Chapter 2

Literature Review

The effect of incorporation of various antibacterial agents as secondary phases, tailored structures (micro to nano-sized) and techniques such as, application of external stimuli (electrical and magnetic fields) for improving the antibacterial response has been reviewed. In addition, the effect of surface polarization and external electrical stimuli on cytocompatibility of various piezoelectric bioceramics has also been discussed.

2.1 Introduction

The development of suitable prosthetic implants for human health care is one of the stimulating areas in orthopedics [1]. Despite of significant progress in orthopedics, the research is in continuous thrust to address the number of existing issues which lead to the failure of prosthetic implants [2]. The bacterial infections at implant site have been recognized as one of the serious concerns [3]. Although, both, gram positive and gram negative bacteria are responsible for the microbial infection, maximum infection (~ 65%) is caused due to gram-positive cocci [*Staphylococcus aureus (S. aureus)* and *Staphylococcus epidermidis (S. epidermidis)*] [4]. However, gram negative bacteria [*Escherichia coli (E. coli)* and *Pseudomonas aeruginosa (P. aerugenosa)*] cause about 11 % of the bacterial infections in orthopedic implants [5]. Commonly used orthopedic implant materials such as stainless steel, cobalt-chromium, hydroxyapatite, bioglasses, tricalcium phosphate, and polyamide polymethyl methacrylate etc., are susceptible towards microbial infections [6,7]. To overcome the problem of implant associated infections, various techniques such as, modifications in surface chemistry, the compositional variation of prosthetic implants as well as the application of external stimulants are in continuous thrust. Recently, various piezobioceramics have been developed as prospective materials for orthopedic applications due to piezoelectric nature

of bone [8,9]. The piezoelectric $Na_{0.5}K_{0.5}NbO_3$ (NKN) and $BaTiO_3$ demonstrate antibacterial response [10,11]. On the other hand, piezoelectric ceramics enhance the bioactive performance as well as cellular response [12]. The addition of piezoelectric material as a secondary phase in HA matrix enhances the proliferation of human osteoblast cells [13].This chapter reviews various biomaterials and techniques for improving the antibacterial response. In addition, the cellular response of tailored biomaterials has also been reviewed.

2.2. Antimicrobial response of tailored biocomposites

In this section, the effect of addition of various antibacterial agents as secondary phases on antibacterial response of composite systems has been discussed along with their mechanisms.

2.2.1. Effect of addition of Ag on antibacterial response of biocomposites

Ag possesses a good antibacterial property, which is used as an antibacterial agent since ancient time. [14,15]. It has been reported that the growth of *E. coli* bacteria is inhibited by ~ 62 %, 88 %, and 100 %, with addition of 10, 50, and 100 μg/cm^3 Ag in HA, respectively, after incubation of 24 h [16]. In another study, it has been observed that the addition of 1000 ppm of Ag as secondary phase in HA increases the diameter of inhibition zone by about 41 and 46 % for *S. aureus* and *E. coli* bacteria, respectively. Pandey et al. [17] reported that the viability of *E. coli* and *S. aureus* bacteria reduces by ~ 61 % and 53 %, respectively, with the addition of 2.5 wt. % Ag in HA matrix.

The formation of reactive oxygen species (ROS) as well as Ag^+ ions are responsible for the antibacterial response of Ag based composites [18]. The Ag^+ ions are produced by its oxidation, these ions are highly reactive with bacteria [19]. The produced Ag^+ ions can bind to DNA, RNA and proteins, present in bacterial cells to inhibit their growth [33]. The Ag^+ ions distorted the structure of bacterial cells. On the other hand, the ROS such

as, superoxide's, hydroxyl radicals, hydrogen peroxide radicals etc., interact with the cell membranes of bacteria and damage them [20]. Fig. 2.1 demonstrates consequences of the interaction of silver ions with the cell wall [21].

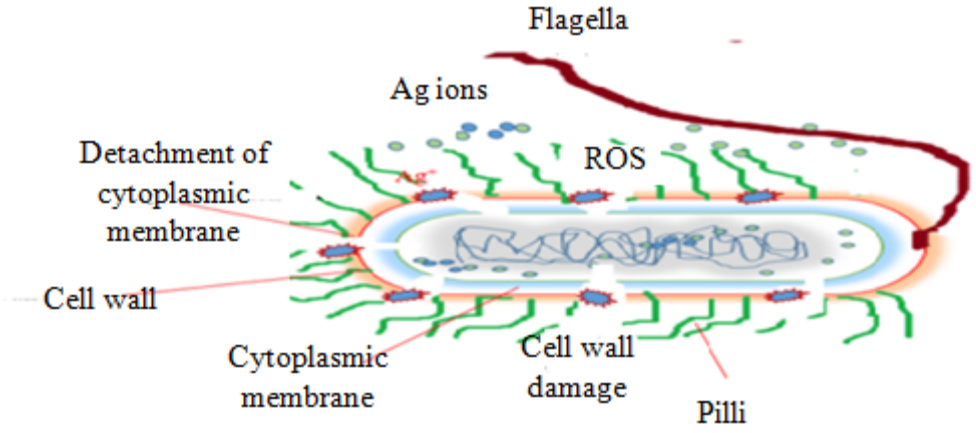

Fig. 2.1. Schematic illustrating the mechanism of antibacterial response due to silver ions. [Reproduced with permission from ref. (21): Copyright (2018), American Chemical Society].

2.2.2. Effect of addition of TiO_2 on antibacterial response of biocomposites

TiO_2 is a non-toxic metal oxide with good antibacterial property [22]. Recently, TiO_2 has been used as secondary phase in base materials (HA) for improving the antibacterial response of develpoed composite system [32]. The diameter of inhibition zone for HA-60 wt. % TiO_2 has been increased by ~ 31 and 36 % as compared to control sample, while cultured with *S. aureus* and *E. coli* bacteria, respectively [23]. It has been reported that colony formation unit (CFU/ml) of *bacillus subtilis* bacteria decreases by ~ 99.90 % on AgCl/TiO_2 composite, while exposed to visible light for 3 h. However, for *pseudomonas aeruginosa* bacteria biofilm formation has been reduces to 57 % for the same substrate and similar exposure conditions [24].

It has been demonstrated that TiO_2 oxidises in presence of visible light and produces free radicals such as hydrogen peroxide (H_2O_2), superoxide's, peroxides (O_2^{-2}), hydroxyl

radicals (OH^-) etc. These free radicals are responsible for antibacterial response of TiO_2 based bio-composite systems [25,26]. TiO_2 shows photo-catalytic behavior under UV light, however, surface modification of TiO_2 (with iodine) enhances the visible absorbance [27]. In presence of visible light, TiO_2 surface, irradiated by photon energy, produces electron (e^-) and hole (h^+) pairs at conduction and valence bands, respectively [Eq.(2.1)]. The generated electrons and holes have strong oxidizing power. These electrons and holes may recombine and produce ROS as shown in equations (2.2) and (2.3), these radicals can damage the outer layer of bacteria. [28]. Fig.2.2 schematically represents the mechanisms, responsible for the antibacterial activity of TiO_2 based bio-composites [29].

$$\mathbf{TiO_2} + \mathbf{h\nu} \rightarrow \mathbf{e^-} + \mathbf{h^+} \qquad (\mathbf{2.1})$$

$$\mathbf{O_2} + \mathbf{e^-} \rightarrow \mathbf{O_2^-} \qquad (\mathbf{2.2})$$

$$\mathbf{H_2O} + \mathbf{h^+} \rightarrow \mathbf{(OH)^-} + \mathbf{h^+} \qquad (\mathbf{2.3})$$

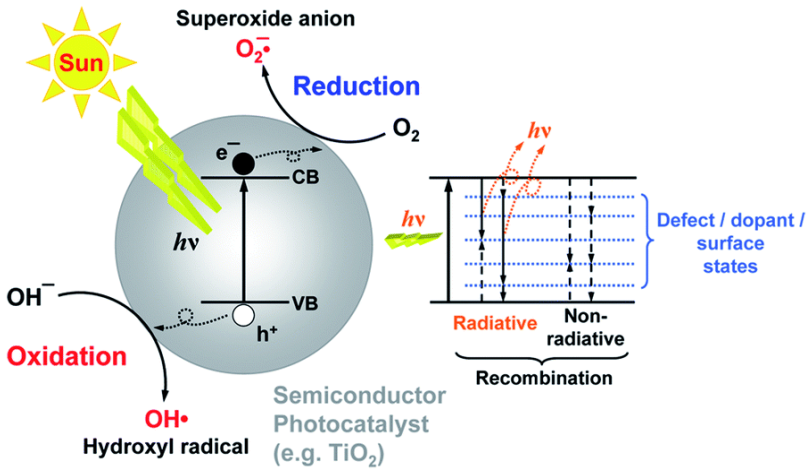

Fig. 2.2. Schematic diagram of proposed mechanisms for antibacterial response of TiO_2 [Reproduced with permission from ref (29): Copyright (2014), Royal Society of Chemistry].

