than pure chitosan under relevant conditions, regardless of the bacterial species tested. Specifically, the diameter of ZoI was calculated as 14.67 and 15.67 mm for the chitosan-AgNP against *S. aureus* and *P. aeruginosa* strains which was reduced to 11.88 and 14.11 mm when tested with pure chitosan

against respective strains. Interestingly, The MIC values of all bacterial isolates treated with pure amikacin (antibiotic) were lowered from 48 to 2 μg ml^{-1} after combining amikacin with chitosan-based silver nanoparticles, indicating their synergistic role. The proposed nano-formulation having 4 μg ml^{-1} amikacin with chitosan-AgNP hydrogel (5 ppm Ag in 6.9 mg ml^{-1} chitosan) was recommended for the treatment of MRSA and *P. aeruginosa* chronic wound infection without emergence of any nephrocytotoxicity or liver biochemical functions.

Another study hypothesized the concept of multifunctional biomaterial as a synthetic bone draft by encapsulating AgNPs (60–80 nm) within porous methacrylate hydrogels containing Na_2HPO_4 and $CaCl_2$ micro particles (Gonzalez-Sanchez et al. 2015). The antimicrobial efficacy of AgNP-hydrogel composites were determined on the basis of variation in the apparent lag phase and growth rate of *S. aureus* and *S. epidermidis* cells. Results indicate that hydrogel with 0.1 mM AgNP concentration demonstrated a better antibacterial activity than hydrogel having lower (0.5 mM) AgNP content. The maximum antibacterial effect of AgNP-hydrogel was achieved in 48 h while a further increase in contact time marked no increment in its antibacterial activity. Moreover, the presence of AgNPs did not pose either any cytotoxic effects on osteoblast cells or rheological characteristics of hydrogel. With both osteoconductive and antibacterial features, such hybrid gel could effectively be used in biomedical and dentistry applications as bone graft material.

In recent years, catheters with hydrogel coatings loaded with antimicrobial agents have been used to reduce the burden of catheters related infections. In a recent article, Loo et al. (2014) demonstrated the importance of AgNP-PVA hydrogels as antimicrobial coating on commercial endotracheal tubes. The antibacterial potential of hydrogel nanocomposite was evaluated on the basis of their degree of colonization on its surface after desired durations. Results showed that the density of *P. aeruginosa* colonization on pure PVA hydrogels was estimated to be in the range of 2.2–5.5 × 10^3 CFU cm^{-2} after 6 h of incubation whereas, no adherent bacterial colony was found on AgNP loaded PVA hydrogel for initial 6 h. After 18h, the bacterial colonization in pure PVA was increased up to 2.0–3.0 × 10^5 CFU cm^{-2} however AgNP-PVA hydrogel severely inhibited biofilm formation with density of colonization ranging from 1.2–9.0 × 10^4 CFU cm^{-2}. Similar trend was observed in case of *S. aureus* strains attachment to the hydrogels surface. Moreover, exposing hydrogel to human normal bronchial epithelial (BEAS2B) cells showed no cytotoxicity consequences. In another perspective, incorporating AgNPs into PVA matrix enhanced Young's modulus and ultimate tensile strength whereas its elongation at break was decreased than pristine PVA hydrogel. The mechanical property of hydrogel was found to comparable with commercially available endotracheal tubes and hence their utilization as antibacterial coatings for preventing nosocomial infections was envisaged by the authors. The details of other silver-based hydrogel nanocomposites and their potential biomedical applications have been summarized in Table 3.

Table 3 Silver nanoparticles hydrogel nanocomposites and their biomedical applications

AgNP based hydrogel nanocomposites	AgNP Size (nm)	Activity	Organisms tested	Evaluation parameter	Potential biomedical application	References
Ag/methacrylamide	<20	AB	E. coli, S. aureus, P. auregenosa	ZoI: 129–157% increase in diameters	Antimicrobial wound dressing	GhavamiNejad et al. (2016)
AgNPs/Dextran	20–30	AB	Bacillus cereus	ND	Antimicrobial material	Ma et al. (2009)
Ag/Polyacrylamide/PVP	3–5	AB	E. coli	100% reduction rate	Antimicrobial material	Murthy et al. (2008)
Ag/cellulose acetate aerogel	2.8	AB	ND	ND	Antimicrobial membranes	Luong et al. (2008)
Ag/carboxymethyl cellulose	8–14	AB	E. coli, K. pneumoniae, P. aeruginosa, P. vulgaris, S. aureus, P. mirabilis	ZoI: 14.8–16.2 mm	Antimicrobials against UTI infections	Alshehri et al. (2016)
Ag/Chitosan-PVA	13.3	AB	E. coli	83.5% inhibition rate	Disinfectants	Agnihotri et al. (2012)
Ag/Nap-FFC peptide	15	AB	MRSA, Acinetobacter baumannii	MIC: 40 µg/ml	Wound dressings	Simon et al. (2016)
Ag/hyaluronan/PVA	20–50	AB	E. coli	70–95% inhibition rate	Wound dressings	Zhang et al. (2012b)
Ag/Chitosan–Chitlac-blend	20	AB	S. aureus, E. coli, S. epidermidis, P. aeruginosa	3 Log reduction in bacterial counts	Wound dressings	Sacco et al. (2015)
Ag/Polyacrylamide-co-acrylic acid	1–12	AB, AF	E. coli, S. aureus, B. subtilis, C. albicans	Maximum ZOI: 20 mm	Antimicrobial material	Aggor et al. (2010)
Ag/Chitosan-PVA	16.5	AB, AF	E. coli, P. aeruginosa, C. albicans, Micrococcus, Staphylococcus	Maximum ZOI: 2.1 mm	Wound dressing	Vimala et al. (2011)

(continued)

Table 3 (continued)

AgNP based hydrogel nanocomposites	AgNP Size (nm)	Activity	Organisms tested	Evaluation parameter	Potential biomedical application	References
Ag/PHEMA/IA)/PVP hybrid hydrogels	ND	AB, AF	*S. aureus, C. albicans, E. coli*	70–95% inhibition rate	Tissue scaffold	Jovašević et al. (2011)
Ag/methylcellulose	10	AB, AF	*S. aureus, C. albicans, E. coli, P. auregenosa, S. epidermis*	MIC: 25 mg/L	Topical burns, Wounds healing	Panacek et al. (2014)

AB Antibacterial; *AF* Antifungal, *ND* Not Determined

4 Nanomaterials Based on Chitosan/Chitin

After cellulose, chitin is the most abundant mucopolysaccharide on earth. Chemically, it is a long chain polymer of poly (β-(1-4)-N-acetyl-d-glucosamine) virtually present in the exoskeleton of crustaceans/invertebrates as internal supporting structure and in the cell walls of fungus and yeasts (Jayakumar et al. 2010). Chitosan is a deacetylated form of chitin derivative which is a linear copolymer of N-acetyl glucosamine and glucosamine. Owing to its poor solubility in both aqueous and organic solvents, chitin polymer limits its practical applications and was widely accepted in the form of chitosan, which provided ample opportunities for further development (Dash et al. 2011). Moreover, chitosan offers some extraordinary properties such as innate biocompatibility, biodegradability, intrinsic antimicrobial efficacy, bone forming capability, and wound healing knack difficult to achieve with any other natural or synthetic polymer, making it a promising biomaterial to be used in various biomedical applications (Dash et al. 2011). With the shift of dimension from macro to nano, chitosan nanomaterials have also been shown to have expansive antibacterial, antiviral, and antifungal activity (Rabea et al. 2003), which depend upon several factors, including pH, degree of deacetylation, and the type of solvent (Tavaria et al. 2013; Chung et al. 2003). Moreover, the average molecular weight is also an important parameter that signifies the solubility of chitosan in various solvents.

In past two decades, chitosan has been proved to be a safer carrier for drug formulations (Felt et al. 1998). A number of delivery vehicles based on colloidal chitosan have been recently cited for delivering drugs, peptides, proteins, vaccines, DNA and siRNA (Almeida and Souto 2007; Mao et al. 2010). Due to its excellent mucoadhesive nature to a variety of hard and soft tissues, chitosan based hybrid materials may serve as a temporary skeleton in bone tissue engineering (Cañas et al. 2016). Fortunately, most of the chitosan based formulations has not been reported to provoke either inflammatory responses or allergic consequence within human body which has established its wide acceptability and utilization as biocompatible implants, injection, oral ingestions, topical applications for diverse biomedical purposes. Chitosan films/membranes has been tested as an efficient biomaterial for wound healing applications (skins, burns) and coating implants, thanks to its innate antimicrobial nature which inhibits biofilm formation yet promoting cell (e.g., fibroblasts, keratinocytes) proliferation for epidermal regeneration (Blažević et al. 2016). For example, Qi et al. (2004) synthesized chitosan and copper loaded chitosan nanoparticles (CSNPs) based on ionic gelation interaction between positively charged chitosan and negatively charged tripolyphosphate molecules. The processing was operated at room temperature and copper ions were absorbed on to CSNPs via ion exchange resins and/or surface chelation respectively. The average size of pure CSNPs and Cu-loaded CSNPs was calculated as 40 and 257 nm, respectively through AFM. These NPs showed effective antibacterial activity than their pristine counterparts against *E. coli*, *S. choleraesuis*, *S. typhimurium*, and *S. aureus* with MIC values ranging between 0.01 and 0.13 μg ml^{-1}. AFM analyses

revealed that CSNPs severely killed *S. choleraesuis* cells via membrane disruption such that membrane permeability was severely damaged resulting in the leakage of intracellular components. In another study, a synergistic antibacterial activity of Cu loaded CSNPs was observed against *E. coli* K88 strain with MIC (9 μg ml^{-1}) and MBC values 21–42 folds lower than the individual antibacterial entities i.e., copper ions and chitosan nanoparticles (Du et al. 2008). Therefore, it is anticipated that CSNPs often integrated with metallic nanoparticles can be used as potential antibacterial agents in biomedicine.

Anitha et al. (2009) synthesized CSNPs (average size, 40–50 nm) and their water soluble derivatives i.e., O-carboxymethyl chitosan (O-CMC, 90–100 nm) and N,O-carboxymethyl chitosan (N,O-CMC, 80–85 nm) nanoparticles, to compare their antibacterial efficacy against *S. aureus*. They found that among modified NPs; N,O-CMC NPs showed maximum antibacterial efficiency than O-CMC and CSNPs, with 100% inhibition rate at a maximum concentration of 1 mg ml^{-1}. The greater antibacterial effect of N,O-CMC NPs was attributed to their relatively higher degree of substitution of carboxymethyl groups on chitosan than unmodified chitosan, (Sun et al. 2006). Earlier, It was hypothesized that the introduction of carboxymethyl groups strengthen the overall positive charge on chitosan molecules, resulting in greater interaction with negatively charged components (lipopolysaccharides, proteins) present in the bacterial membrane, thereby causing membrane disruption and release of major content of intracellular material outside cells (Sudarshan et al. 1992).

Yien et al. (2012) evaluated the antifungal activity of CSNPs prepared from both low and high molecular weight chitosan against *Candida albicans, Fusarium solani* and *Aspergillus niger* species. Results showed a significant antimycotic activity with MIC values ranging from 0.6–1.0 and 0.5–1.2 mg ml^{-1} against *C. albicans* while 0.25–0.86 and 0.86–1.2 mg ml^{-1} against *F. solani* for high mol. wt. and low mol. wt. CSNPs, respectively. Among all the tested strains, *A. niger* appeared as the most resistant strain since there was an increase in MIC values by ten times (2–3 mg ml^{-1}). Therefore, these types of NPs could be incorporated into biomaterials for natural antifungal effect.

Biofilm formation due to the growth of *Streptococcus mutans* bacterial colonies in oral cavities is a major concern because it can cause diseases like caries, gum inflammation (gingivitis), and dilapidation of periodontal tissues i.e., periodontitis (Marsh 2004, 2005). In order to combat this problem nanoscale systems with antibacterial properties are being developed as a biological carrier materials to inhibit biofilms formation, maturation and growth. For the above mentioned reasons endodontic irrigants like sodium hypochlorite have been used for successful elimination of biofilms but these irrigants when retained in higher amounts for longer period of time may even cause more structural damage into the dentin (Zhang et al. 2010). Also, they leave smear layers because of incomplete elimination of bacteria and hence there is a need of chelating agents used as final irrigant to get rid of smear layers from the root canals (Çalt and Serper 2002). However the chelates may cause additional damage by compromising the mechanical integrity and amplified bacterial adherence on collagen (Kishen et al. 2008). Inspired by this approach, de Paz et al.

(2011) tested the antibacterial efficiency of chitosan nanoparticles prepared from chitosan (high and low mol. wt.) against *Streptococcus mutans* biofilms. Confocal scanning laser microscopy (CSLM) image analysis showed high antibacterial potency of low MW CSNPs with more than 95% destruction rate of bacterial cells as compared to 25% killing rate for high MW CSNPs. In line to this, a recent study has demonstrated the chelating and antibacterial effect of CSNPs to remove the smear layer and inhibit bacterial colonization on bovine dentin (del Carpio-Perochena et al. 2011). Results showed noteworthy chelation and antibacterial potency of NaOCl-EDTA, NaOCl-EDTA-CNPs and NaOCl-CNPs in comparison with that of the control and NaOCl groups, with 73% of live cells in case of NaOCl-EDTA-CNPs compared to 92% of control. This establishes the fact that CSNPs can be used as anti-biofilm and chelating agent in dental applications, however, further work on the above issues is needed.

Degradation of root canal system and the periradicular spot by bacterial infection and its toxin release can cause apical periodontitis and tissue demolition. These defects can be treated with guided tissue regeneration (GTR) method using collagen membrane barriers which prevents the apical migration of gingival epithelial into the bereaved root surface with improved healing and bone closure (Stoecklin-Wasmer et al. 2013) however, bacterial colonization still persists. Therefore, Barreras et al. (2016) used chitosan nanoparticles with chlorhexidine to demonstrate its antibacterial activity against *E. faecalis* in infected collagen membrane. Results show that CSNPs displayed significant antibacterial efficacy on conjugating with chlorhexidine since no bacterial growth was observed even at its lowest concentration, i.e., 0.08%. Thus, CSNPs/chlorhexidine nanosystems can be applied into membrane barriers to prevent periodontal infections.

Regarding fabrication of a drug delivery vehicle, Lee et al. (2016a) developed a method to load two drugs, tetracycline and lovastatin into PLGA/CSNPs (Average size, 107.8 nm) for fighting against bacterial infection concurrently with minimizing bone material loss. Preliminary studies conducted in dogs for potential antibacterial, bone formation/regeneration ability showed promising results against *A. actinomycetemcomitans* and *P. nigrescens* pathogens with a distinct ZoI appearing in PLGA/CSNPs/lovastatin-tetracycline (0.3%). Histopathological examination of tissue treated with prepared nanocomposite showed no sign of inflammation, though new deposits of cementum on the root surface and active plasmacytoid osteogenic activity were observed than in the control group. Therefore this material can be applied for controlled release of tetracycline and lovastatin into the periodontic defect for antibacterial and osteogenic activity.

Joint replacement procedures are being done now with high success rate however microbial colonization on artificial biomaterial still remains a problem causing serious implant rejections. Poly(methyl methacrylate) (PMMA) has been being used as bone cement for joint replacements however due to no intrinsic antibacterial activity, it is susceptible to infections due to biofilm formation over its surface (Hendriks et al. 2004). The addition of antibacterial agent in bone cement can really help to solve the persisting problem. Therefore, doping of CSNPs into PMMA and quaternary ammonium chitosan derivative nanoparticles (QCS NPs) has been done
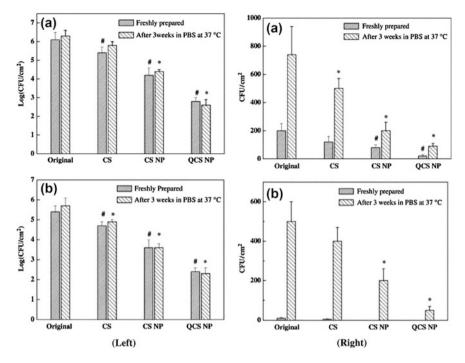
Fig. 11 Number of viable adherent *S. aureus* (**a**) and *S. epidermidis* (**b**) cells on the different substrates based on Smart set bone cement without (*Left*) and with gentamicin (*Right*). (Reproduced with permission from Shi et al. (2006), Elsevier)

to check their potential use as bactericidal agents (Shi et al. 2006). CSNPs & QCS NPs embedded cements showed decrease in viability of *S. aureus* and *S. epidermidis* with two and three orders of magnitude, respectively (Fig. 11). Moreover, the cytotoxicity assays of CSNPs and QCSNP-loaded bone cements showed no adverse effects on 3T3 mouse fibroblasts compared to pure PMMA cement.

Regarding the development of antibacterial coatings for medical devices and wound healing applications, a few reports have been published in recent times. For example, Romainor et al. (2014) demonstrated the potential of CSNPs (216 nm) doped cellulose films as an antibacterial wound dressing material. Disk diffusion assays revealed that while pure cellulose film could not exhibit any inhibitory effects on bacterial growth, no colonies were able to grown on the surface in contact with either chitosan-doped or chitosan nanoparticles-doped cellulose films. It was hypothesized that being polar in nature, both chitosan and CSNPs were able to diffuse slowly from films to agar plate, facilitating a direct contact killing action. The polar nature of film was further increased after cross linking with citric acid, which demonstrated a larger diameter of zone of inhibition i.e., an enhanced bactericidal potency of CSNP-doped cellulose films, validating the above hypothesis. The antibacterial assays showed the highest activity against *E. coli* with 85% and

81% inhibition rate in bacterial growth at 5% and 10% doping concentration of CSNPs and chitosan in cellulosic films, respectively. The value MIC and MBC values of CSNPs/cellulose films were determined as 10 and 13 µg ml^{-1} respectively, which was significantly lower than the bulk chitosan/cellulose films (MIC: 16.37 µg ml^{-1}; MBC: 19.70 µg ml^{-1}). Similarly, Jamil et al. (2016) developed cefazolin loaded chitosan nanoparticles (CSNPs) and tested their antimicrobial activity against *K. pneumoniae*, *P. aeroginosa* and extended spectrum beta lactamase (ESBL) positive *E. coli*. Antibiotic loaded CSNPs showed ZoI ranging from 15 to 22 mm with increasing concentration of drug from 200 to 2000 µg ml^{-1}. It indicates that CSNPs can be used to fabricate antibacterial agent for effective therapeutic solutions against MDR bacteria and can be employed in antimicrobial coatings on medical devices.

5 Other Nano-Antimicrobials

Despite the highest antibacterial potency manifested by silver, an overwhelming demand of nano-based products in biomedical and healthcare sector has obliged researchers to explore a few other materials, such as gold, copper/copper oxide, ZnO due to their inherent antibacterial properties. A number of publications describing antimicrobial applications of these nanomaterials for targeted drug delivery, antimicrobial coatings, biocidal medical devices and wound dressings have risen exponentially. While most research focused on claiming antimicrobial potential of these nanomaterials have employed either the colloidal state or often conjugated with antibiotics/drugs, the hypotheses of using them as next generation antimicrobial agents has limited clinical relevance. It is worth mentioning that for treating biomaterial associated infections, it is an important concern that a biocidal agent would not only kill the invaded microbes, but it should also prevent further bacterial adhesion and colonization on biomaterial surface. At the same time, antimicrobial agent must also encourage tissue integration at the implant site (Subbiahdoss et al. 2013). The incorporation of nanomaterials on to some support materials would thus be the most promising approach for developing novel nano-antimicrobial surfaces with multiple functionalities, durability with an enhanced biocidal response against clinical pathogens. In this section, only those studies are included where the antimicrobial potency of gold, copper oxide, and zinc oxide nanomaterials were explicitly assessed for specific biomedical applications.

5.1 *Gold Based Antimicrobial Nanomaterials*

Gold in bulk form is generally considered as an inert metal with feeble antimicrobial properties. However, it can be modified to introduce antimicrobial

properties when synthesized as nano sized particles. Similar to nano silver, a variety of biological synthesis approaches for gold nanoparticles (GNPs) has been published in recent times. For example, MubarakAli et al. (2011) used *Mentha piperita* plant extract to synthesize GNPs (150 nm) which were found to be active against clinically isolated human *E. coli* pathogen. Similarly, Ramamurthy et al. (2013) reported a simple and economical approach for synthesizing gold nanoparticles using aqueous extract of *Solanum torvum* fruit for treating several oxidative stress diseases and controlling human and veterinary infections. Gold nanoparticles demonstrated a noticeable zone of inhibition against *E. coli*, *Pseudomonas* and *Bacillus* while serving as a strong hydroxyl, superoxide, nitric oxide radical scavengers. Some recent reports employing fungal and bacterial mediated routes have shown the existence of phytochemicals in biological extracts, which might play a major role in improving the antibacterial efficacy of biogenic GNPs than conventional antibiotics (Prema et al. 2016; Balakumaran et al. 2016).

Another promising aspects of using GNPs in nanomedicine as carrier of antimicrobial agents and antibiotics is currently under investigation (Dykman and Khlebtsov 2012). GNPs are appropriate to deliver drugs to cellular addresses due to their ease in fabrication, functionalization, biocompatibility and their ability to cross cellular barriers while interacting with cell surface lipids (Huang et al. 2009). On the other hand, antibacterial efficacies of GNPs can be increased by adding antibiotics (Grace and Pandian 2007; Rai et al. 2010) whereas on conjugating with target-specific biomolecules, GNPs can be used as powerful therapeutics to destroy even cancerous cells. Gu et al. (2003) synthesized vancomycin-conjugated GNPs exploiting the strong binding affinity of gold to thiol (–SH) groups such that the antimicrobial activity of vancomycin was significantly improved on coating with gold nanoparticles (5 nm) against vancomycin resistant *Enterococci* (VRE) well as *E. coli*. These gold NP conjugates were more effective than vancomycin itself against various bacterial strains. Similar findings were presented in another study (Huang et al. 2007) where polygonal shaped GNPs after immobilizing vancomycin were used as an effective photothermal agents for the selective killing of VRE, MRSA, and other potentially drug-resistant microorganisms. Authors revealed that the dual functionalities of GNPs (to absorb near-infrared radiations) and vancomycin (binding with the terminal D-Ala-D-Ala moieties of the peptide units of bacterial cell wall) contributed towards its photothermal destruction with high efficiency without eliciting any toxic effects on human cells.

Rosemary et al. (2006) also demonstrated that ciprofloxacin-encapsulated gold-silica nanoshells mediated enhanced antibacterial activity as compared to free ciprofloxacin against *E. coli* DH5. The ability of Cefaclor, a second-generation antibiotic for synthesizing GNPs showed potent antimicrobial activities on both Gram positive *S. aureus* and Gram-negative bacteria *E. coli* strains (Rai et al. 2010). As compared to individual components, GNPs-antibiotic conjugate facilitated more severe perforations in bacterial cell wall followed by disrupting the bacterial DNA leading to cell death. Demurtas and Perry (2014) created a proficient drug delivery/carrier system by conjugating stable GNPs with antibiotic amoxicillin (a member of the penicillin family) which also reduced the chloroauric acid to form

nanoparticles (30–40 nm) and simultaneously coated them to afford the function-alized nanomaterial. Figure 12 shows the comparative antibacterial performances of pure amoxicillin, pure GNPs and amox-GNPs conjugates with various proportions over duration of 4 h. Results indicate that amoxicillin-conjugated GNPs showed an enhancement in antibacterial potency against *E. coli* as compared to the antibiotics and GNPs alone. The conjugated form exhibited 100% inhibition of *E. coli* growth in minimum time (2 h) with an MIC value of 300 μg ml^{-1}. Authors claimed that these GNPs conjugates can be used to coat a wide variety of biomaterial surfaces for instance implants, fabrics for treatment of wounds and glass surfaces to maintain hygienic conditions in the home, in hospitals and other infected prone areas (Das et al. 2009).

In a recent article, Naveena and Prakash (2013) evaluated ciprofloxacin-conjugated GNPs for its antibacterial activity against *S. aureus*, *E. faecalis*, *E. aerogenes* and *E. coli* pathogenic bacteria and demonstrated the highest ZoI in case of antibiotic conjugated GNPs with *E. coli* (24 mm) and *E. aerogenes* (21 mm), and *S. aureus* (19 mm) bacteria whereas a reasonable activity was observed against *E. faecalis* (14 mm). The combined antibacterial and antifungal activities of GNPs on conjugating with 5-fluorouracil (5-FU, anti-cancer drug) was also testified against *Micrococcus luteus*, *S. aureus*, *P. aeruginosa*, *E. coli*, *Aspergillus fumigatus* and *Aspergillus niger* (Selvaraj and Alagar 2007). 5-FU conjugated GNPs were
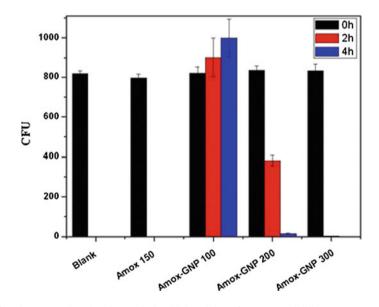

Fig. 12 Histogram plot showing antimicrobial activity of pure amoxicillin (Amox), pure gold nanoparticles (GNP) and amoxicillin-conjugated GNPs against *E. coli* after different incubation times (0, 2, and 4 h). All concentrations are in units of μg ml^{-1}. The 'blank' without addition of amoxicillin or amoxicillin-conjugated gold nanoparticles is shown for $t = 0$ only where it was possible to measure the colony forming units. (Reproduced with permission from Demurtas and Perry (2014), Springer)

found to be more effective on Gram negative bacteria than Gram positive due to their easier permeability into the cells. Additionally, they showed antifungal activity on *A. fumigates* and *A. niger*.

Apart from nano colloids, gold based nanocomposites have also been employed in biomedical applications as they prove to be a promising multifunctional platform, combining various diagnostic, therapeutic and antimicrobial modalities. For example, Chen et al. (2010) synthesized lysozyme-protected gold nano clusters combining the individual antibacterial properties of lysozyme and gold nanoclusters which inhibited the growth of antibiotic-resistant bacteria, such as *Acinetobacter baumannii* and vancomycin-resistant *E. faecalis* (VRE). Zaporojtchenko et al. (2006) produced an antibacterial metal/polymer nanocomposite coating system employing Ag/Au together with polytetrafluorethylene (PTFE) film having thickness between 100 and 300 nm. Higher antimicrobial effect of Ag–Au/PTFE nanocomposite coatings was estimated as compared to either individual Ag/PTFE or Au/PTFE as manifested by evaluating the extent of inhibition of *S. aureus* and *S. epidermidis* model bacterial strains. Marsich et al. (2011) prepared a nanocomposite hydrogel based on natural polysaccharides alginate and chitlac with incorporated GNPs (Average size, <20 nm). A good antimicrobial efficacy of these hydrogels was tested against *S. aureus* and *P. aeruginosa* though the GNPs containing nanocomposites showed some cytotoxic effects towards eukaryotic cell lines HepG2 and MG63. Recently, a novel biodegradable hydrogels based on gold nanocomposites was synthesized using acrylamide and wheat protein isolate through an environmentally benign route (Jayaramudu et al. 2013). GNPs were synthesized by reducing HAuCl$_4$ using neem leaf extract (*Azadirachta indica)* within the hydrogels network with an average size of 10 nm. The gold-nanocomposite hydrogel showed potential applications for wound/burns dressings as it exhibited a strong antibacterial activity against *S. pyogenes* and *E. coli* with ZoI 0.9 cm and 1 cm respectively, however it was entirely absent for hydrogel without GNPs. Zhou et al. (2014) synthesized cellulose nanofiber mats by alternatively depositing negatively charged GNPs (Average size 18.7 nm) and positively charged lysozyme through layer-by-layer (LBL) self-assembly technique. Multiple functional moieties present in lysozyme provided the necessary electrostatic interactions required for binding GNPs and lysozyme to the supporting substrate. The resulting GNPs/lys/cellulose LBL multilayer assembly was found to be highly stable while exposing them under dilute acid, alkali and surfactant solutions. These film coated mats were tested against *E. coli* and *S. aureus* which showed good antimicrobial potency for food packing, tissue engineering, wound dressings applications. The fabrication process for GNPs coated cellulose mats is shown in Fig. 13.