2.2.3. Effect of addition of ZnO on antibacterial response of biocomposites

In general, few of the metal oxides such as, ZnO, MgO, CuO etc., illustrate antibacterial effect [30]. In recent years, number of biocomposites with ZnO is recognized for their potential biocompatibility, biodegradability and antibacterial nature [31, 32]. The colonies of *E. coli* bacteria reduce by ~ 13 % to 50.45 % for HA- ZnO biocomposite while increasing the ZnO content by1.5 % to 30 wt. %, after incubation of 4 h. However, the incubation of 24 h reduces the colonies of *E. coli* bacteria by ~ 26 % to 60 % for similar material composition and bacteria [33]. The incorporation of 25 wt. % ZnO in HA reduces the biofilm formation by ~ 98 % and 99 % while cultured with *S. aureus* and *E. coli* bacteria, respectively [34].

The release Zn^{2+} ions and generation of ROS are responsible for antibacterial response of ZnO based bio-composites [35]. The ROS and Zn^{2+} ions are toxic in nature which can destroy the outer membranes of bacterial cells [36]. The ROS reacts with lipid layer of bacterial cell (for gram-negative bacteria) and destroy cell wall structure and consequently, the death of bacterial cell [37]. On the other hand, Zn^{2+} ions diffuse in the cell walls (for gram-positive bacteria) and disrupt the amino acid metabolism and enzymes which leads to cell death [38-40]. Fig. 2.3 illustrates the mechanisms, responsible for antibacterial response of ZnO containing composites [41].

The effect of incorporation of various antibacterial agents on antibacterial response of HA has been summarized in Table 2.1.

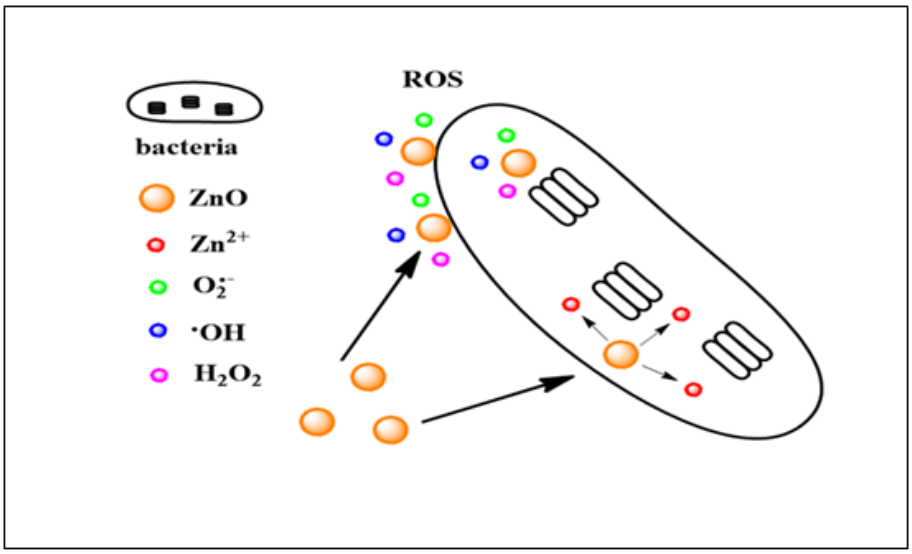

Fig. 2.3. Schematic presentation of mechanisms for antibacterial response of ZnO based biocomposites. [Reproduced from ref. (41): open access]

Table 2.1 Effect of addition of secondary phase on antibacterial response of HA

S.N.	Material Composition		Pathogen (Incubation Time :12 h)	Effects	Ref.
	Base material	**Secondary phase**			
1.	HA	Ag	*S. mutans*	Diameter of inhibition zone 6.33 mm (for 5wt.% Ag in HA) 8.66 mm (for 10 wt.% Ag in HA)	6
			S. anguinis	7.66 mm (for 5wt.% Ag in HA) 9.66 mm (for 10 wt.% Ag in HA)	
			L. acidophilus	5.66 mm (for 5wt.% Ag in HA) 7.66 mm (for 10 wt.% Ag in HA)	
2.	HA	1000 ppm of Ag	*S. aureus*	17 mm	16
			Pneumococcus	17.5 mm	
			E. coli	18 mm	

3.	HA	TiO_2 (10-60 wt. %)	*E. coli*	14 mm (60 wt.% TiO_2)	33
			S. aureus	21 mm (60 wt.% TiO_2)	
4.	HA	ZnO (1.5-30 wt. %)	*E. coli*	Bacterial colonies decreased by 35% (for 10 wt.% ZnO) 60 % (for 30 wt. % ZnO)	34
5.	HA	-	*E. coli*	14.7 mm	42
			S. epidermidis	10 mm	
6.	HA	Collagen/poly lactic acid with antibiotic vancomycin (VCM/nHAC/PLA)	*S. aureus* (72 h)	21.07 mm	43
	Fluro Hydroxy apatite (FHA)	FHA -40Zr-20Ce composite. (Compositions are in wt. %)	*S. aureus*	42 mm	
7.	ZnO	Cr	*E. coli*	10-13 mm	44
			S. aureus	15-17 mm	
	ZnO	-	*S. aureus*	6-9 mm	
			E. coli	No inhibition zone found	
8.	HA	AgCl/ TiO_2	*E. coli* (for 3 h)	Colony forming unit (CFU) decreases by 99.10 % when exposed to visible light irradiation	45
9.	FHA	Zr- Ce (40	*E. coli*	Diameter of inhibition zone	46

		wt.% Zr & 20 % Ce)		37 mm	
			S. aureus	42 mm	
10.	HA	0.2 wt. % Zn, 0.25 wt. % Ag, and 0.025 wt. % Au	*E. coli*	15 mm	47
			S. aureus	16 mm	
			Bacillus cereus	14 mm	

2.3. Effect of nanostructured materials on antibacterial response

Nanomaterials gained a good attention in biomedical field due to their higher surface area, catalytic activity, dissolution rate etc. [48]. They demonstrate different properties than their respective counter bulk materials. The nano structured Fe, TiO_2, and ZnO etc. possess good antibacterial properties as compared to their bulk counterpart [49]. In another study, it has been found that Fe_2O_3 nanoparticles show antibacterial activity whereas such response in its bulk counterpart is not observed [50].