In a more recent study, Regiel-Futyra et al. (2015) have developed chitosan-gold nanocomposite (CS-GNPs) films, where biodegradable chitosan polymer was used both as reducing and stabilizing agent for GNPs. Three different grades of chitosan with low, medium, high average molecular weight & having different degrees of deacetylation (DD) were used for nanoparticles synthesis. Films based on chitosan with medium molecular weight and the highest DD exhibited the highest antibacterial activity against multi-drug resistant pathogens *S. aureus* and *P. aeruginosa*.
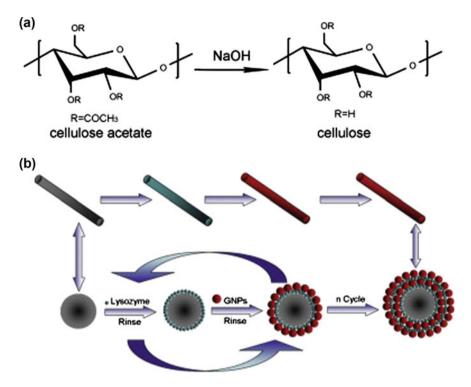
Fig. 13 a Hydrolysis scheme of cellulose acetate and **b** Schematic diagram illustrating the fabrication process of the layer by layer film of GNP/lysozyme coated cellulose mats (**b**). (Reproduced with permission from Zhou et al. (2014), Elsevier)

Moreover, small sized GNPs (16 nm) did not pose any cytotoxic effects on A549 (human lung adenocarcinoma epithelial cell line) and HaCaT (human keratinocyte) cell lines thereby these nanocomposites can be used for wound dressings as an adhesive bandages, or as antimicrobial coatings.

5.2 Copper/Copper-Oxide Based Antimicrobial Nanomaterials

The antimicrobial properties of copper have been known to us since ancient times contemporary with silver (Longano et al. 2012b). Later, the use of copper for treating sores and skin infections was well accepted by Greeks and Americans while a similar approach is still functional in many parts of Africa and Asia (Dollwet and Sorenson 1988). Copper materials combined with metals like cadmium and lead have been considered for sanitary and hygienic purposes since 1980s because of its biocidal activity against a varieties of microbes (Domek et al.

1984; Gould et al. 2009). CuNPs are currently gaining enormous interests due its low cost, availability and are considered to be practically more safe for humans (Grass et al. 2011; Longano et al. 2012b). In addition to this, CuNPs exhibit excellent antimicrobial activity against a varieties of clinically relevant pathogens including bacteria, fungi, and algae while a recent few reports have demonstrated their antimicrobial and catalytic efficacies similar to other metallic nanoparticles, i.e., silver and gold (Wei et al. 2010; Usman 2013) with intrinsic antimicrobial activities. Nano copper is considered as a potential candidate for new generation of antimicrobials since in trace amounts it is necessary for the execution of several metabolic processes in organisms (Krupanidhi et al. 2008) and at the same time it shows bactericidal activity at a relatively higher dose due to membrane disruption, nucleic acid and protein damage (Gant et al. 2007) and ROS production (Pelgrift and Friedman 2013; Longano et al. 2012b).

As compared to other popular antimicrobial nanoparticles of silver, a few studies in accordance with antimicrobial property of copper and CuO NPs have been reported. This is because of the fact that the antimicrobial potency of CuNPs is inferior as compared to silver or ZnO and hence, a higher concentration of CuNPs would be required to show similar biocidal effect as of other potential NPs (Ren et al. 2009). Nevertheless, being more economical than silver, CuO NPs are useful in combating against nosocomial infections and after immobilizing on to some support matrix, they can be utilized effectively with enhanced antibacterial properties (Xu et al. 1999; Longano et al. 2012b).

Nicola Cioffi and coworkers have done pioneer research for exploiting the nano copper based nanocomposites for antimicrobial applications (Longano et al. 2012a, b; Cioffi et al. 2004, 2005a, b). In 2005, they first reported the synthesis of bioactive coatings made from polymeric thin films loaded with copper nanoparticles for antibacterial and antifungal applications (Cioffi et al. 2005b). Copper nanoparticles (average size, 3.2 nm) were synthesized employing a novel electrochemical method under an inert and stabilizing environment. The synthesized CuNP were embedded in polymer matrices of polyvinylmethyl ketone (PVMK), poly(vinyl chloride) (PVC), and polyvinylidene fluoride (PVDF) followed by spin casting the resulting solution on to some substrate in order to get Cu-polymeric mixture in form of films with an average thickness of 400–500 nm. The bioactivity of three nanocomposites was screened against *S. cerevisiae* (yeast), *E. coli*, *S. aureus*, *Lysteria monocyto-genes*, and molds, where CuNPs-PVMK films exhibited the strongest biostatic effect with more than 99.9 and 95% inhibition of bacterial and molds colonies, respectively. The higher antimicrobial activity of Cu nanocomposites was attributed to their higher release kinetics of Cu into the solution which was in turn linearly correlated with CuNPs loading. CuNPs-PVDF nanocomposites showed minimum antibacterial activity since the amount of Cu loading among all three coatings followed order as PVMK > PVC > PVDF. Authors envisaged the application of these CuNP based nanomaterials for the preparation of antibacterial coatings in household, biomedical and hospital, which are prone to receive infections. In another study, (Cioffi et al. 2005a), same research group investigated the electro-chemical synthesis of copper and silver core-shell nanoparticles (range, 1.7–

6.3 nm) using tetraoctylammonium (TOA) salts as both base electrolyte and stabilizing agents to NPs. The nanocoatings formed after incorporating them onto PVMK polymer showed an extraordinary inhibitory effect on both eukaryotes and prokaryotes microbes, thanks to the synergistic action of nanoparticles and tetraoctylammonium as potential disinfectants. The enhanced physicochemical properties with good stability of Cu nanocoatings thus prompted the authors to find applications in antifouling paint and coating formulations. In a different study, CuNPs synthesized using laser ablation method were deposited on to polylactic acid after drop casting so as to make a self assembled antimicrobial film. The resulting nanocomposite showed good bioactivity against *Pseudomonas* spp. which is the most causative pathogen in food processing (Longano et al. 2012a).

A few other researchers have exploited the antimicrobial potential of CuNPs after incorporating onto some support material. For example, Grace et al. (2009) embedded CuNPs (37.5 nm) on alginate-cotton Cellulose (CACC) fibers for fabricating an antimicrobial package for wound dressing applications. The hybrid nanofibers demonstrated a noticeable antibacterial activity against *E. coli* with a MIC value of 5 CFU cm^2 when composites were loaded with 4% wt. of copper had. In addition to this, the presence of cellulose provided the required mechanical strength to alginate nanofibers for holding CuNPs for intended applications. In another study, polyurethane nanofibrous scaffold (Fig. 14) was used as a template for incorporating CuNPs (5–10 nm) for making antimicrobial wound dressing material (Sheikh et al. 2011). Ahmad et al. (2012) synthesized a CuO NPs doped polyurethane coatings by infusing CuO NPs (50 nm) into polyurethane (PU) elastomer and showed 90% reduction in growth of methicillin resistant *S. aureus* after an incubation period of 4 h with CuO (10% w/w)as a dopant. Owing to its good mechanical stability and excellent biocompatible properties, the prospects of CuO-PU nanocomposite in designing new antibacterial dental fillers, coatings, and tissue engineering constructs was discussed.

Similarly, Cady et al. (2011) synthesized CuNPs/cellulose nanofibrous composites as wound care materials exhibiting strong antibacterial activity against a multi-drug resistant pathogen, *A. baumannii*. The nanocomposite was fabricated by an alternative deposition of copper ions onto functionalized (negatively charged) cotton nanofibers followed by its reduction resulting in the synthesis of copper nanoparticles (Average size, 5 nm) onto cellulose substrate as a self assembled multilayer coatings. The antibacterial properties of CuNPs coated cotton substrates were assessed in solid media using zone of inhibition assay and growth inhibition assay in liquid broth. ZoI results indicated while Acticoat^{TM} (a commercial silver dressings) exhibited the largest zone of inhibition, which is an indicative of high Ag$^+$ ions release from sample into the solid agar medium, CuNP-cotton nanocomposite did not shown any distinct ZoI, thanks to non-leachable CuNPs that were bound firmly to the functionalized cotton surface. Contrary to this, CuNP-cotton caused a 8 log reduction in growth of *A. baumannii* in liquid assay within 10 min of incubation as compared to merely 1 log reduction for bacterial cells treated with AgNP-cotton samples. Moreover, CuNPs coated cotton exhibited
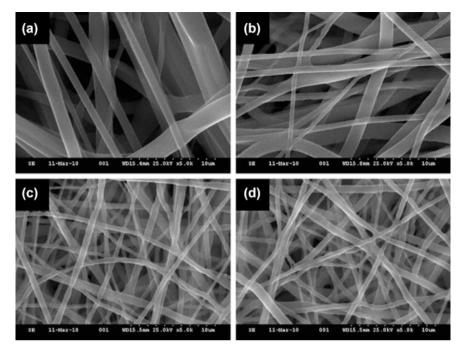
Fig. 14 SEM images for nanofibers that contain different amounts of Cu: **a** 0%, **b** 5%, **c** 7% and **d** 10%. (Reproduced with permission from Sheikh et al. (2011), Elsevier)

more effective bacterial killing than Acticoat™ despite having approximately 90% less metal cm^{-2}. Authors envisaged the enhanced bactericidal killing of Cu-coated cotton samples to be predominantly contact killing activity, a similar mechanism of action proposed by (Agnihotri et al. 2013) in a different study related to immobilized silver nanoparticles. Several examples of Cu/CuO nanoparticles based nanocomposites and their biomedical applications are summarized in Table 4.

5.3 Zinc/Zinc-Oxide Based Antimicrobial Nanomaterials

Zinc oxide is a wurtzite-type semiconductor material with unparalleled physical and chemical properties such as piezoelectric behavior, high chemical stability, capability to absorb broad range radiations and photocatalytic activity. In recent years, It is being widely accepted in a variety of applications related to sensors, UV-light shielding, semiconductors, piezoelectric devices, field emission displays, pharmaceuticals, agriculture, photocatalytic degradation of pollutants, and as antimicrobial agents (Kołodziejczak-Radzimska and Jesionowski 2014; Wang 2004). Owing to its innate broad range antimicrobial features, ZnO nanoparticles represent another class of antimicrobial biomaterial which are considered to be safe, biocompatible,

Table 4 Copper/Copper oxide based nanocomposite and their biomedical applications

Cu/CuO based nanocomposites	CuNP size (nm)	Activity	Organisms tested	Evaluation parameter	Potential biomedical application(s)	References
CuNPs/Alginate-Cotton Cellulose fibers	37.5	AB	E. coli	5 CFU cm^{-2} at 4 wt% CuNPs	Antimicrobial dressing materials	Grace et al. (2009)
Cu/polypropylene & CuO/polypropylene	10–40	AB	E. coli	99.9% reduction in 4 h	Antimicrobial filler	Delgado et al. (2011)
CuNPs/polyurethane	5–10	AB	E. coli; B. subtilis	ND	Wound dressings	Sheikh et al. (2011)
CuNPs/cellulose	5	AB	A. baumannii	8 log reduction in 4 h	Antimicrobial dressing materials	Cady et al. (2011)
Cu doped diamond-like carbon films	10	AB	E. coli	99.9% reduction rate	Surface coatings in cardiovascular applications.	Chan et al. (2011)
CuNPs/polyurethane	50	AB	MRSA	90% reduction rate at 10% w/w CuO	Antibacterial dental fillers, Tissue engg. constructs, coatings,	Ahmad et al. (2012)
CuNPs/Soda lime glass/Ceramic coating	ND	AB, AF	E. coli, Micrococcus luteus, Issatchenkia orientalis (yeast)	MIC: 10–15 µg/cm^2	Biocide coatings on medical devices	Esteban-Tejeda et al. (2012)
CuO/TiO$_2$ nanorods	100	AB	E. coli; S. aureus	Reduction in viable counts	Antibacterial bone and dental implants	Hassan et al. (2013)
CuNPs/montmorillonite/epoxy nanocomposites	10–20	AB	K. pneumonia, E. coli	ZOI: 23–26 mm	Antimicrobial coating	Das et al. (2014)

Table 4 (continued)

Cu/CuO based nanocomposites	CuNP size (nm)	Activity	Organisms tested	Evaluation parameter	Potential biomedical application(s)	References
CuO/Carboxymethyl cellulose hydrogels	40–75	AB	E. coli, S. aureus	ZOI: E. coli - 14 mm S. aureus-19 mm	Antibacterial material	Yadollahi et al. (2015)
Copper/bioactive glass/eggshell membrane	40–50	AB	E. coli	90% reduction rate	Wound dressing	Li et al. (2016)
Sn, Cu, Hg, and Ag composite nanopowders	<100	AB	Streptococcus mutans, L. acidophilus	MIC-12 mg/ml ZoI: 6, 13 mm	Disinfectant in dental filling materials	Lee et al. (2016b)
CuO/Chitosan hydrogel	10–25	AB	E. coli, S. aureus	ZOI: 8–11 mm	Antibacterial dressings	Farhoudian et al. (2016)

AB Antibacterial; AF Antifungal; ND Not determined

economically viable and have been used in our daily products such as cosmetics, delivery vehicles, and even as nanofillers in medical implants. Furthermore, ZnO posses some remarkable features such as low toxicity, biocompatibility and biodegradability which makes it a multifunctional material of interest for biomedical applications (Stoimenov et al. 2002).

ZnO NPs tend to have an extensive range of antimicrobial activity against various microorganisms which in turn dependent on concentration, size, shape, surface charge, porosity, surface functionalization and ligand binding ability of ZnO NPs (Yamamoto 2001). For instance, Narayanan et al. (2012) synthesized ZnO NPs of size ranging between 41–167 nm by precipitation method using zinc nitrate and NaOH. The antimicrobial activity was tested against common human pathogens such as *S. aureus, E. coli, K. pneumoniae, E. faecalis*, and *P. aeruginosa* with an average ZoI of nearly 21, 17, 13, 16, and 30 mm for respective strains at 100 μg concentration of ZnO NPs. In addition to this, the biogenic synthesis of nanoparticles can alleviate various limitations associated with the involvement of toxic chemicals and reagents through chemical approaches. For example, the antimicrobial efficacies of ZnO nanoparticles synthesized using green (average size, 40 nm) and chemical (average size, 25 nm) approaches were tested against various bacterial and fungal pathogens viz. *S. aureus, Serratia marcescens*, *Proteus mirabilis, Citrobacter freundii,* and fungal strains *Aspergillus flavus, Aspergillus nidulans, Trichoderma harzianum*, and *Rhizopus stolonifer* (Gunalan et al. 2012). Amongst the various tested bacterial strains, ZnO NPs showed the highest antimicrobial activity against *S. aureus* as demonstrated through a larger ZoI (26 mm) than *P. mirabilis* (27 mm), *S. marcescens* (24 mm) and *C. freundii* (19 mm). Among fungal pathogens, the order of antifungal effect was noticed as *R. stolonifer > A. flavus > A. nidulans > T. harzianum*. Interestingly, ZnO NPs synthesized through green route exhibited enhanced antibacterial & antifungal activity than chemical ones, despite having larger nanoparticle size. Several other studies have also reported the improved antimicrobial potency of ZnO NPs synthesized through green routes over chemical approaches (Gnanasangeetha and Thambavani 2013; Salem et al. 2015; Sharma et al. 2010).

In order to design antimicrobial coatings based on ZnO NPs, surface functionalization or immobilization to a support material would be utmost concern for facilitating their ease in handling, utilization and applicability. Antimicrobial activity and stability of ZnO nanoparticles can be improved by incorporating them into some carrier matrix. Moreover, ZnO in the form of nanorods can even act as an immobilizing template for other nano-antimicrobials like AgNPs and may contribute towards an unprecedented antimicrobial performance. For instance, Agnihotri et al. (2015) described a facile approach for dense immobilization of silver nanoparticles (AgNPs) on ZnO nanorods using arginine molecule as an eco-friendly cross linker. Various characterization studies indicated that arginine molecules provided numerous nucleation sites on ZnO nanorods forming stable silver-arginine complexes, which was subsequently reduced into silver nanoparticles (Fig. 15). The resulting Ag/ZnO hybrid nanocomposite (HNC) demonstrated an extraordinary antibacterial activity against *E. coli* and *B. subtilis* strains under the
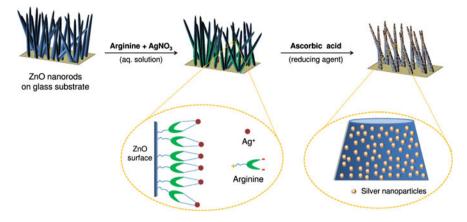
Fig. 15 Schematic representation shows in situ synthesis and immobilization of silver nanoparticles on ZnO nanorods using arginine as a linker. (Reproduced with permission from Agnihotri et al. (2015), Royal Society of Chemistry)

given test conditions. A dual mode of bactericidal action of HNCs, i.e., mediated through direct-contact as well as release of silver ions was hypothesized. Ag/ZnO HNCs showed no significant reduction in antibacterial efficacy even after being recycled multiple times. A good extent of immobilization was confirmed by measuring the amount of Ag and Zn release in potable water which was found to be well below the USEPA recommended standard. Interestingly, the Ag/ZnO HNC did not show any cytotoxic effects on the human hepatocarcinoma cell line (HepG2) and no significant generation of ROS was observed by the treated cells after an exposure of 24 h. The immobilized substrate thus showed good biocompatibility and sustained bactericidal activity and has good potential as a nano-antimicrobial biomaterial.

A hybrid nanocomposite based on ZnO & soluble starch (stabilizer) for coating onto cotton fabrics in order to make antimicrobial textiles was described earlier (Vigneshwaran et al. 2006). Incorporation of 1% ZnO NPs (average size, 38 nm) exhibited good antibacterial activity against *S. aureus* and *K. pneumonia* strains and inhibited biofilm formation by 99.9% along with preventing damage of cotton fabrics under UV illumination. In another study, dental composites made from polymer resin and ZnO NPs (as nanofillers) were employed to inhibit dental plaque (biofilm) formation that contribute to dental caries and often leads to degradation of resin composite (Aydin Sevinç and Hanley 2010). The plaque inhibition potency of polymer/ZnO nanocomposites were examined against various strains *Streptococcus sobrinus*, which is known to cause dental caries and possesses a superior adherence on tooth surfaces as compared to other *Streptococcus* species. The MIC and MBC values of ZnO NPs against *S. sobrinus* were found to be 50 and 150 μg ml^{-1}, respectively while the incorporation of 10% ZnO-NPs in polymeric resin reduced biofilm growth by 80% as compared to polymeric resin without ZnO NPs. Moreover, the adherence of *S. sobrinus* cells was found to be significantly higher on

pristine polymer resin as compared to 10% ZnO/polymer nanocomposites. On a similar account, higher biofilm coverage was observed on pure polymer resin after 3 days of incubation whereas, 10% ZnO/polymer nanocomposite showed significantly less colonies of *S. sobrinus* attached on its surface which discouraged the formation of a continuous biofilm layer.

Shalumon et al. (2011) synthesized an electrospun hybrid nanofibrous scaffold from sodium alginate/polyvinyl alcohol co-polymeric mixture containing dispersed ZnO NPs. Three nanocomposites were designed with varying amount (0.5, 1, 2 and 5%) of nano ZnO (average size, 160 nm) incorporated inside the polymeric nanofibers. The addition of ZnO NPs leads to increase in diameter of electrospun magnifiers from 190–240 to 220–360 nm. The evaluation of antibacterial activity on the basis of disc diffusion assays revealed that a larger ZoI was obtained for nanocomposites with higher ZnO concentration. Among the two strains, *S. aureus* appeared to be more sensitive to nanofibrous composite where the diameter of ZoI was calculated in the range of 15–16 mm as compared to 14–15 mm in case with *E. coli*. For assessing cytocompatibility, the nanofibrous scaffolds with low concentrations of ZnO (up to 0.5%) showed good adherence and spreading of L929 cells up to 96 h while a further increase in ZnO concentrations, the cell spreading is severely affected (Fig. 16). Moreover, a significant change in cell morphology was also observed and the existence of more rounded cells was attributed to cytotoxic consequences of ZnO NPs. Thus, nanofibrous composite with 5% ZnO was considered as a suitable material for wound dressing applications by providing good antibacterial activities yet remaining non cytotoxic.

Similarly, Ul-Islam et al. (2014) synthesized a potential wound dressing/healing nanocomposite films using bacterial cellulose with different concentrations of ZnO NPs (size range, 70–90 nm). The antimicrobial efficacies of nanostructured films were tested against *E. coli* where an introduction of 1 and 2% ZnO to cellulosic
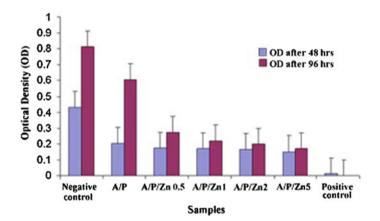

Fig. 16 Cytotoxicity studies on sodium alginate/PVA/ZnO mats using L929 at 48 and 96 h of attachment. A, P and Zn respectively represents alginate, PVA and ZnO. (Reproduce with permission from Shalumon et al. (2011), Elsevier)

Table 5 Zinc oxide based nanocomposites and their biomedical applications

ZnO based nanocomposites	NPs size (nm)	Activity	Organisms tested	Evaluation parameter	Potential biomedical applications	References
ZnO/sodium alginate/PVA nanofibers	160	AB	S. aureus, E. coli	ZoI: 15–16 mm	Wound dressings	Shalumon et al. (2011)
ZnO/Chitosan/PEG/Ag	25–65	AB	E. coli, S. aureus, P. aeruginosa, B. subtilis	ZoI: 11.9–17.2 mm	Wounds/burns dressings	Liu and Kim (2012)
ZnO/parylene-glass	15	AB	E. coli, S. aureus,	Reduction rate: 100% (E. coli) & 76% (S. aureus)	Biomedical coatings Disinfectants	Applerot et al. (2010)
ZnO/TiO$_2$ coatings	20–80	AB	E. coli, S. aureus,	Reduction rate: 100% (E. coli) & 99.8% (S. aureus)	Antibacterial orthopedic and dental implants.	Hu et al. (2012)
ZnO/Polyvinylchloride	20	AB	S. aureus	Reduction rate: 100% at 30% ZnO conc.	Antibacterial endotracheal tube	Geilich and Webster (2013)
ZnO/Polyurethane membranes	<100	AF	Aspergillus	ND	Antibacterial scaffold	Vlad et al. (2012)
ZnO/Polycaprolactone	60	AB	E. coli, S. aureus,	ZoI: 9.8–10. 2 mm	Antibacterial scaffold for tissue engineering	Augustine et al. (2014)
ZnO/Poly(D,L-lactide) nanofiber mats	8–20	AB	E. coli, S. aureus	Reduction rate: 35% (E. coli) & 95% (S. aureus)	Antimicrobial wound dressings.	Rodríguez-Tobías et al. (2014)
ZnO/Polypyrrole/chitosan	ND	AB	E. coli, S. aureus, B. cereus, P. aeruginosa	ZoI: 17.7–29.6 mm	Surgical devices, biosensor and drug-delivery vehicles	Ebrahimiasl et al. (2015)

AB Antibacterial; AF Antifungal; ND Not determined

depicted a ZoI of 34 and 41 mm, respectively. Also, there was a significant up gradation in thermal, mechanical and biological properties of composites with addition of ZnO NPs. The resulting nanocomposites were also found to be non toxic and biocompatible to be used in biomedical applications. In a similar sense, several other polymeric and inorganic nanocomposites have been translated as an effective antimicrobial coating material for medical implants, devices after incorporating ZnO NPs which remain active for months without posing any cytotoxicity to human cells (Sudheesh Kumar et al. 2012; Applerot et al. 2010; Schwartz et al. 2012). A summary of various ZnO based nanocomposite and their biomedical applications are shown in Table 5.

6 Conclusions

The demands for high living standards and hygienic disciplines call new challenges for exploring some advanced, reliable but effective antimicrobial agents that should be environmentally benign and extremely safe for human use. Moreover, there is a daunting concern about the reoccurrence of drug-resistant microorganisms in infection prone areas such as hospitals, where the development of new antimicrobial materials for therapeutics, antisepsis or disinfection purposes are anticipating new strategies for employing them under clinically relevant conditions. In this context, nanotechnology is playing major role in some high priorities areas such as biomedical implants, surgical devices, catheters, stents, and antimicrobial coatings where the application of nano-antimicrobials based on metallic nanoparticles (silver, gold, copper/copper oxide), zinc oxide, chitosan nanoparticles along with their hybrid nanocomposites have been evaluated at various parameters such as their broad spectrum antimicrobial nature, efficacy, reusability and potential cytotoxicity towards mammalian cells. Each nanomaterial has shown its advantages and limitations therefore, accepting an ideal strategy or procedure for developing the antimicrobial biomaterials is not feasible. However, in future the involvement of "translational research" for making nano-antimicrobial biomaterials needs to be urgently constructed so as to implement the actual transformation of laboratory research for the realization of product for commercial applications.

References

Afzal MA, Kalmodia S, Kesarwani P, Basu B, Balani K (2013) Bactericidal effect of silver-reinforced carbon nanotube and hydroxyapatite composites. J Biomater Appl 27 (8):967–978. doi:10.1177/0885328211431856

Aggor FS, Ahmed EM, El-Aref A, Asem M (2010) Synthesis and characterization of poly (Acrylamide-co-Acrylic acid) hydrogel containing silver nanoparticles for antimicrobial applications. J Am Sci 12:6

Agnihotri S, Bajaj G, Mukherji S, Mukherji S (2015) Arginine-assisted immobilization of silver nanoparticles on ZnO nanorods: an enhanced and reusable antibacterial substrate without human cell cytotoxicity. Nanoscale 7(16):7415–7429

Agnihotri S, Mukherji S, Mukherji S (2012) Antimicrobial chitosan–PVA hydrogel as a nanoreactor and immobilizing matrix for silver nanoparticles. Appl Nanosci 2(3):179–188

Agnihotri S, Mukherji S, Mukherji S (2013) Immobilized silver nanoparticles enhance contact killing and show highest efficacy: elucidation of the mechanism of bactericidal action of silver. Nanoscale 5(16):7328–7340. doi:10.1039/C3nr00024a

Agnihotri S, Mukherji S, Mukherji S (2014) Size-controlled silver nanoparticles synthesized over the range 5–100 nm using the same protocol and their antibacterial efficacy. RSC Adv 4 (8):3974–3983

Ahamed MI, Sankar S, Kashif PM, Basha SK, Sastry TP (2015) Evaluation of biomaterial containing regenerated cellulose and chitosan incorporated with silver nanoparticles. Int J Biol Macromol 72:680–686. doi:10.1016/j.ijbiomac.2014.08.055

Ahmad MB, Shameli K, Darroudi M, Yunus W, Ibrahim NA, Hamid AA, Zargar M (2009) Antibacterial activity of silver/clay/chitosan bionanocomposites. Res J Biol Sci 4(11):1156–1161

Ahmad Z, Vargas-Reus MA, Bakhshi R, Ryan F, Ren GG, Oktar F, Allaker RP (2012) Antimicrobial properties of electrically formed elastomeric polyurethane-copper oxide nanocomposites for medical and dental applications. Methods Enzymol 509:87–99. doi:10.1016/b978-0-12-391858-1.00005-8

Akhavan O, Abdolahad M, Abdi Y, Mohajerzadeh S (2011) Silver nanoparticles within vertically aligned multi-wall carbon nanotubes with open tips for antibacterial purposes. J Mater Chem 21(2):387–393

Allen AB, Priddy LB, Li MT, Guldberg RE (2015) Functional augmentation of naturally-derived materials for tissue regeneration. Ann Biomed Eng 43(3):555–567. doi:10.1007/s10439-014-1192-4

Almajhdi FN, Fouad H, Khalil KA, Awad HM, Mohamed SH, Elsarnagawy T, Albarrag AM, Al-Jassir FF, Abdo HS (2014) In-vitro anticancer and antimicrobial activities of PLGA/silver nanofiber composites prepared by electrospinning. J Mater Sci Mater Med 25(4):1045–1053

Almeida AJ, Souto E (2007) Solid lipid nanoparticles as a drug delivery system for peptides and proteins. Adv Drug Del Rev 59(6):478–490

Alshehri SM, Aldalbahi A, Al-hajji AB, Chaudhary AA, in het Panhuis M, Alhokbany N, Ahamad T (2016) Development of carboxymethyl cellulose-based hydrogel and nanosilver composite as antimicrobial agents for UTI pathogens. Carbohydr Polym 138:229–236

Anitha A, Rani VD, Krishna R, Sreeja V, Selvamurugan N, Nair S, Tamura H, Jayakumar R (2009) Synthesis, characterization, cytotoxicity and antibacterial studies of chitosan, O-carboxymethyl and N, O-carboxymethyl chitosan nanoparticles. Carbohydr Polym 78 (4):672–677

Anjum S, Abbasi BH (2016) Thidiazuron-enhanced biosynthesis and antimicrobial efficacy of silver nanoparticles via improving phytochemical reducing potential in callus culture of *Linum usitatissimum* L. Int J Nanomedicine 11:715