2.3.1. Antibacterial response of zinc oxide (ZnO) nanoparticles

The nanostructured ZnO have been used in biomedical fields for bio-sensing, imaging, drug delivery and bio-implant applications [51]. The nano structured Zn demonstrates excellent antibacterial properties [52].The antibacterial activity of ZnO nanoparticles increases with reducing the particle size, even in nano-size range [53]. The content of ZnO in the solution/base materials is the influential factor for improving the antibacterial property [54]. The colonies of *E. coli* bacteria on ZnO nanoparticles (~ 40 nm) reduced by ~ 86 % after incubation of 24 h [54]. However, for *K. pneumonia,* the bacterial colonies have been decreased by ~ 75.38 % at the concentration of 0.75 mM for similar material and incubation period [47]. The antibacterial activity of Ti-ZnO nanorods increased by 65.5 % and 55.4 %, against *S. aureus* and *E. coli* bacteria, respectively, after incubation of 12 h [55]. However, the hybrid ZnO/polydopamine (PDA) / arginine-

glycine-aspartic acid crystine (RGDC) nanorods have found to be ~ 72.2 % and 74.7 % efficient against *S.aureus* and *E. coli* bacteria, respectively, while incubated with similar condition [56].

The release of zinc ions, owing to the dissolution of ZnO as well as formation of ROS are the suggested mechanisms responsible for the antibacterial activity of ZnO nanoparticles [57]. The nanosized ZnO particulates penetrate the cell wall easily and produces comparatively higher amount of ROS than that of bulk-sized ZnO [58]. The point defects in nanoparticles increase the abrasive nature of its surface which injured the bacterial cells [59]. However, surface defects, produced by the partial dissolution of nanosized ZnO in water, result in an uneven surface texture that damage the bacterial cells [60]. In another study, it has been demonstrated that physical interaction of ZnO nanoparticles with the bacterial cells also play an important role in bactericidal effects [61]. The strong electrostatic interaction takes place between bacterial cell surface (negative charge) and Zn^{2+} ions [62]. As a result, ZnO nanoparticles (≤ 10 nm) accumulate at the outer layer of plasma membranes and neutralize the surface charge of bacterial cells [56]. This leads to increase in surface tension, membrane permeability, change in membrane texture, morphology, and generation of oxidative stress which results in bacterial cell death [63]. The ZnO nanoparticles (≤ 10 nm) passage through the cytoplasm membrane and accumulate inside bacterial cells (via particle internalization) which damage the intercellular constituents, including nucleic acids etc. [64,65]. The possible mechanisms for antibacterial response of ZnO nanoparticles are illustrated in Fig. 2.4 [62].

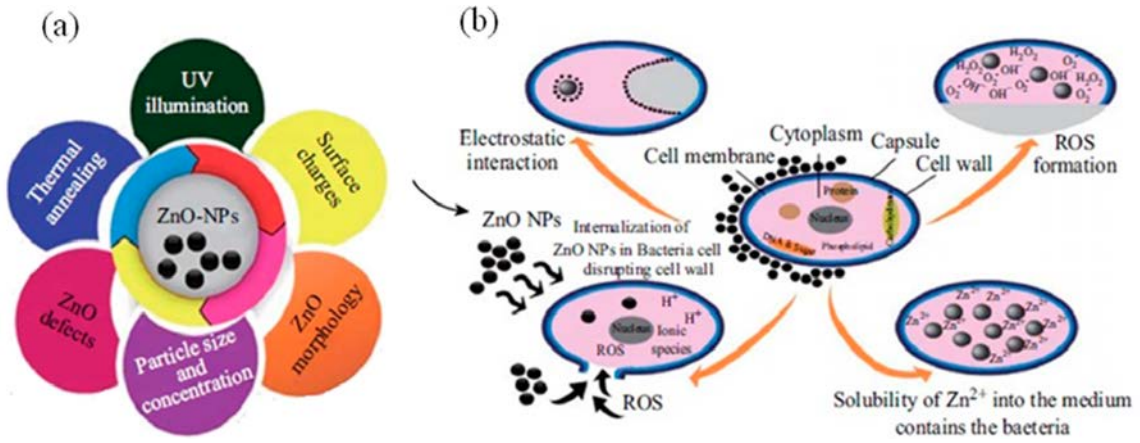

Fig. 2.4. Schematic illustration represents (a) various modes of antibacterial response due to ZnO nanoparticles and (b) possible mechanisms for ZnO nanoparticles mediated antibacterial response.[Reproduced from ref (63): open access].

2.3.2. Antibacterial response of silver (Ag) nanoparticles

Ag is used as an antibacterial agent since ancient time due to its inherent antibiotic properties. [66]. The nano-structured Ag has better antibacterial properties as compared to their bulk counterpart. [67]. Recently, Ag nanoparticles have been introduced as a quite appealing choice for the development of new generation bactericidal material [68]. The antibacterial response of Ag nanoparticles depends upon particle size as well as shape [69,. In addition the concentration of Ag in growth medium and exposure time are also important factors for improving the antibacterial response [70]. In another study, it has been reported that triangular shape Ag nanoparticles has better antibacterial response (as compared to other shapes) even at low concentrations [71]. The growth of *E. coli* bacteria in Luria-Bertina growth media has been reduced by about 70 % and 100 %, respectively, while increasing the concentration of Ag nanoparticle (~12 nm) from 10 $\mu g/cm^3$ to 60 $\mu g/cm^3$, after incubation of 24 h at 37 ºC [72]. However, 100 µg/ml concentration of Ag nanoparticle inhibit the growth of *S. aureus* bacteria completely [73]. The viability of *E. coli* and *S. aureus* bacteria reduced to 96.3 % and 99.4 %

respectively, while cultured on graphene oxide and silver nanoparticles composite [74]. The antibacterial response of nanoparticles against various pathogens is summarized in Table 2.2.

The mechanism, responsible for the antibacterial response of Ag nanoparticles is very complicated. Catalina et al. [75] proposed three mechanisms for the antibacterial response of Ag nanoparticles. (i) The release of silver ions that disrupts ATP production and DNA replication. (ii) Formation of reactive oxygen species (ROS) and (iii) Direct damage of cell membrane by silver nanoparticles due to its inherent antibacterial property.

Ag nanoparticles interact with bacterial cells and penetrate inside (particle internalization) through pits and holes which disrupt the cell walls [Fig. 2.5 (a and b)]. It has been hypothesized that Ag^+ ions are released by dissolving silver nanoparticles in growth media (Eqs. 2.4 and 2.5). [76].

$$\mathbf{2Ag + H_2O_2 + 2H^+ \rightarrow 2Ag^+ + 2H_2O, E^0 = 0.17V} \qquad (\mathbf{2.4})$$

$$\mathbf{4Ag + O_2 + H_2O \rightarrow 4Ag^+ + 4OH^-} \qquad (\mathbf{2.5})$$

Choi et al. [77] reported that silver nanoparticles oxidize in presence of oxygen and release silver ions as well as produce hydroxyl radicals. These silver ions interact with peptidoglycan layer in the bacterial membrane and consequently, disrupt the cell metabolic processes. This mechanism is dominated in case of gram-positive bacteria, owing to their thick peptidoglycan layers as compared to gram-negative bacteria [78]. In addition, the formation of ROS also leads to the death of bacterial cells [79]. Fig. 2.5 (c and d) represents the schematic diagrams of mechanisms, responsible for the generation of Ag ions [62].

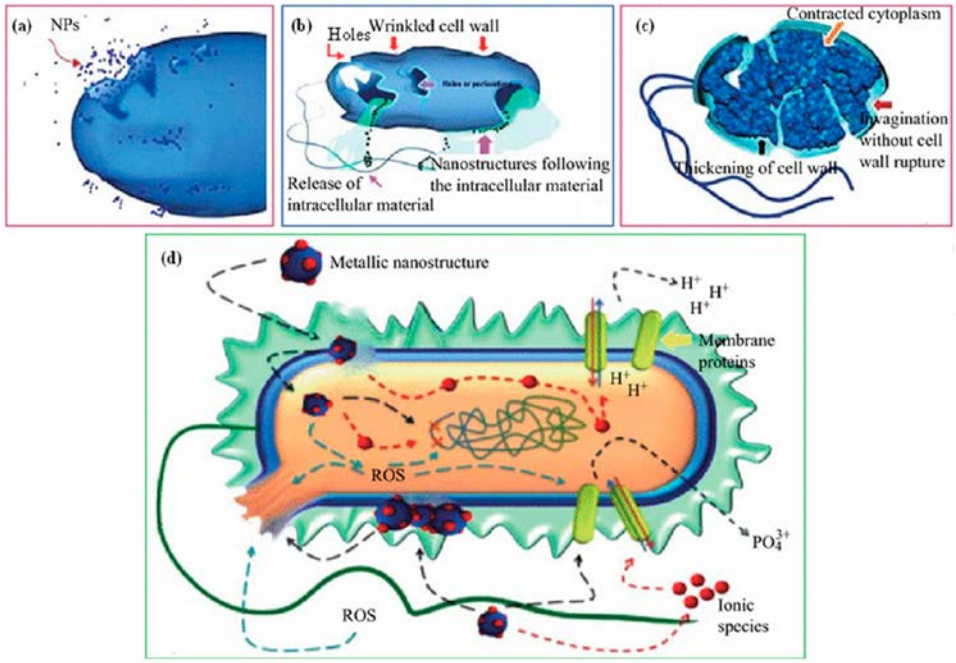

Fig. 2.5. Schematic illustrating (a) nanoparticles internalization into the cell wall, (b) disruption of cell wall and release of intracellular materials, (c) thickenings of cell wall and release of cytoplasm, and (d) mechanisms of dissolution of nanoparticles in the bacterial membrane, release of metal ions, ROS, and disruption of DNA. [Reproduced from ref (63): open access]

2.3.3. Antibacterial response of copper and copper oxide nanoparticles

Recently, nano-sized Cu and CuO attracted attention due to their better antibacterial response as compared to its bulk counterpart [80]. However, the application of Cu nanoparticles is limited due to its rapid oxidation during air exposure [81]. The antibacterial response of Cu and CuO nanoparticles depends upon on the concentration of Cu in growth media for both, gram-positive and gram-negative bacteria [82]. The diameter of inhibition zone for *E. coli* and *S. aureus* bacteria has been increased by ~ 43 % and 48 % while increasing the concentration of Cu nanoparticles (~20 nm) by 25 % respectively, after incubation of 24 h [83]. In another study, it has been demonstrated that viability of *E. coli* bacteria reduced by ~ 48 %, after incubation of 12 h, while cultured on Cu nanoparticles (~ 20 nm) [84]. Carboxyl methyl chitosan (CMC) alginate

(Alg) scaffolds with Cu nanoparticles (CMC/Alg/Cu) shows better antibacterial response against *S. aureus* bacteria as compared to carboxyl methyl chitosan alginate (CMC/Alg) after incubation of 2 and 4 days [85]. Table 2.2 summarizes the antibacterial response of CuO nanoparticles against various pathogens.

It is assumed that Cu ions, produced by Cu nanoparticles, interact with sulfydryl groups that destroy the bacterial cells, enzymes and proteins [86]. The interaction of bacterial cells with Cu nanoparticles affects the membrane integrity due to decrease in transmembrane potential [87]. The nano-structured CuO produces more Cu^{+} ions and ROS as compared to its bulk form [88]. It has been reported that CuO nanoparticles are more toxic than bulk CuO due to the solubility of CuO nanoparticles and release of Cu ions [89]. The released Cu ions interact with the bacterial cell membrane and generate oxidative stress, which damage the outer cell membranes and proteins of bacteria [90]. ROS production starts with the reduction of O_2 and synthesis of sulphoxide anion. ROS interact with the cellular membranes that lead to the damage of DNA, enzymes and other proteins [91]. Fig. 2.6 demonstrates the mechanisms responsible for antibacterial response of Cu nanoparticles [87].

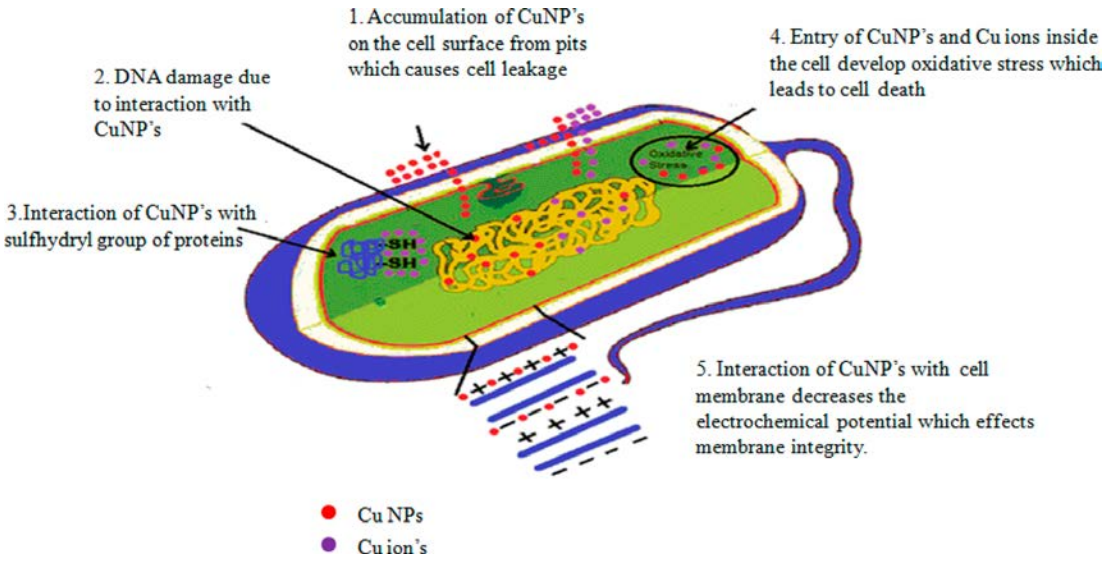

Fig. 2.6. Schematic illustration of antibacterial response due to Cu nanoparticles.[Reproduced from ref (87): open access]

2.3.4. Antibacterial response of iron oxide nanoparticles

Iron oxide has been widely used biomedical field due to its reasonable antibacterial properties [92]. The antibacterial response of iron oxide nanoparticles also depends upon the particle size, concentration of iron oxide in growth media as well as exposure time [93]. It has been demonstrated that the size of inhibition zone increases by 61.53 %, 60.17 % and 55.17 % for *E. coli, P. aerugenosa*, and *S. aureus* bacteria, respectively while increasing the concentration of iron oxide nanoparticles (~10 nm) from 0.01 to 0.15 mg/ml in growth media, after incubation of 24 h [94]. However, the size of inhibition zone has increased by 21.42 % and 29.41 % against *E. coli* and *S. aureus* bacteria, respectively when standard antibiotics neomycin (30 µg/disc) was used with iron oxide nanoparticles (~ 66 nm and concentration 50 mg/ml) and incubated for 24 h [58]. The CFU of *S. aureus* bacteria for porous iron-carboxylate metal-organic framework [MOF-53 (Fe)] nanoparticles decreases by 16.26 % while concentration of [MOF-53(Fe)] Fe ions increases to 87.5 %, after incubation of 24 h However, CFU reduction for vancomycin loaded MOF-53 (Van) decreases by 99 % while concentration

of vancomycin increases to 87.7 % for similar bacteria and incubation conditions [95]. The antibacterial response of iron-oxide nanoparticles with various pathogens are summarized in Table 2.2.

The mechanisms responsible for the antibacterial response of iron oxide nanoparticles are similar to other metal oxides such as ZnO, TiO_2 and CuO etc. [96]. However, the primary cause of antibacterial response of iron oxide is oxidative stresses, produced via the formation of ROS which includes superoxide's, hydroxyl radicals, hydrogen peroxide and singlet oxygen molecules. ROS can be generated by Fenton reaction as [97],

$$\mathbf{Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\cdot} + OH^{-}} \qquad (\mathbf{2.6})$$

$$\mathbf{Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO^{\cdot} + H^{+}} \qquad (\mathbf{2.7})$$

ROS is toxic in nature which damages the proteins and DNA in the bacteria [98].