Anna R, Silvia I, Agnieszka K, Manuel A, Jesus S (2013) Preparation and characterization of chitosan–silver nanocomposite films and their antibacterial activity against *Staphylococcus aureus*. Nanotechnology 24(1):015101

Applerot G, Abu-Mukh R, Irzh A, Charmet J, Keppner H, Laux E, Guibert G, Gedanken A (2010) Decorating parylene-coated glass with ZnO nanoparticles for antibacterial applications: a comparative study of sonochemical, microwave, and microwave-plasma coating routes. ACS Appl Mater Interfaces 2(4):1052–1059

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

Augustine R, Malik HN, Singhal DK, Mukherjee A, Malakar D, Kalarikkal N, Thomas S (2014) Electrospun polycaprolactone/ZnO nanocomposite membranes as biomaterials with antibacterial and cell adhesion properties. J Polym Res 21(3):1–17

Aydin Sevinç B, Hanley L (2010) Antibacterial activity of dental composites containing zinc oxide nanoparticles. J Biomed Mater Res Part B Appl Biomater 94(1):22–31

Azizi S, Ahmad MB, Hussein MZ, Ibrahim NA, Namvar F (2014) Preparation and properties of poly(vinyl alcohol)/chitosan blend bionanocomposites reinforced with cellulose nanocrystals/ZnO–Ag multifunctional nanosized filler. Int J Nanomed 9:1909–1917. doi:10.2147/ijn.s60274

Bakare R, Hawthrone S, Vails C, Gugssa A, Karim A, Stubbs J 3rd, Raghavan D (2016) Antimicrobial and cell viability measurement of bovine serum albumin capped silver nanoparticles (Ag/BSA) loaded collagen immobilized poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) film. J Colloid Interface Sci 465:140–148. doi:10.1016/j.jcis.2015.11.041

Balakumaran M, Ramachandran R, Balashanmugam P, Mukeshkumar D, Kalaichelvan P (2016) Mycosynthesis of silver and gold nanoparticles: Optimization, characterization and antimicrobial activity against human pathogens. Microbiol Res 182:8–20

Barbinta-Patrascu ME, Ungureanu C, Iordache SM, Iordache AM, Bunghez IR, Ghiurea M, Badea N, Fierascu RC, Stamatin I (2014) Eco-designed biohybrids based on liposomes, mint-nanosilver and carbon nanotubes for antioxidant and antimicrobial coating. Mater Sci Eng C Mater Biol Appl 39:177–185. doi:10.1016/j.msec.2014.02.038

Barreras US, Méndez FT, Martínez REM, Valencia CS, Rodríguez PRM, Rodríguez JPL (2016) Chitosan nanoparticles enhance the antibacterial activity of chlorhexidine in collagen membranes used for periapical guided tissue regeneration. Mater Sci Eng C Mater Biol Appl 58:1182–1187

Bazaka K, Jacob MV, Crawford RJ, Ivanova EP (2012) Efficient surface modification of biomaterial to prevent biofilm formation and the attachment of microorganisms. Appl Microbiol Biotechnol 95(2):299–311

Blažević F, Milekić T, Romić MD, Juretić M, Pepić I, Filipović-Grčić J, Lovrić J, Hafner A (2016) Nanoparticle-mediated interplay of chitosan and melatonin for improved wound epithelialisation. Carbohydr Polym 146:445–454

Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, Scheld M, Spellberg B, Bartlett J (2009) Bad bugs, no drugs: no ESKAPE! An update from the infectious diseases society of America. Clin Infect Dis 48(1):1–12

Busscher HJ, Van Der Mei HC, Subbiahdoss G, Jutte PC, Van Den Dungen JJ, Zaat SA, Schultz MJ, Grainger DW (2012) Biomaterial-associated infection: locating the finish line in the race for the surface. Sci Transl Med 4 (153):153rv110–153rv110

Cady NC, Behnke JL, Strickland AD (2011) Copper-based nanostructured coatings on natural cellulose: Nanocomposites exhibiting rapid and efficient inhibition of a Multi-Drug Resistant wound pathogen, A. baumannii, and mammalian cell biocompatibility in vitro. Adv Funct Mater 21(13):2506–2514

Çalt S, Serper A (2002) Time-dependent effects of EDTA on dentin structures. J Endod 28(1):17–19

Campoccia D, Montanaro L, Arciola CR (2013a) A review of the biomaterials technologies for infection-resistant surfaces. Biomaterials 34(34):8533–8554

Campoccia D, Montanaro L, Arciola CR (2013b) A review of the clinical implications of anti-infective biomaterials and infection-resistant surfaces. Biomaterials 34(33):8018–8029

Cañas AI, Delgado JP, Gartner C (2016) Biocompatible scaffolds composed of chemically crosslinked chitosan and gelatin for tissue engineering. J Appl Polym Sci 133 (33)

Cao X, Tang M, Liu F, Nie Y, Zhao C (2010) Immobilization of silver nanoparticles onto sulfonated polyethersulfone membranes as antibacterial materials. Colloids Surf B Biointerfaces 81(2):555–562. doi:10.1016/j.colsurfb.2010.07.057

Chan Y-H, Huang C-F, Ou K-L, Peng P-W (2011) Mechanical properties and antibacterial activity of copper doped diamond-like carbon films. Surf Coat Technol 206(6):1037–1040

Chen Q, Jiang H, Ye H, Li J, Huang J (2014) Preparation, antibacterial, and antioxidant activities of silver/chitosan composites. J Carbohydr Chem 33(6):298–312

Chen WY, Lin JY, Chen WJ, Luo L, Wei-Guang Diau E, Chen YC (2010) Functional gold nanoclusters as antimicrobial agents for antibiotic-resistant bacteria. Nanomed (Lond) 5 (5):755–764. doi:10.2217/nnm.10.43

Chen X, Schluesener H (2008) Nanosilver: a nanoproduct in medical application. Toxicol Lett 176 (1):1–12

Chen YN, Hsueh YH, Hsieh CT, Tzou DY, Chang PL (2016) Antiviral activity of Graphene-silver nanocomposites against non-enveloped and enveloped viruses. Int J Environ Res Public Health 13(4). doi:10.3390/ijerph13040430

Cheng L, Weir MD, Xu HH, Antonucci JM, Kraigsley AM, Lin NJ, Lin-Gibson S, Zhou X (2012) Antibacterial amorphous calcium phosphate nanocomposites with a quaternary ammonium dimethacrylate and silver nanoparticles. Dent Mater 28(5):561–572. doi:10.1016/j.dental.2012.01.005

Chernousova S, Epple M (2013) Silver as antibacterial agent: ion, nanoparticle, and metal. Angew Chem Int Ed 52(6):1636–1653

Chopra I (2007) The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern? J Antimicrob Chemother 59(4):587–590

Chung Y-C, Wang H-L, Chen Y-M, Li S-L (2003) Effect of abiotic factors on the antibacterial activity of chitosan against waterborne pathogens. Bioresour Technol 88(3):179–184

Cioffi N, Ditaranto N, Torsi L, Picca RA, De Giglio E, Sabbatini L, Novello L, Tantillo G, Bleve-Zacheo T, Zambonin PG (2005a) Synthesis, analytical characterization and bioactivity of Ag and Cu nanoparticles embedded in poly-vinyl-methyl-ketone films. Anal Bioanal Chem 382(8):1912–1918. doi:10.1007/s00216-005-3334-x

Cioffi N, Torsi L, Ditaranto N, Sabbatini L, Zambonin PG, Tantillo G, Ghibelli L, D'Alessio M, Bleve-Zacheo T, Traversa E (2004) Antifungal activity of polymer-based copper nanocomposite coatings. Appl Phys Lett 85(12):2417–2419

Cioffi N, Torsi L, Ditaranto N, Tantillo G, Ghibelli L, Sabbatini L, Bleve-Zacheo T, D'Alessio M, Zambonin PG, Traversa E (2005b) Copper nanoparticle/polymer composites with antifungal and bacteriostatic properties. Chem Mater 17(21):5255–5262

Costerton JW, Stewart PS, Greenberg E (1999) Bacterial biofilms: a common cause of persistent infections. Science 284(5418):1318–1322

Curtis L (2008) Prevention of hospital-acquired infections: review of non-pharmacological interventions. J Hosp Infect 69(3):204–219

Dahlin RL, Kasper FK, Mikos AG (2011) Polymeric nanofibers in tissue engineering. Tissue Eng Part B Rev 17(5):349–364

Damm C, Münstedt H, Rösch A (2007) Long-term antimicrobial polyamide 6/silver-nanocomposites. J Mater Sci 42(15):6067–6073

Dang JM, Leong KW (2006) Natural polymers for gene delivery and tissue engineering. Adv Drug Deliv Rev 58(4):487–499. doi:10.1016/j.addr.2006.03.001

Das G, Kalita RD, Gogoi P, Buragohain AK, Karak N (2014) Antibacterial activities of copper nanoparticle-decorated organically modified montmorillonite/epoxy nanocomposites. Appl Clay Sci 90:18–26

Das SK, Das AR, Guha AK (2009) Gold nanoparticles: microbial synthesis and application in water hygiene management. Langmuir 25(14):8192–8199. doi:10.1021/la900585p

Dash M, Chiellini F, Ottenbrite R, Chiellini E (2011) Chitosan—A versatile semi-synthetic polymer in biomedical applications. Prog Polym Sci 36(8):981–1014

de Mel A, Chaloupka K, Malam Y, Darbyshire A, Cousins B, Seifalian AM (2012) A silver nanocomposite biomaterial for blood-contacting implants. J Biomed Mater Res A 100 (9):2348–2357. doi:10.1002/jbm.a.34177

de Paz LEC, Resin A, Howard KA, Sutherland DS, Wejse PL (2011) Antimicrobial effect of chitosan nanoparticles on Streptococcus mutans biofilms. Appl Environ Microbiol 77 (11):3892–3895

del Carpio-Perochena AE, Bramante CM, Duarte MA, Cavenago BC, Villas-Boas MH, Graeff MS, Bernardineli N, de Andrade FB, Ordinola-Zapata R (2011) Biofilm dissolution

and cleaning ability of different irrigant solutions on intraorally infected dentin. J Endod 37 (8):1134–1138

Delgado K, Quijada R, Palma R, Palza H (2011) Polypropylene with embedded copper metal or copper oxide nanoparticles as a novel plastic antimicrobial agent. Lett Appl Microbiol 53 (1):50–54. doi:10.1111/j.1472-765X.2011.03069.x

Demurtas M, Perry CC (2014) Facile one-pot synthesis of amoxicillin-coated gold nanoparticles and their antimicrobial activity. Gold Bulletin 47(1–2):103–107

Dollwet H, Sorenson J (1988) Roles of copper in bone maintenance and healing. Biol Trace Elem Res 18(1):39–48

Domek MJ, Lechevallier MW, Cameron SC, McFeters GA (1984) Evidence for the role of copper in the injury process of coliform bacteria in drinking water. Appl Environ Microbiol 48 (2):289–293

Donlan RM, Costerton JW (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 15(2):167–193

Dorobantu LS, Goss GG, Burrell RE (2015) Effect of light on physicochemical and biological properties of nanocrystalline silver dressings. RSC Adv 5(19):14294–14304

Drury JL, Mooney DJ (2003) Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials 24(24):4337–4351

Du WL, Xu YL, Xu ZR, Fan CL (2008) Preparation, characterization and antibacterial properties against E. coli K(88) of chitosan nanoparticle loaded copper ions. Nanotechnology 19 (8):085707. doi:10.1088/0957-4484/19/8/085707

Dunn K, Edwards-Jones V (2004) The role of Acticoat™ with nanocrystalline silver in the management of burns. Burns 30:S1–S9

Dykman L, Khlebtsov N (2012) Gold nanoparticles in biomedical applications: recent advances and perspectives. Chem Soc Rev 41(6):2256–2282

Ebrahimiasl S, Zakaria A, Kassim A, Basri SN (2015) Novel conductive polypyrrole/zinc oxide/chitosan bionanocomposite: synthesis, characterization, antioxidant, and antibacterial activities. Int J Nanomed 10:217

Egger S, Lehmann RP, Height MJ, Loessner MJ, Schuppler M (2009) Antimicrobial properties of a novel silver-silica nanocomposite material. Appl Environ Microbiol 75(9):2973–2976. doi:10.1128/aem.01658-08

El-Naggar MY, Gohar YM, Sorour MA, Waheeb MG (2016) Hydrogel dressing with a nano-Formula against methicillin-resistant Staphylococcus aureus and Pseudomonas aeruginosa diabetic foot bacteria. J Microbiol Biotechnol 26(2):408–420. doi:10.4014/jmb.1506.06048

Elbeshehy EK, Elazzazy AM, Aggelis G (2015) Silver nanoparticles synthesis mediated by new isolates of Bacillus spp., nanoparticle characterization and their activity against Bean Yellow Mosaic Virus and human pathogens. Front Microbiol 6

Esteban-Tejeda L, Malpartida F, Díaz LA, Torrecillas R, Rojo F, Moya JS (2012) Glass-(nAg, nCu) biocide coatings on ceramic oxide substrates. PLoS ONE 7(3):e33135

Ewald A, Hosel D, Patel S, Grover LM, Barralet JE, Gbureck U (2011) Silver-doped calcium phosphate cements with antimicrobial activity. Acta Biomater 7(11):4064–4070. doi:10.1016/j.actbio.2011.06.049

Fan Z, Liu B, Wang J, Zhang S, Lin Q, Gong P, Ma L, Yang S (2014) A novel wound dressing based on Ag/graphene polymer hydrogel: effectively kill bacteria and accelerate wound healing. Adv Funct Mater 24(25):3933–3943

Farhoudian S, Yadollahi M, Namazi H (2016) Facile synthesis of antibacterial chitosan/CuO bio-nanocomposite hydrogel beads. Int J Biol Macromol 82:837–843

Fei X, Jia M, Du X, Yang Y, Zhang R, Shao Z, Zhao X, Chen X (2013) Green synthesis of silk fibroin-silver nanoparticle composites with effective antibacterial and biofilm-disrupting properties. Biomacromolecules 14(12):4483–4488. doi:10.1021/bm4014149

Felt O, Buri P, Gurny R (1998) Chitosan: a unique polysaccharide for drug delivery. Drug Dev Ind Pharm 24(11):979–993. doi:10.3109/03639049809089942