2.3.5. Antibacterial response of magnesium oxide (MgO) nanoparticles

Magnesium (Mg) is a quite appealing biodegradable implant [99,100]. The nano-structured MgO exhibit anti-oxidation and anti-inflammatory properties, due to which, it is potentially used as antibacterial agent [101]. In general, nano-sized inorganic metal oxides have potential to kill bacteria because they react with intracellular oxygen and produce ROS [102]. The antibacterial response of MgO nanoparticles depends upon the annealing temperature, concentration of MgO and incubation time [103]. It has been reported that viability of *E. coli* bacteria decreases by 83 % and 95 % with the exposure of MgO nanoparticles (~ 8 nm) for 1 and 4 h, respectively. Similarly, for *S. aureus* bacteria, it has been reduced by 82 % and 99.9 %, respectively, for the similar particles and exposure conditions [104]. The antibacterial efficiency of Mg alloy (AZ 31) doped with silver nanoparticles and polymethyl trimethoxysilane (PMTMS) against *S. aureus* bacteria increases by 85 %, after incubation of 16 h. However, it is increased by 98.40 %

for silver nanoparticles dipped in Polyethylenimine (PEI) solution for similar bacteria and incubation condition [105]. Sundrarajan et al. [106] reported that the diameter of inhibition zone for nano-structured MgO samples decreases with increasing the annealing temperature.The inhibition zone for *S. aureus* and *E. coli* bacteria has been found to be 23 and 21 mm on MgO nanoparticles (~ 4.6 nm), annealed at 300 °C.

Despite of a number of proposed mechanisms, the exact mechanism responsible for the antibacterial response of MgO nanoparticles is still unclear [107]. It has been hypothesized that oxidation and reduction reactions occur at the surface of MgO nanoparticles which produce ROS [108]. Peter et al. [109] suggested that antibacterial response of MgO nanoparticles is due to the presence of defects (oxygen vacancies) on the surface of nanoparticles. MgO nanoparticles hydrated to form Mg $(OH)_2$ at the surface that leads to the generation of electrons and holes. The oxygen molecules present on the surface react with electrons and produce superoxide's which leads to the destruction of cells. Due to hygroscopic nature, MgO nanoparticles absorb moisture and form a thin water layer around it. The local pH of this thin layer is higher than its equilibrium value in the growth media. When nanoparticles come in contact with bacterial cells, the higher value of pH damage the membrane which results in cell death [110,111]. Therefore, MgO nanoparticles induced pH variation of the growth media is also one of the possible reasons for the antibacterial response of MgO. Fig. 2.7 represents the mechanisms for antibacterial response of MgO nanoparticles [112].

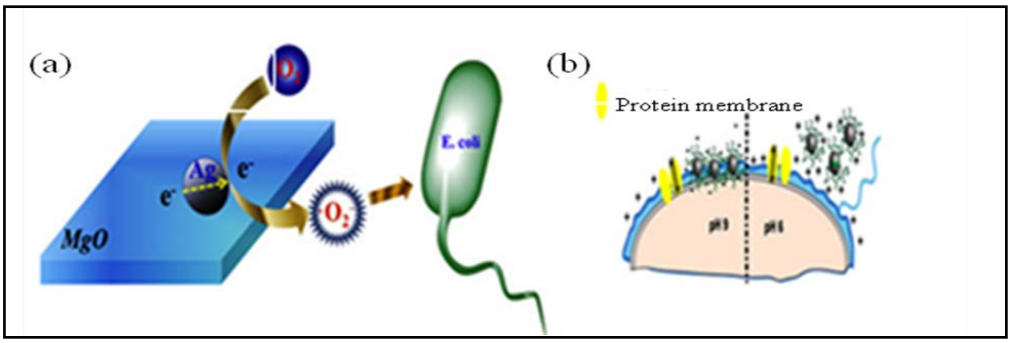

Fig. 2.7. Schematic diagram representing the (a) interaction of bacterial cells with MgO and generation of ROS and (b) protein disruption with pH variation. [Reproduced from ref (112): open access]

2.3.6. Antibacterial response of gold (Au) nanoparticles

Gold nanoparticles have widely used in various biomedical applications such as imaging, gene therapy, water remediation, drug delivery, as well as antibacterial agents [113]. The size of inhibition zone increases by 33.33 % and 29.31 % against *S. aureus* and *E. coli* bacteria respectively, for Au nanoparticles with the concentration of 50 µg/ml in media after incubation of 24 h. However, size of inhibition zone decreases by 29.41 % against *Basilus subtilus* bacteria with the concentration of 75 µg/ml for similar particles and exposure time [114]. Table 2.2. summarizes the antibacterial response of gold nanoparticles with various bacterial cells.

There are basically two mechanisms, which are responsible for the antibacterial activity of Au nanoparticles. One of them is by collapsing the membrane potential that inhibits ATPase activity and secondly by inhibiting the subunit of ribosome for tRNA binding [115]. In contrast to the earlier discussed antibacterial agents, Au nanoparticles showed ROS independent antibacterial response [116]. Fig. 2.8 demonstrates the mechanisms for antibacterial response of Au nanoparticles [117].

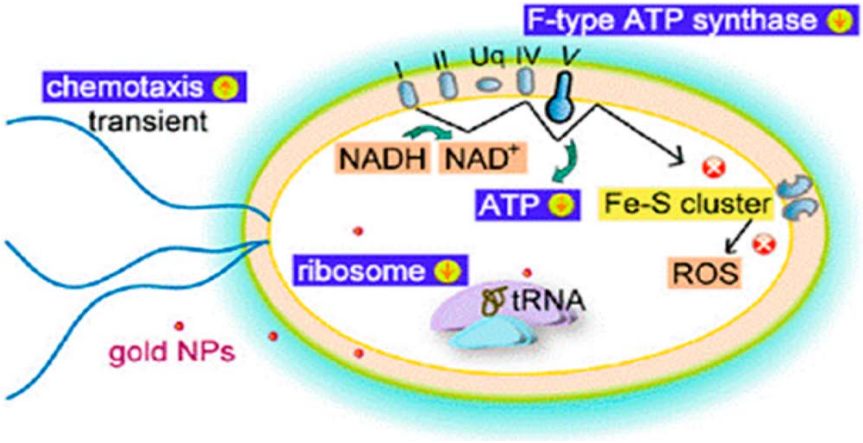

Fig. 2.8. Schematic illustrating the possible mechanism for antibacterial response of gold nanoparticles. [Reproduced with permission from ref (117): Copyright (2015) Springer Nature]

Table 2.2. Antibacterial response of nanoparticles against various pathogens.

S.N.	**Nonomaterials**	**Average particle size**	**Pathogens** (Incubation time: 12 h)	**Concentration in growth medium**	**Effects**		**Ref.**
1.	ZnO nanoparticles	6.8 nm	*E. coli*	0.1 mg/mL	**Bacterial cell death** 99.8 %		74
			S. aureus	0.1 mg/mL	98 %		
2.	Iron oxide nanoparticles	66 nm		Fe_3O_4 concentration	**Diameter of inhibition zone (mm)**		107
					With out antibiotic	With standard antibiotic	
			E. coli	50 mg/mL	11	14	
			S. Epidermidis	50 mg/mL	14	15	
			S. aureus	50 mg/mL	12	16	
			Bacillus subtilis	50 mg/mL	20	16	
3.	Silver nanoparticles	12 nm	*E. coli*	10 µg/cm^3 60 µg/cm^3	bacterial growth inhibited by 70 % 100 %		81
			S. aureus	100 µg/cm^3	100 %		
4.	ZnO nanoparticles	48 nm	*B. subtilis*	10 ppm	90 %		118
			E. coli	1000 ppm	48 %		
			S.aureus	0.1 mg/mL	98 %		
5.	Ag-ZnO nanocomposites	64 nm	*E. coli*	550 µg/mL	100 %		119
			S.aureus	60 µg/mL	100 %		
			E. coli	0.01-0.15 mg/mL	61 %		
			P. aerugenosa	0.01-0.15 mg/mL	60 %		
			S.aureus	0.01-0.15 mg/mL	55.17 %		
6.	Iron oxide nanoparticles	35 nm	*E. coli*	10-50 mg/mL	30 %		120
			S.aureus	10-50 mg/mL	25 %		
7.	TiO_2 nanoparticles	25 nm	*E. coli*	20-100 µg/mL	41.17 %		121

8.	TiO_2 nanoparticles	22.41 nm			**Diameter of inhibition zone (mm)**	122
			E. coli	100 mg/mL	12	
			S. epidermidis	144 mg/mL	15	
9.	CuO nanoparticles	7.8 nm	*E. coli*	103 µg/mL	15.8	123
			S.aureus	120 µg/mL	13.2	
		4.8 nm	*E. coli*	103 µg/mL	16.8	
			S.aureus	120 µg/mL	15	
10.	MgO nanoparticles	43-91 nm	*S.aureus*	1 mg/mL	27	124
			P. aerugenosa	1 mg/mL	24	
11.	$MnFe_2O_4$/ Ag	216 nm	*E. coli*	1 mg/mL	35.8	125
			S.aureus	1 mg/mL	42.8	
12	Silver nanoparticles	16 nm	*E. coli*	60 µg/mL	Completely inhibit the CFU	126
13	Silver nanoparticles	14 nm	*B. subtilis*	13.5 µg/mL	76 % reduction in CFU	127
14.	CuO nanoparticles	23.17 nm	*E. coli*	31.25 µg/mL	Completely inhibit the bacterial growth	128
			S.aureus	125 µg/mL	Completely inhibit the bacterial growth	
			P. aerugenosa	62.5 µg/mL	Completely inhibit the bacterial growth	
15.	Iron oxide nanoparticles	10 nm			**Diameter of inhibition zone increased by**	129
			E. coli	0.01-0.15 mg/mL	61 %	
			P. aerugenosa	0.01-0.15 mg/mL	60 %	
			S.aureus	0.01-0.15 mg/mL	55.17 %	

2.4. Effect of external stimuli on antibacterial response

Apart from and nanoparticles and incorporation of secondary phase in base materials, the application of external stimuli such as magnetic and electric fields are the potential alternatives for reducing the bacterial infections [130-132].

2.4.1. Effect of magnetic field on antibacterial response

The application of magnetic field has been recognized in various biomedical fields, such as cancer therapy and drug delivery as well as antimicrobial agent [133]. The intensity of applied magnetic field as well as exposure time is the main influencing factor to enhance the antibacterial response [134]. It has been reported that low-intensity DC magnetic field (2.7-10 mT) is not bacteriostatic on gram-negative *E. coli* bacteria [101]. However, the growth of *S. aureus* bacteria decreases with the exposure to low-intensity DC magnetic field (0.1-0.3 mT) [135]. The CFU for *E. coli* bacteria decreases by ~ 57 %, 68 %, and 76 % while exposed to static magnetic fields of 30, 50 and 80 mT, respectively, for 24 h. However, the CFU of *S. aureus* bacteria decreased by ~ 18 %, 32.47 %, and 61.73 %, respectively, for similar treatment (magnetic field and exposure time). In contrast, the growth rate of *bacillus subtilis* bacteria increases by 72 %, 75 %, and 81 %, respectively, after exposure to similar magnetic field intensity and time duration [136]. It has been reported that inhomogeneous (5.2~6.1T and 3.2~6.7 T) magnetic field is more effective than homogeneous (7T) magnetic field, as far as the viability of *E. coli* cells in the magnetic field is concerned [137]. The viability of bacterial cells decreases by 60 % and 70 % for *S. aureus* and *E. coli* bacteria, respectively, after exposure to the pulsed magnetic field of 4T for 30 mS [138]. Almost similar effect has been reported for exposure to the static magnetic field on antibacterial response, while both, gram-positive and gram-negative bacteria were cultured on various bio-ceramics surfaces. The viability of both, gram positive and gram negative bacteria, adhered on the surface of HA and HA-Fe_3O_4 composites, decreases with increasing the duration of exposure (0.5-4 h) in the magnetic field (100 mT) as well as the Fe_3O_4 content in the bio-composites [139]. There are basically two mechanisms which are responsible for antibacterial response due to the application of the external magnetic field. Fig.2.10 schematically represents the

mechanism of antibacterial response in the magnetic field [139]. Electromagnetic field affects the permeability of the ionic channels in the cell membrane. The other possible effects include the formation of free radicals such as, hydroxyl radicals, superoxide's etc., due to magnetic field exposure that increases the production of reactive oxygen species (ROS) which is bactericidal [140 - 144].

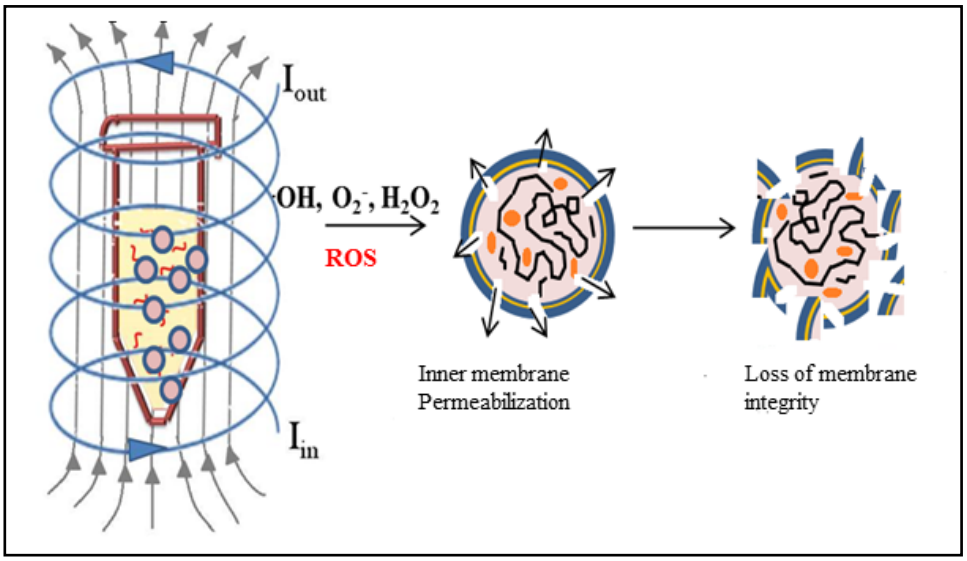

Fig. 2.9: Schematic representation of mechanism for antibacterial activity due to production of free radicals in magnetic field. [Reproduced with permission from ref (139): Copyright (2015) Springer Nature]

2.4.2. Effect of electrical stimuli on antibacterial response

The external electric field induced antibacterial response depends on the intensity of electric field, exposure time and pulse duration (in case of pulsed electric field) etc. [145, 146]. The number of CFU for *E. coli* and *S. aureus* bacteria decreases by 33 % and 31 %, respectively, after exposure to the static electric field of 4.5 kV/cm for the duration of 30 min. However, CFU decreases by 56 and 54%, respectively, for similar bacteria and electric field, after exposure for 2.5 h. The antibacterial response also depends upon the frequency of the applied electric field. Mirzaii et al. [147] reported that the number of

CFU decreases by 3 % and 47.17 % against *S. aureus* bacteria, upon exposure to an electric field with strength of 6 V/cm^2 and frequencies of 1 MHz and 20 MHz respectively, for 6 h. However, the similar treatment (electric field and frequency) leads to the reduction of CFU by 10 % and 27 % for *P. aerugenosa* bacteria, when exposed for 4 h. Table 2.3 summarizes the antibacterial activity for both, gram positive and gram negative bacterial cells under the exposure to the electric field. Pulsed electric field (PEF) is used for killing the bacteria in food preservation. Malicki et al. [148] reported that the maximum reduction (4.7 log unit) in CFU/ml against *E. coli* bacteria is obtained after the exposure of pulsed electric field (32.89 kV/cm) with 180 pulses for the duration of 30 μs. However, CFU/ml decreasesby 1.2 log unit against *bacillus cereus* bacteria, through exposure of 16.7 kV/cm PEF for 2 mS (50 pulses) [149].