Fouda MM, El-Aassar MR, Al-Deyab SS (2013) Antimicrobial activity of carboxymethyl chitosan/polyethylene oxide nanofibers embedded silver nanoparticles. Carbohydr Polym 92 (2):1012–1017. doi:10.1016/j.carbpol.2012.10.047

Gaikwad S, Ingle A, Gade A, Rai M, Falanga A, Incoronato N, Russo L, Galdiero S, Galdiero M (2013) Antiviral activity of mycosynthesized silver nanoparticles against herpes simplex virus and human parainfluenza virus type 3. Int J Nanomed 8:4303

Gajbhiye S, Sakharwade S (2016) Silver nanoparticles in cosmetics. J Cosmet Dermatol Sci Appl 6(01):48

Gant VA, Wren MW, Rollins MS, Jeanes A, Hickok SS, Hall TJ (2007) Three novel highly charged copper-based biocides: safety and efficacy against healthcare-associated organisms. J Antimicrob Chemother 60(2):294–299

Geilich BM, Webster TJ (2013) Reduced adhesion of Staphylococcus aureus to ZnO/PVC nanocomposites. In: Bioengineering Conference (NEBEC), 2013 39th Annual Northeast, 2013. IEEE, pp 7–8

Ghanbari H, Radenkovic D, Marashi SM, Parsno S, Roohpour N, Burriesci G, Seifalian AM (2016) Novel heart valve prosthesis with self-endothelialization potential made of modified polyhedral oligomeric silsesquioxane-nanocomposite material. Biointerphases 11(2):029801. doi:10.1116/1.4939036

GhavamiNejad A, Park CH, Kim CS (2016) In situ synthesis of antimicrobial silver nanoparticles within antifouling zwitterionic hydrogels by catecholic redox chemistry for wound healing application. Biomacromolecules 17(3):1213–1223

Gnanasangeetha D, Thambavani DS (2013) Biogenic production of zinc oxide nanoparticles using Acalypha Indica. J Chem Bio Phys Sci 4(1):238

Gonzalez-Sanchez MI, Perni S, Tommasi G, Morris NG, Hawkins K, Lopez-Cabarcos E, Prokopovich P (2015) Silver nanoparticle based antibacterial methacrylate hydrogels potential for bone graft applications. Mater Sci Eng C Mater Biol Appl 50:332–340. doi:10.1016/j.msec.2015.02.002

Gould SW, Fielder MD, Kelly AF, Morgan M, Kenny J, Naughton DP (2009) The antimicrobial properties of copper surfaces against a range of important nosocomial pathogens. Ann Microbiol 59(1):151–156

Grace AN, Pandian K (2007) Antibacterial efficacy of aminoglycosidic antibiotics protected gold nanoparticles—A brief study. Colloids Surf Physicochem Eng Aspects 297(1):63–70

Grace M, Chand N, Bajpai SK (2009) Copper alginate-cotton cellulose (CACC) fibers with excellent antibacterial properties. J Eng Fiber Fabr 4(3):1–14

Grass G, Rensing C, Solioz M (2011) Metallic copper as an antimicrobial surface. Appl Environ Microbiol 77(5):1541–1547

Gristina AG (1987) Biomaterial-centered infection: microbial adhesion versus tissue integration. Science 237(4822):1588–1595

Gu H, Ho PL, Tong E, Wang L, Xu B (2003) Presenting vancomycin on nanoparticles to enhance antimicrobial activities. Nano Lett 3(9):1261–1263. doi:10.1021/nl034396z

Gunalan S, Sivaraj R, Rajendran V (2012) Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. Prog Nat Sci Mater Int 22(6):693–700

Gupta A, Silver S (1998) Molecular genetics: silver as a biocide: will resistance become a problem? Nat Biotechnol 16(10):888

Hall-Stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol 2(2):95–108

Hall-Stoodley L, Stoodley P (2009) Evolving concepts in biofilm infections. Cell Microbiol 11(7):1034–1043

Harding JL, Reynolds MM (2014) Combating medical device fouling. Trends Biotechnol 32(3):140–146

Hassan MS, Amna T, Kim HY, Khil M-S (2013) Enhanced bactericidal effect of novel CuO/TiO$_2$ composite nanorods and a mechanism thereof. Compos B Eng 45(1):904–910

Hench LL, Polak JM (2002) Third-generation biomedical materials. Science 295(5557):1014–1017

Hendriks J, Van Horn J, Van Der Mei H, Busscher H (2004) Backgrounds of antibiotic-loaded bone cement and prosthesis-related infection. Biomaterials 25(3):545–556

Herkendell K, Shukla VR, Patel AK, Balani K (2014) Domination of volumetric toughening by silver nanoparticles over interfacial strengthening of carbon nanotubes in bactericidal hydroxyapatite biocomposite. Mater Sci Eng C Mater Biol Appl 34:455–467. doi:10.1016/j.msec.2013.09.034

Hill JW (2009) Colloidal silver: medical uses, toxicology and manufacture. Clear Springs Press

Hoffman AS (2012) Hydrogels for biomedical applications. Adv Drug Del Rev 64:18–23

Hoop M, Shen Y, Chen XZ, Mushtaq F, Iuliano LM, Sakar MS, Petruska A, Loessner MJ, Nelson BJ, Pané S (2015) Magnetically driven silver-coated nanocoils for efficient bacterial contact killing. Adv Funct Mater 26(7):1063–1069. doi:10.1002/adfm.201504463

Hsu SH, Tseng HJ, Lin YC (2010) The biocompatibility and antibacterial properties of waterborne polyurethane-silver nanocomposites. Biomaterials 31(26):6796–6808. doi:10.1016/j.biomaterials.2010.05.015

Hu H, Zhang W, Qiao Y, Jiang X, Liu X, Ding C (2012) Antibacterial activity and increased bone marrow stem cell functions of Zn-incorporated TiO_2 coatings on titanium. Acta Biomater 8(2):904–915

Hu R, Li G, Jiang Y, Zhang Y, Zou JJ, Wang L, Zhang X (2013) Silver-zwitterion organic-inorganic nanocomposite with antimicrobial and antiadhesive capabilities. Langmuir 29(11):3773–3779. doi:10.1021/la304708b

Hu W, Peng C, Luo W, Lv M, Li X, Li D, Huang Q, Fan C (2010) Graphene-based antibacterial paper. ACS Nano 4(7):4317–4323. doi:10.1021/nn101097v

Huang JF, Zhong J, Chen GP, Lin ZT, Deng Y, Liu YL, Cao PY, Wang B, Wei Y, Wu T, Yuan J, Jiang GB (2016) A hydrogel-based hybrid theranostic contact lens for fungal keratitis. ACS Nano. doi:10.1021/acsnano.6b00601

Huang WC, Tsai PJ, Chen YC (2007) Functional gold nanoparticles as photothermal agents for selective-killing of pathogenic bacteria. Nanomed (Lond) 2(6):777–787. doi:10.2217/17435889.2.6.777

Huang X, Neretina S, El-Sayed MA (2009) Gold nanorods: from synthesis and properties to biological and biomedical applications. Adv Mater 21(48):4880–4910

Hubbell JA (1995) Biomaterials in tissue engineering. Biotechnol (N Y) 13(6):565–576

Ifuku S, Tsukiyama Y, Yukawa T, Egusa M, Kaminaka H, Izawa H, Morimoto M, Saimoto H (2015) Facile preparation of silver nanoparticles immobilized on chitin nanofiber surfaces to endow antifungal activities. Carbohydr Polym 117:813–817. doi:10.1016/j.carbpol.2014.10.042

Ingle A, Gade A, Pierrat S, Sonnichsen C, Rai M (2008) Mycosynthesis of silver nanoparticles using the fungus Fusarium acuminatum and its activity against some human pathogenic bacteria. Curr Nanosci 4(2):141–144

Jamil B, Habib H, Abbasi S, Nasir H, Rahman A, Rehman A, Bokhari H, Imran M (2016) Cefazolin loaded chitosan nanoparticles to cure multi drug resistant Gram-negative pathogens. Carbohydr Polym 136:682–691

Jayakumar R, Menon D, Manzoor K, Nair S, Tamura H (2010) Biomedical applications of chitin and chitosan based nanomaterials—A short review. Carbohydr Polym 82(2):227–232

Jayaramudu T, Raghavendra GM, Varaprasad K, Sadiku R, Raju KM (2013) Development of novel biodegradable Au nanocomposite hydrogels based on wheat: for inactivation of bacteria. Carbohydr Polym 92(2):2193–2200

Johnsson B, Lofas S, Lindquist G (1991) Immobilization of proteins to a carboxymethyldextran-modified gold surface for biospecific interaction analysis in surface plasmon resonance sensors. Anal Biochem 198(2):268–277

Jones V, Grey JE, Harding KG (2006) Wound dressings. BMJ 332(7544):777–780. doi:10.1136/bmj.332.7544.777

Jones V, Milton T (2000) When and how to use hydrogels. Nurs Times 96(23):3–4

Jovašević J, Dimitrijević S, Filipović J, Tomić SLj MM (2011) Swelling, mechanical and antimicrobial studies of Ag/P (HEMA/IA)/PVP semi-IPN hybrid hydrogels. Acta Phys Pol A 2:279–283

Jukes L, Mikhail J, Bome-Mannathoko N, Hadfield SJ, Harris LG, El-Bouri K, Davies AP, Mack D (2010) Rapid differentiation of *Staphylococcus aureus*, *Staphylococcus epidermidis* and other coagulase-negative staphylococci and methicillin susceptibility testing directly from growth-positive blood cultures by multiplex real-time PCR. J Med Microbiol 59(12):1456–1461

Kamrupi I, Phukon P, Konwer B, Dolui S (2011) Synthesis of silver–polystyrene nanocomposite particles using water in supercritical carbon dioxide medium and its antimicrobial activity. J Supercrit Fluids 55(3):1089–1094

Kang S, Herzberg M, Rodrigues DF, Elimelech M (2008) Antibacterial effects of carbon nanotubes: size does matter! Langmuir 24(13):6409–6413. doi:10.1021/la800951v

Kannan RY, Salacinski HJ, De Groot J, Clatworthy I, Bozec L, Horton M, Butler PE, Seifalian AM (2006) The antithrombogenic potential of a polyhedral oligomeric silsesquioxane (POSS) nanocomposite. Biomacromolecules 7(1):215–223. doi:10.1021/bm050590z

Kar S, Bagchi B, Kundu B, Bhandary S, Basu R, Nandy P, Das S (2014) Synthesis and characterization of Cu/Ag nanoparticle loaded mullite nanocomposite system: A potential candidate for antimicrobial and therapeutic applications. Biochimica et Biophysica Acta (BBA)-General Subjects 1840(11):3264–3276

Khalil KA, Fouad H, Elsarnagawy T, Almajhdi FN (2013) Preparation and characterization of electrospun PLGA/silver composite nanofibers for biomedical applications. Int J Electrochem Sci 8:3483–3493

Kim J, Lee J, Kwon S, Jeong S (2009) Preparation of biodegradable polymer/silver nanoparticles composite and its antibacterial efficacy. J Nanosci Nanotechnol 9(2):1098–1102

Kishen A, Sum C-P, Mathew S, Lim C-T (2008) Influence of irrigation regimens on the adherence of *Enterococcus faecalis* to root canal dentin. J Endod 34(7):850–854

Kołodziejczak-Radzimska A, Jesionowski T (2014) Zinc oxide—from synthesis to application: a review. Materials 7(4):2833–2881

Krupanidhi S, Sreekumar A, Sanjeevi C (2008) Copper & biological health. Indian J Med Res 128 (4):448

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

Lavorgna M, Attianese I, Buonocore GG, Conte A, Del Nobile MA, Tescione F, Amendola E (2014) MMT-supported Ag nanoparticles for chitosan nanocomposites: structural properties and antibacterial activity. Carbohydr Polym 102:385–392. doi:10.1016/j.carbpol.2013.11.026

Lee B-S, Lee C-C, Wang Y-P, Chen H-J, Lai C-H, Hsieh W-L, Chen Y-W (2016a) Controlled-release of tetracycline and lovastatin by poly (d, l-lactide-co-glycolide acid)-chitosan nanoparticles enhances periodontal regeneration in dogs. Int J Nanomed 11:285

Lee J-H, Velmurugan P, Park J-H, Lee K-J, Jin J-S, Park Y-J, Bang K-S, Oh B-T (2016b) In vitro fabrication of dental filling nanopowder by green route and its antibacterial activity against dental pathogens. J Photochem Photobiol B: Biol 159:229–236

Lee KY, Mooney DJ (2001) Hydrogels for tissue engineering. Chem Rev 101(7):1869–1880

Li C, Wang X, Chen F, Zhang C, Zhi X, Wang K, Cui D (2013) The antifungal activity of graphene oxide-silver nanocomposites. Biomaterials 34(15):3882–3890. doi:10.1016/j.biomaterials.2013.02.001

Li J, Zhai D, Lv F, Yu Q, Ma H, Yin J, Yi Z, Liu M, Chang J, Wu C (2016) Preparation of copper-containing bioactive glass/eggshell membrane nanocomposites for improving angiogenesis, antibacterial activity and wound healing. Acta Biomater 36:254–266

Li X, Lenhart JJ (2012) Aggregation and dissolution of silver nanoparticles in natural surface water. Environ Sci Technol 46(10):5378–5386

Li Z, Lee D, Sheng X, Cohen RE, Rubner MF (2006) Two-level antibacterial coating with both release-killing and contact-killing capabilities. Langmuir 22(24):9820–9823. doi:10.1021/la0622166

Lin JJ, Lin WC, Li SD, Lin CY, Hsu SH (2013) Evaluation of the antibacterial activity and biocompatibility for silver nanoparticles immobilized on nano silicate platelets. ACS Appl Mater Interfaces 5(2):433–443. doi:10.1021/am302534k

Liu X, Mou Y, Wu S, Man H (2013) Synthesis of silver-incorporated hydroxyapatite nanocomposites for antimicrobial implant coatings. Appl Surf Sci 273:748–757

Liu Y, Kim H-I (2012) Characterization and antibacterial properties of genipin-crosslinked chitosan/poly (ethylene glycol)/ZnO/Ag nanocomposites. Carbohydr Polym 89(1):111–116

Longano D, Ditaranto N, Cioffi N, Di Niso F, Sibillano T, Ancona A, Conte A, Del Nobile M, Sabbatini L, Torsi L (2012a) Analytical characterization of laser-generated copper nanoparticles for antibacterial composite food packaging. Anal Bioanal Chem 403(4):1179–1186