The mechanism, responsible for electric field induced antibacterial activity, includes electrolysis of molecules on the surface of bacterial cells that produce the toxic substances like H_2O_2, oxidizing radicals, and chlorine molecule [150,151]. Fig. 2.10 demonstrates the mechanisms, suggested for antimicrobial response in electric field [152].

The following reactions occur at cathode and anode [153]. Production of H_2O_2 at cathode,

$$\mathbf{H_2O + 2e^- \rightarrow H_2 + 2OH^-} \qquad (\mathbf{2.8})$$

$$\mathbf{H_2 + 2H^- + 2e^- \rightarrow H_2O_2} \qquad (\mathbf{2.9})$$

Production of Cl_2 at anode,

$$\mathbf{2H_2O \rightarrow O_2 + 4H^+ + 2e^-} \qquad (\mathbf{2.10})$$

$$\mathbf{2Cl^- \rightarrow Cl_2 + 2e^-} \qquad (\mathbf{2.11})$$

The application of electric field induces a potential across the cell membrane for short duration which causes the loss of membrane resistance [154,155]. The strength of the

applied electric field is also an important parameter for pulse electric stimulation induced bactericidal effect. Usually, the electric field of strength of 20-80 kV/cm is applied for a short duration (generally in microseconds) for killing the bacterial cells [156]. The mechanism, responsible for antibacterial response due to the application of electric current is the generation of chemical oxidant as well as the hydrophilicity of suspension medium [157-159]. Application of electric current to bacterial suspension generates few chemical oxidants such as chloride on electrodes due to electrolysis, which is bactericidal [160]. Oxidative stress develops in the solution due to ions which cause the significant reduction in hydrophilicity i.e., reduction in biofilm formation [161]. A high-intensity electric current can alter the orientation of membrane lipids and also oxidize the cellular constituents which destruct the cell membrane [162].

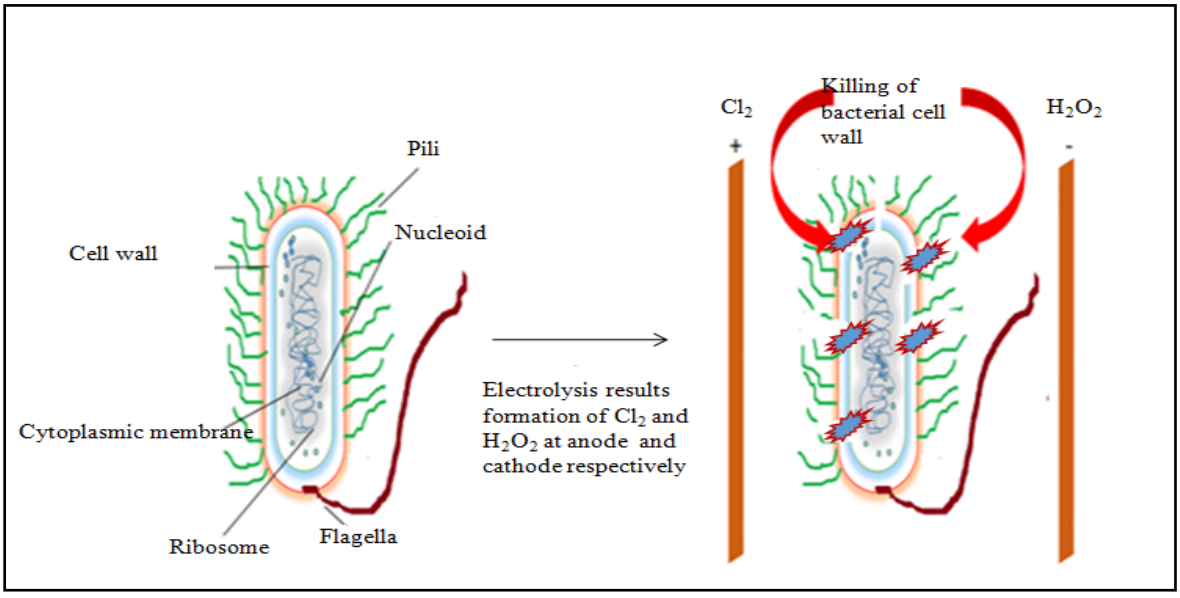

Fig. 2.10: Schematic representation for the mechanisms of antibacterial response due to electric field. [Reproduced with permission from ref. (21): Copyright (2018), American Chemical Society].

Table 2.3: Effect of incorporation of secondary phase as well as external stimulant on antibacterial response of bioceramic substrates

S.N.	Material		External stimulant (Magnetic field/ electric field/ surface charge)	Types of pathogens and exposure time	Effects	Ref.
	Base material	Secondary phase				
1.	HA	-	Static Magnetic field of 100 mT	*E. coli* for 4 h	Cell viability decrease by 60%	163
				S. epidermidis for 4 h	17 %	
2.	HA	Fe_3O_4 (10-40 wt. %)	Static Magnetic field of 100 mT	*S. aureus* for 4 h	Bacterial adhesion is reduced by 8% (HA) 25% (HA-40 wt.% Fe_3O_4)	164
3.	Glass substrate	-	Low-intensity electric field (1.5-30V/cm)	*S. aureus* for 4h	Bacterial adherence Recesses to 98%	165
				S. epidermidis 4 h	No effect on bacterial adhesion	
4.	HA	ZnO (10 wt. %)	DC electric field of intensity 1V/cm	*S. aureus* for 24 h	%Viability reduction 60 % (pure HA) 70% (10wt.% ZnO in HA)	166
5..	HA	-	Positively charged surface	*S. aureus* for 72h	Bacterial cells per colony decreases by 42.10 %	167
			Negatively	*S .aureus* for 72h	28 %	

			charged surface			
				S. epidermidis 4 h	No effect on bacterial adhesion	
6.	Glass ceramic (Cerec Blocs S3-M14)	-	Polarized surface (DC electric field of 0.1 -1 kV/cm in air at 250 ˚C for 1 h)	*Streptococcus mutans* for 24 h	Reduction in bacterial adhesion.	168

2.5. Effect of addition of piezoelectric secondary on antibacterial response of bioceramics

It has been reported that piezoelectric materials demonstrate antibacterial response [169]. Recently, various piezobioceramics are emerged as prospective materials for bone implant, due to the piezoelectric nature of bone [170]. The piezoelectric $Na_{0.5}K_{0.5}NbO_3$ (NKN) and $BaTiO_3$ demonstrate antibacterial response [171]. It has been reported that incorporation of BT (40 and 60 wt. %) in HA matrix increases the diameter of inhibition zones by (123,154 %) and (136,140 %) for *S. aureus* and *E. coli* bacteria, respectively [172]. The viability of *S. aureus*, *E. coli* and *P. aeruginosa* bacteria reduces by almost 43, 35 and 34 % on HA - 40 wt. % $BaTiO_3$, respectively. However, the addition of 60 wt. % $BaTiO_3$ in HA reduces the viability by 56, 66 and 51 %, while cultured with similar bacteria and incubation condition [173]. Overall, the litrature reports suggested that the incorporation of piezoelectric secondary phases in HA increases the antibacterial properties.

2.6. Cellular response of tailored biocomposites

Apart from antibacterial properties, the cytocompatibility of developed implant materials is very serious concern. In this section the cellular response of various bioceramics as wll as effect of external stimuli on cellular response have been reviewed.