Longano D, Ditaranto N, Sabbatini L, Torsi L, Cioffi N (2012b) Synthesis and antimicrobial activity of copper nanomaterials. In: Cioffi N, Rai M (eds) Nano-antimicrobials: progress and prospects. Springer, Berlin, Heidelberg, pp 85–117. doi:10.1007/978-3-642-24428-5_3

Loo CY, Young PM, Lee WH, Cavaliere R, Whitchurch CB, Rohanizadeh R (2014) Non-cytotoxic silver nanoparticle-polyvinyl alcohol hydrogels with anti-biofilm activity: designed as coatings for endotracheal tube materials. Biofouling 30(7):773–788. doi:10.1080/08927014.2014.926475

Lu L, Sun R, Chen R, Hui C-K, Ho C-M, Luk JM, Lau G, Che C-M (2008) Silver nanoparticles inhibit hepatitis B virus replication. Antiviral Ther 13(2):253

Luong ND, Lee Y, Nam J-D (2008) Highly-loaded silver nanoparticles in ultrafine cellulose acetate nanofibrillar aerogel. Eur Polym J 44(10):3116–3121

Ma Y-Q, Yi J-Z, Zhang L-M (2009) A facile approach to incorporate silver nanoparticles into dextran-based hydrogels for antibacterial and catalytical application. J Macromol Sci, Pure Appl Chem 46(6):643–648

Maathuis PG, Neut D, Busscher HJ, van der Mei HC, van Horn JR (2005) Perioperative contamination in primary total hip arthroplasty. Clin Orthop Relat Res 433:136–139

Mack D, Davies AP, Harris LG, Jeeves R, Pascoe B, Knobloch JK-M, Rohde H, Wilkinson TS (2013) Staphylococcus epidermidis in biomaterial-associated infections. In: Moriarty FT, Sebastian ZAJ, Busscher HJ (eds) Biomaterials associated infection: Immunological aspects and antimicrobial strategies. Springer, New York, pp 25–56. doi:10.1007/978-1-4614-1031-7

Mandal A, Meda V, Zhang WJ, Farhan KM, Gnanamani A (2012) Synthesis, characterization and comparison of antimicrobial activity of PEG/TritonX-100 capped silver nanoparticles on collagen scaffold. Colloids Surf B Biointerfaces 90:191–196. doi:10.1016/j.colsurfb.2011.10.021

Maneerung T, Tokura S, Rujiravanit R (2008) Impregnation of silver nanoparticles into bacterial cellulose for antimicrobial wound dressing. Carbohydr Polym 72(1):43–51

Mao S, Sun W, Kissel T (2010) Chitosan-based formulations for delivery of DNA and siRNA. Adv Drug Del Rev 62(1):12–27

Marsh P (2004) Dental plaque as a microbial biofilm. Caries Res 38(3):204–211

Marsh P (2005) Dental plaque: biological significance of a biofilm and community life-style. J Clin Periodontol 32(s6):7–15

Marsich E, Travan A, Donati I, Di Luca A, Benincasa M, Crosera M, Paoletti S (2011) Biological response of hydrogels embedding gold nanoparticles. Colloids Surf B Biointerfaces 83(2):331–339

Mauter MS, Elimelech M (2008) Environmental applications of carbon-based nanomaterials. Environ Sci Technol 42(16):5843–5859

Mohan R, Shanmugharaj AM, Sung Hun R (2011) An efficient growth of silver and copper nanoparticles on multiwalled carbon nanotube with enhanced antimicrobial activity. J Biomed Mater Res B Appl Biomater 96(1):119–126. doi:10.1002/jbm.b.31747

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

Mori Y, Ono T, Miyahira Y, Nguyen VQ, Matsui T, Ishihara M (2013) Antiviral activity of silver nanoparticle/chitosan composites against H1N1 influenza A virus. Nanoscale Res Lett 8(1):93. doi:10.1186/1556-276x-8-93

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

MubarakAli D, Thajuddin N, Jeganathan K, Gunasekaran M (2011) Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. Colloids Surf B Biointerfaces 85(2):360–365

Mukherji S, Ruparelia J, Agnihotri S (2012) Antimicrobial activity of silver and copper nanoparticles: variation in sensitivity across various strains of bacteria and fungi. In: Cioffi N, Rai M (eds) Nano-Antimicrobials: Progress and Prospects. Springer, Berlin, Heidelberg, pp 225–251

Muñoz-Bonilla A, Fernández-García M (2012) Polymeric materials with antimicrobial activity. Prog Polym Sci 37(2):281–339. doi:10.1016/j.progpolymsci.2011.08.005

Murthy PK, Mohan YM, Varaprasad K, Sreedhar B, Raju KM (2008) First successful design of semi-IPN hydrogel–silver nanocomposites: a facile approach for antibacterial application. J Colloid Interface Sci 318(2):217–224

Murugan E, Vimala G (2011) Effective functionalization of multiwalled carbon nanotube with amphiphilic poly(propyleneimine) dendrimer carrying silver nanoparticles for better dispersability and antimicrobial activity. J Colloid Interface Sci 357(2):354–365. doi:10.1016/j.jcis.2011.02.009

Nadagouda MN, Varma RS (2007) Synthesis of thermally stable carboxymethyl cellulose/metal biodegradable nanocomposites for potential biological applications. Biomacromolecules 8(9):2762–2767. doi:10.1021/bm700446p

Nanda A, Saravanan M (2009) Biosynthesis of silver nanoparticles from Staphylococcus aureus and its antimicrobial activity against MRSA and MRSE. Nanomed Nanotechnol Biol Med 5(4):452–456

Narayan R, Abernathy H, Riester L, Berry C, Brigmon R (2005) Antimicrobial properties of diamond-like carbon-silver-platinum nanocomposite thin films. J Mater Eng Perform 14(4):435–440

Narayanan P, Wilson WS, Abraham AT, Sevanan M (2012) Synthesis, characterization, and antimicrobial activity of zinc oxide nanoparticles against human pathogens. BioNanoScience 2(4):329–335

Naveena BE, Prakash S (2013) Biological synthesis of gold nanoparticles using marine algae Gracilaria corticata and its application as a potent antimicrobial and antioxidant agent. Asian J Pharm Clin Res 6:179–182

Norowski PA, Bumgardner JD (2009) Biomaterial and antibiotic strategies for peri-implantitis: A review. J Biomed Mater Res Part B Appl Biomater 88(2):530–543

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

Panacek A, Kilianova M, Prucek R, Husickova V, Vecerova R, Kolar M, Kvitek L, Zboril R (2014) Preparation and in vitro bactericidal and fungicidal efficiency of nanosilver/methylcellulose hydrogel. Int J Chem Mol Nucl Mater Metall Eng 8(6):493–498

Panáček A, Kolář M, Večeřová R, Prucek R, Soukupová J, Kryštof V, Hamal P, Zbořil R, Kvítek L (2009) Antifungal activity of silver nanoparticles against Candida spp. Biomaterials 30(31):6333–6340. doi:10.1016/j.biomaterials.2009.07.065

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

Parsek MR, Singh PK (2003) Bacterial biofilms: an emerging link to disease pathogenesis. Ann Rev Microbiol 57(1):677–701

Pelgrift RY, Friedman AJ (2013) Nanotechnology as a therapeutic tool to combat microbial resistance. Adv Drug Del Rev 65(13):1803–1815

Peng S, Jin G, Li L, Li K, Srinivasan M, Ramakrishna S, Chen J (2016) Multi-functional electrospun nanofibers for advances in tissue regeneration, energy conversion & storage, and water treatment. Chem Soc Rev

Peppas NA, Hilt JZ, Khademhosseini A, Langer R (2006) Hydrogels in biology and medicine: from molecular principles to bionanotechnology. Adv Mater 18(11):1345–1360

Percival SL, Suleman L, Vuotto C, Donelli G (2015) Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. J Med Microbiol 64(4):323–334

Pinto RJ, Marques PA, Neto CP, Trindade T, Daina S, Sadocco P (2009) Antibacterial activity of nanocomposites of silver and bacterial or vegetable cellulosic fibers. Acta Biomater 5(6):2279–2289. doi:10.1016/j.actbio.2009.02.003

Pishbin F, Mourino V, Gilchrist JB, McComb DW, Kreppel S, Salih V, Ryan MP, Boccaccini AR (2013) Single-step electrochemical deposition of antimicrobial orthopaedic coatings based on a bioactive glass/chitosan/nano-silver composite system. Acta Biomater 9(7):7469–7479. doi:10.1016/j.actbio.2013.03.006

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

Prema P, Iniya P, Immanuel G (2016) Microbial mediated synthesis, characterization, antibacterial and synergistic effect of gold nanoparticles using Klebsiella pneumoniae (MTCC-4030). RSC Adv 6(6):4601–4607

Qi L, Xu Z, Jiang X, Hu C, Zou X (2004) Preparation and antibacterial activity of chitosan nanoparticles. Carbohydr Res 339(16):2693–2700

Rabea EI, Badawy ME-T, Stevens CV, Smagghe G, Steurbaut W (2003) Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules 4(6):1457–1465

Raghavendra GM, Jayaramudu T, Varaprasad K, Sadiku R, Ray SS, Raju KM (2013) Cellulose–polymer–Ag nanocomposite fibers for antibacterial fabrics/skin scaffolds. Carbohydr Polym 93(2):553–560

Raghunath J, Zhang H, Edirisinghe MJ, Darbyshire A, Butler PE, Seifalian AM (2009) A new biodegradable nanocomposite based on polyhedral oligomeric silsesquioxane nanocages: cytocompatibility and investigation into electrohydrodynamic jet fabrication techniques for tissue-engineered scaffolds. Biotechnol Appl Biochem 52(1):1–8. doi:10.1042/ba20070256

Raghupathi KR, Koodali RT, Manna AC (2011) Size-dependent bacterial growth inhibition and mechanism of antibacterial activity of zinc oxide nanoparticles. Langmuir 27(7):4020–4028

Rai A, Prabhune A, Perry CC (2010) Antibiotic mediated synthesis of gold nanoparticles with potent antimicrobial activity and their application in antimicrobial coatings. J Mater Chem 20(32):6789–6798

Rai M, Yadav A, Gade A (2009) Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 27(1):76–83

Ramamurthy C, Padma M, Mareeswaran R, Suyavaran A, Kumar MS, Premkumar K, Thirunavukkarasu C (2013) The extra cellular synthesis of gold and silver nanoparticles and their free radical scavenging and antibacterial properties. Colloids Surf B Biointerfaces 102:808–815

Rangari VK, Mohammad GM, Jeelani S, Hundley A, Vig K, Singh SR, Pillai S (2010) Synthesis of Ag/CNT hybrid nanoparticles and fabrication of their nylon-6 polymer nanocomposite fibers for antimicrobial applications. Nanotechnology 21(9):095102. doi:10.1088/0957-4484/21/9/095102

Rao KK, Reddy PR, Lee Y-I, Kim C (2012) Synthesis and characterization of chitosan–PEG–Ag nanocomposites for antimicrobial application. Carbohydr Polym 87(1):920–925

Ratner BD, Hoffman AS, Schoen FJ, Lemons JE (2004) Biomaterials science: an introduction to materials in medicine. Academic Press, Cambridge

Regiel-Futyra A, Kus-Liskiewicz M, Sebastian V, Irusta S, Arruebo M, Stochel G, Kyziol A (2015) Development of noncytotoxic chitosan-gold nanocomposites as efficient antibacterial materials. ACS Appl Mater Interfaces 7(2):1087–1099. doi:10.1021/am508094e

Ren G, Hu D, Cheng EW, Vargas-Reus MA, Reip P, Allaker RP (2009) Characterisation of copper oxide nanoparticles for antimicrobial applications. Int J Antimicrob Agents 33(6):587–590

Rodrigues AG, Ping LY, Marcato PD, Alves OL, Silva MC, Ruiz RC, Melo IS, Tasic L, De Souza AO (2013) Biogenic antimicrobial silver nanoparticles produced by fungi. Appl Microbiol Biotechnol 97(2):775–782

Rodríguez-Tobías H, Morales G, Ledezma A, Romero J, Grande D (2014) Novel antibacterial electrospun mats based on poly (D, L-lactide) nanofibers and zinc oxide nanoparticles. J Mater Sci 49(24):8373–8385

Rogers JV, Parkinson CV, Choi YW, Speshock JL, Hussain SM (2008) A preliminary assessment of silver nanoparticle inhibition of monkeypox virus plaque formation. Nanoscale Res Lett 3(4):129–133

Romainor ANB, Chin SF, Pang SC, Bilung LM (2014) Preparation and characterization of chitosan nanoparticles-doped cellulose films with antimicrobial property. J Nanomater 2014:130

Rosemary MJ, MacLaren I, Pradeep T (2006) Investigations of the antibacterial properties of ciprofloxacin@SiO_2. Langmuir 22(24):10125–10129. doi:10.1021/la061411h

Rujitanaroj P-O, Pimpha N, Supaphol P (2008) Wound-dressing materials with antibacterial activity from electrospun gelatin fiber mats containing silver nanoparticles. Polymer 49(21):4723–4732

Ruparelia JP, Chatterjee AK, Duttagupta SP, Mukherji S (2008) Strain specificity in antimicrobial activity of silver and copper nanoparticles. Acta Biomater 4(3):707–716

Rusen E, Mocanu A, Nistor LC, Dinescu A, Calinescu I, Mustatea G, Voicu SI, Andronescu C, Diacon A (2014) Design of antimicrobial membrane based on polymer colloids/multiwall carbon nanotubes hybrid material with silver nanoparticles. ACS Appl Mater Interfaces 6(20):17384–17393. doi:10.1021/am505024p

Russell A, Path F, Sl FP, Hugo W (1994) Antimicrobial activity and action of silver. Prog Med Chem 31:351

Sacco P, Travan A, Borgogna M, Paoletti S, Marsich E (2015) Silver-containing antimicrobial membrane based on chitosan-TPP hydrogel for the treatment of wounds. J Mater Sci Mater Med 26(3):128. doi:10.1007/s10856-015-5474-7

Salem W, Leitner DR, Zingl FG, Schratter G, Prassl R, Goessler W, Reidl J, Schild S (2015) Antibacterial activity of silver and zinc nanoparticles against *Vibrio cholerae* and enterotoxic *Escherichia coli*. Int J Med Microbiol 305(1):85–95

Salwiczek M, Qu Y, Gardiner J, Strugnell RA, Lithgow T, McLean KM, Thissen H (2014) Emerging rules for effective antimicrobial coatings. Trends Biotechnol 32(2):82–90

Sandri G, Bonferoni MC, Ferrari F, Rossi S, Aguzzi C, Mori M, Grisoli P, Cerezo P, Tenci M, Viseras C, Caramella C (2014) Montmorillonite-chitosan-silver sulfadiazine nanocomposites for topical treatment of chronic skin lesions: in vitro biocompatibility, antibacterial efficacy and gap closure cell motility properties. Carbohydr Polym 102:970–977. doi:10.1016/j.carbpol. 2013.10.029

Sastri VR (2013) Materials used in medical devices. In: Sastri V R (eds), Plastics in medical devices: properties, requirements, and applications. William Andrew Publishing, Oxford, pp 19–31. doi:10.1016/B978-1-4557-3201-2.00003-3

Sawant SN, Selvaraj V, Prabhawathi V, Doble M (2013) Antibiofilm properties of silver and gold incorporated PU, PCLm, PC and PMMA nanocomposites under two shear conditions. PLoS ONE 8(5):e63311. doi:10.1371/journal.pone.0063311

Schexnailder P, Schmidt G (2009) Nanocomposite polymer hydrogels. Colloid Polym Sci 287 (1):1–11

Schwartz VB, Thétiot F, Ritz S, Pütz S, Choritz L, Lappas A, Förch R, Landfester K, Jonas U (2012) Antibacterial surface coatings from zinc oxide nanoparticles embedded in poly (n-isopropylacrylamide) hydrogel surface layers. Adv Funct Mater 22(11):2376–2386

Selvaraj V, Alagar M (2007) Analytical detection and biological assay of antileukemic drug 5-fluorouracil using gold nanoparticles as probe. Int J Pharm 337(1–2):275–281. doi:10.1016/j. ijpharm.2006.12.027

Seo Y, Hwang J, Kim J, Jeong Y, Hwang MP, Choi J (2014) Antibacterial activity and cytotoxicity of multi-walled carbon nanotubes decorated with silver nanoparticles. Int J Nanomed 9:4621–4629. doi:10.2147/ijn.s69561

Shalumon K, Anulekha K, Nair SV, Nair S, Chennazhi K, Jayakumar R (2011) Sodium alginate/poly (vinyl alcohol)/nano ZnO composite nanofibers for antibacterial wound dressings. Int J Biol Macromol 49(3):247–254

Shameli K, Ahmad MB, Yunus WM, Ibrahim NA, Rahman RA, Jokar M, Darroudi M (2010) Silver/poly (lactic acid) nanocomposites: preparation, characterization, and antibacterial activity. Int J Nanomed 5:573–579

Shameli K, Bin Ahmad M, Zargar M, Yunus WM, Ibrahim NA, Shabanzadeh P, Moghaddam MG (2011) Synthesis and characterization of silver/montmorillonite/chitosan bionanocomposites by chemical reduction method and their antibacterial activity. Int J Nanomed 6:271–284. doi:10.2147/ijn.s16043

Shanthi S, Jayaseelan BD, Velusamy P, Vijayakumar S, Chih CT, Vaseeharan B (2016) Biosynthesis of silver nanoparticles using a probiotic Bacillus licheniformis Dahb1 and their antibiofilm activity and toxicity effects in Ceriodaphnia cornuta. Microb Pathog

Sharma D, Rajput J, Kaith B, Kaur M, Sharma S (2010) Synthesis of ZnO nanoparticles and study of their antibacterial and antifungal properties. Thin Solid Films 519(3):1224–1229

Sharma VK, Yngard RA, Lin Y (2009) Silver nanoparticles: green synthesis and their antimicrobial activities. Adv Colloid Interf 145(1):83–96

Sheikh FA, Kanjwal MA, Saran S, Chung W-J, Kim H (2011) Polyurethane nanofibers containing copper nanoparticles as future materials. Appl Surf Sci 257(7):3020–3026

Shi Z, Neoh K, Kang E, Wang W (2006) Antibacterial and mechanical properties of bone cement impregnated with chitosan nanoparticles. Biomaterials 27(11):2440–2449

Siegel JD, Rhinehart E, Jackson M, Chiarello L (2007) 2007 guideline for isolation precautions: preventing transmission of infectious agents in health care settings. Am J Infect Control 35(10): S65–S164

Simon T, Wu C-S, Liang J-C, Cheng C, Ko F-H (2016) Facile synthesis of a biocompatible silver nanoparticle derived tripeptide supramolecular hydrogel for antibacterial wound dressings. New J Chem

Singh M, Singh S, Prasad S, Gambhir I (2008) Nanotechnology in medicine and antibacterial effect of silver nanoparticles. Dig J Nanomater Biostruct 3(3):115–122

Son WK, Youk JH, Park WH (2006) Antimicrobial cellulose acetate nanofibers containing silver nanoparticles. Carbohydr Polym 65(4):430–434

Song J, Kim H, Jang Y, Jang J (2013) Enhanced antibacterial activity of silver/polyrhodanine-composite-decorated silica nanoparticles. ACS Appl Mater Interfaces 5 (22):11563–11568. doi:10.1021/am402310u

Speshock JL, Murdock RC, Braydich-Stolle LK, Schrand AM, Hussain SM (2010) Interaction of silver nanoparticles with Tacaribe virus. J Nanobiotechnol 8(1):19

Stewart PS, Costerton JW (2001) Antibiotic resistance of bacteria in biofilms. Lancet 358 (9276):135–138

Stickler DJ (2000) Biomaterials to prevent nosocomial infections: is silver the gold standard? Curr Opin Infect Dis 13(4):389–393

Stoecklin-Wasmer C, Rutjes A, Da Costa B, Salvi G, Jüni P, Sculean A (2013) Absorbable collagen membranes for periodontal regeneration: A systematic review. J Dent Res 92(9):773–781

Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ (2002) Metal oxide nanoparticles as bactericidal agents. Langmuir 18(17):6679–6686

Subbiahdoss G, da Silva Domingues JF, Kuijer R, van der Mei HC, Busscher HJ (2013) Bridging the gap between in vitro and in vivo evaluation of biomaterial-associated infections. Springer

Sudarshan N, Hoover D, Knorr D (1992) Antibacterial action of chitosan. Food Biotechnol 6 (3):257–272

Sudheesh Kumar P, Lakshmanan V-K, Anilkumar T, Ramya C, Reshmi P, Unnikrishnan A, Nair SV, Jayakumar R (2012) Flexible and microporous chitosan hydrogel/nano ZnO

composite bandages for wound dressing: in vitro and in vivo evaluation. ACS Appl Mater Interfaces 4(5):2618–2629

Sun L, Du Y, Fan L, Chen X, Yang J (2006) Preparation, characterization and antimicrobial activity of quaternized carboxymethyl chitosan and application as pulp-cap. Polymer 47 (6):1796–1804

Tavaria FK, Costa EM, Gens EJ, Malcata FX, Pintado ME (2013) Influence of abiotic factors on the antimicrobial activity of chitosan. J Dermatol 40(12):1014–1019

Tian T, Shi X, Cheng L, Luo Y, Dong Z, Gong H, Xu L, Zhong Z, Peng R, Liu Z (2014) Graphene-based nanocomposite as an effective, multifunctional, and recyclable antibacterial agent. ACS Appl Mater Interfaces 6(11):8542–8548. doi:10.1021/am5022914

Travan A, Pelillo C, Donati I, Marsich E, Benincasa M, Scarpa T, Semeraro S, Turco G, Gennaro R, Paoletti S (2009) Non-cytotoxic silver nanoparticle-polysaccharide nanocomposites with antimicrobial activity. Biomacromolecules 10(6):1429–1435. doi:10.1021/bm900039x

Ul-Islam M, Khattak WA, Ullah MW, Khan S, Park JK (2014) Synthesis of regenerated bacterial cellulose-zinc oxide nanocomposite films for biomedical applications. Cellulose 21(1):433–447

Usman SM (2013) Synthesis, characterization, and antimicrobial properties of copper nanoparticles. Int J Nanomedicine 8:4467–4479

Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella MF, Rejeski D, Hull MS (2015) Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. Beilstein J Nanotechnol 6:1769–1780. doi:10.3762/bjnano.6.181

Varaprasad K, Mohan YM, Ravindra S, Reddy NN, Vimala K, Monika K, Sreedhar B, Raju KM (2010) Hydrogel–silver nanoparticle composites: A new generation of antimicrobials. J Appl Polym Sci 115(2):1199–1207

Vargas-Villagran H, Romo-Uribe A, Teran-Salgado E, Dominguez-Diaz M, Flores A (2014) Electrospun polylactic acid non-woven mats incorporating silver nanoparticles. Polym Bull 71 (9):2437–2452. doi:10.1007/s00289-014-1200-8

Vigneshwaran N, Kumar S, Kathe A, Varadarajan P, Prasad V (2006) Functional finishing of cotton fabrics using zinc oxide–soluble starch nanocomposites. Nanotechnology 17(20):5087

Vijayakumar PS, Prasad BL (2009) Intracellular biogenic silver nanoparticles for the generation of carbon supported antiviral and sustained bactericidal agents. Langmuir 25(19):11741–11747. doi:10.1021/la901024p

Vimala K, Yallapu MM, Varaprasad K, Reddy NN, Ravindra S, Naidu NS, Raju KM (2011) Fabrication of curcumin encapsulated chitosan-PVA silver nanocomposite films for improved antimicrobial activity. J Biomater Nanobiotechnol 2(01):55

Vlad S, Tanase C, Macocinschi D, Ciobanu C, Balaes T, Filip D, Gostin I, Gradinaru L (2012) Antifungal behaviour of polyurethane membranes with zinc oxide nanoparticles. Dig J Nanomater Bios 7:51–58

Wang N, Hu B, Chen ML, Wang JH (2015) Polyethylenimine mediated silver nanoparticle-decorated magnetic graphene as a promising photothermal antibacterial agent. Nanotechnology 26(19):195703. doi:10.1088/0957-4484/26/19/195703

Wang Z, Liu S, Ma J, Qu G, Wang X, Yu S, He J, Liu J, Xia T, Jiang GB (2013) Silver nanoparticles induced RNA polymerase-silver binding and RNA transcription inhibition in erythroid progenitor cells. ACS Nano 7(5):4171–4186. doi:10.1021/nn400594s

Wang ZL (2004) Zinc oxide nanostructures: growth, properties and applications. J Phys: Condens Matter 16(25):R829

Wei Y, Chen S, Kowalczyk B, Huda S, Gray TP, Grzybowski BA (2010) Synthesis of stable, low-dispersity copper nanoparticles and nanorods and their antifungal and catalytic properties. J Phys Chem C 114(37):15612–15616

Wildgoose GG, Banks CE, Compton RG (2006) Metal nanoparticles and related materials supported on carbon nanotubes: methods and applications. Small 2(2):182–193

Xu J, Ji W, Shen Z, Tang S, Ye X, Jia D, Xin X (1999) Preparation and characterization of CuO nanocrystals. J Solid State Chem 147(2):516–519

Yadollahi M, Gholamali I, Namazi H, Aghazadeh M (2015) Synthesis and characterization of antibacterial carboxymethylcellulose/CuO bio-nanocomposite hydrogels. Int J Biol Macromol 73:109–114

Yallappa S, Manjanna J, Dhananjaya B, Vishwanatha U, Ravishankar B, Gururaj H, Niranjana P, Hungund B (2015) Phytochemically functionalized Cu and Ag nanoparticles embedded in MWCNTs for enhanced antimicrobial and anticancer properties. Nano-Micro Lett 1–11

Yamamoto O (2001) Influence of particle size on the antibacterial activity of zinc oxide. Int J Inorg Mater 3(7):643–646

Yien L, Zin NM, Sarwar A, Katas H (2012) Antifungal activity of chitosan nanoparticles and correlation with their physical properties. Int J Biomater

Yu B, Leung KM, Guo Q, Lau WM, Yang J (2011) Synthesis of Ag–TiO$_2$ composite nano thin film for antimicrobial application. Nanotechnology 22(11):115603

Yu L, Zhang Y, Zhang B, Liu J (2014) Enhanced antibacterial activity of silver nanoparticles/halloysite nanotubes/graphene nanocomposites with sandwich-like structure. Sci Rep 4:4551. doi:10.1038/srep04551

Zaat S, Broekhuizen C, Riool M (2010) Host tissue as a niche for biomaterial-associated infection. Future Microbiol 5(8):1149–1151

Zahran MK, Ahmed HB, El-Rafie MH (2014) Surface modification of cotton fabrics for antibacterial application by coating with AgNPs-alginate composite. Carbohydr Polym 108:145–152. doi:10.1016/j.carbpol.2014.03.005

Zaporojtchenko V, Podschun R, Schürmann U, Kulkarni A, Faupel F (2006) Physico-chemical and antimicrobial properties of co-sputtered Ag–Au/PTFE nanocomposite coatings. Nanotechnology 17(19):4904

Zeng F, Hou C, Wu S, Liu X, Tong Z, Yu S (2007) Silver nanoparticles directly formed on natural macroporous matrix and their anti-microbial activities. Nanotechnology 18(5):055605

Zhang H, Wu M, Sen A (2012a) Silver nanoparticle antimicrobials and related materials. In: Cioffi N, Rai M (eds) Nano-antimicrobials: progress and prospects. Springer, Berlin, Heidelberg, pp 3–45. doi:10.1007/978-3-642-24428-5

Zhang K, Kim YK, Cadenaro M, Bryan TE, Sidow SJ, Loushine RJ, J-q Ling, Pashley DH, Tay FR (2010) Effects of different exposure times and concentrations of sodium hypochlorite/ethylenediaminetetraacetic acid on the structural integrity of mineralized dentin. J Endod 36(1):105–109

Zhang R, Xue M, Yang J, Tan T (2012b) A novel injectable and in situ crosslinked hydrogel based on hyaluronic acid and α, β-polyaspartylhydrazide. J Appl Polym Sci 125(2):1116–1126

Zheng Y, Cai C, Zhang F, Monty J, Linhardt RJ, Simmons TJ (2016) Can natural fibers be a silver bullet? Antibacterial cellulose fibers through the covalent bonding of silver nanoparticles to electrospun fibers. Nanotechnology 27(5):055102

Zhou B, Li Y, Deng H, Hu Y, Li B (2014) Antibacterial multilayer films fabricated by layer-by-layer immobilizing lysozyme and gold nanoparticles on nanofibers. Colloids Surf B Biointerfaces 116:432–438. doi:10.1016/j.colsurfb.2014.01.016

Zhu J, Marchant RE (2011) Design properties of hydrogel tissue-engineering scaffolds. Expert Rev Med Devices 8(5):607–626