Saha et al. [174] reported that the addition of ZnO (< 7.5 wt. %) in HA matrix increases the cellular proliferation. The adhesion of FMM1 fibroblasts cells increases by 150 % on tri calcium phosphate (TCP) – 50 wt. % HA after incubation of 3 days [175]. Rajendran et al. [176] reported that viability of NIH3T3 cells on HA-10 wt % Ag, increases approximately 80 %, after incubation of 7 days. Wu et al. [177] reported that cytocompatibility of MG 63 cells on magnesium calcium phosphate cement (MCPC) was higher than that of calcium phosphate cement (CPC) and magnesium calcium phosphate cement (MPC) while incubated for 7 days. The optical density of MG-63 cells has been enhanced by ~ 150 %, when cultured on MCPC for 7 days. The incorporation of 1.5 wt. % of graphene oxide (GO) in hydroxyapatite nano-rod (NRHA) increases the proliferation of MC3T3-E1 cells, by approximately 120 % as compared to control samples, after incubation of 7 days [178]. Fig. 2.11 represents the viability of L929 cells on optimally processed HA- 40 wt. % $CaTiO_3$ composite [179]. It is clearly observed that the viability of cells significantly increases with increase in incubation period.

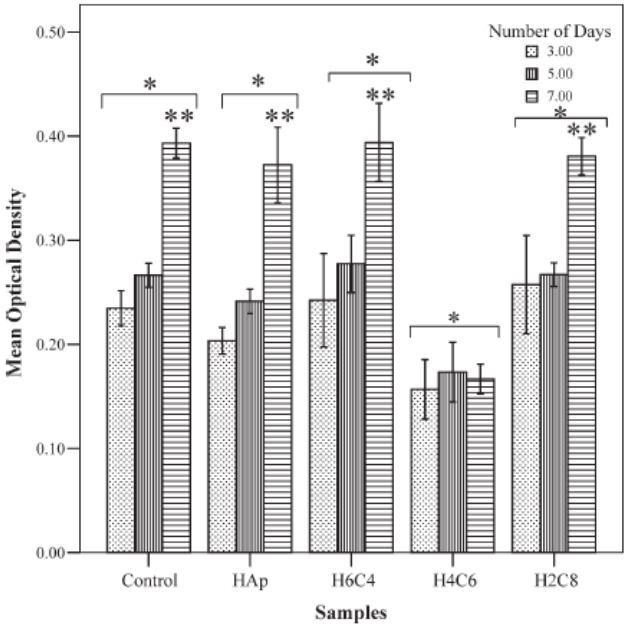

Fig. 2.11. The viability of osteoblast SaOS2 cells, culture on control/ (HA-40 wt. % $CaTiO_3$; H6C4)/ (HA-60 wt. % $CaTiO_3$; H4C6/(HA-80 wt. % $CaTiO_3$; H2C8) samples, after incubation of 3, 5, and 7 days. The symbol (*) represents the statistically significant difference between the samples, at $p < 0.05$. The symbol (**) represents the statistically significant difference between 3, 5, and 7 days of incubation for each composition, at $p < 0.05$. [Reproduced with permission from ref (179): Copyright (2010),Wiley Periodicals, Inc.].

2.6.1. Effect of incorporation of piezoelectric secondary phase on cellular response

The piezoelectric biomaterials have been considered as an appealing alternative for orthopedic applications [180]. Piezoelectricity generates surface charge by application of electrical stimulation which favors ontogenesis [181]. It has been suggested that the incorporation of piezoelectric biomaterials as a secondary phase in HA matrix enhances the proliferation of human osteoblast cells [182]. It has been demonstrated that the proliferation of L929 cells on HA- 70 wt. % of $BaTiO_3$ increases by approximately 201 % as compared to control sample, after incubation of 7days [184]. In another study, the incorporation of 40 wt. % of $BaTiO_3$ in HA has reported to increase the cellular response

[183]. The density of SaOS2 cells, cultured on HA and HA-90 vol. % BT composite, increased by 87 and 97 % respectively, after incubation of 7 days [184].Overall, the literature reports suggested that the addition of piezoelectric secondary phases in HA enhances the proliferation of osteoblast cells.

2.6.2. Effect of external stimuli on cellular response

The application of external stimuli such as, electric field and surface polarization has been recognized as an alternative technique to enhance the cell growth and proliferation [185]. It has been demonstrated that the optimal electrical stimulation significantly enhances the proliferation and differentiation (osteogenic) of human mesenchymal stem cells on biomaterial surfaces [186]. The adhesion of osteoblast cells on negatively polarized surface increases due to electrostatic interaction between negative charge and Ca^{2+} ions [187]. Fig. 2.12 demonstrates the effect of electrical polarization on viability of L929 cells, while cultured on HA-BT composites [188]. The negatively polarized HA-x BT (x = 20 and 40 wt. %) composites increases the density of L929 cells by 40 and 62 %, respectively, while cultured on these surfaces for 48 h [187].The viability of osteoblast SaOS2 cells on negatively charged HA-7.5 wt. % ZnO bio-composite has been reported to increase by ~ 77 %, after incubation of 3 days [189].

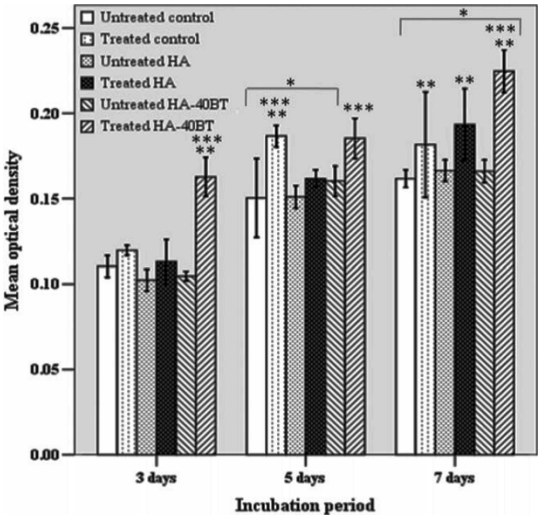

Fig. 2.12. The viability of L929 cells, cultured on control, HA and HA-40 wt. % $BaTiO_3$ composites. The symbol (*) shows the significant difference among the samples, incubated for 5 and 7 days with respect to that incubated for 3 days . The symbols (**) and (***) represent the statistically significant differences among the samples with respect to control disk and HA, respectively, at P < 0.05. [Reproduced with permission from ref. (187): Copyright (2014) Wiley Periodicals, Inc].

It has been reported that polarized HA (negatively charged) enhances the proliferation of MC3T3-E1 osteoblast cells, after incubation of 7 days [190]. In another study, it has been reported that the proliferation of human osteoblast like hFOB cells on N -polarized HA is almost double as compared to P-polarized HA, after incubation of 11 days [191]. The proliferation of rBMSCs cells on polarized NKN increases after incubation of 7 days as compared with incubation of 4 days. The polarized NKN (piezoelectric constant ~ 70 pC/N) samples demonstrate higher proliferation of mouse mesenchymal cells as compared to unpolarized samples [192]. The adhesion of osteoblast cells on polarized (22-24 kV/cm) lithium modified NKN ($Li_{.06}Na_{.5}K_{.44}NbO_3$, LNKN) has been increased by approximately 33 % as compared to unpolarized LNKN [194]. It has been observed that the polarization increases the formation of apatite layer in HA by approximately two times as compared to unpolarized HA [193]. Verma et al. [194] demonstrated that

incorporation of sodium potassium niobate (NKN) in 1393 bioglass (1393 BG) enhances the cell proliferation. It has been concluded that the application of external stimuli and surface polarization enhances the proliferation of osteoblast cells.

The adhesion of cells with substrate depends on the interaction of substrate with protein (present in growth media) [195]. The cations such as, Ca^{+}, Na^{+}, Mg^{+} and K^{+} are adsorbed of on N-polarized surfaces which promotes the formation of bone like apatite layer and increase the cell proliferation [196]. However, anionic groups such as $HPO_4^{2-}$and HCO_3^{2-} attracted towards p-polarized surfaces which act as anti-adhesive agents [188]. These anions do not promote the formation of apatite layer. Fig. 2.13 illustrates the interaction of polarized HA surfaces with MC3T3-E1 cells in growth media. It has been suggested that negatively polarized surfaces promote the formation of bone like apatite layer [197].

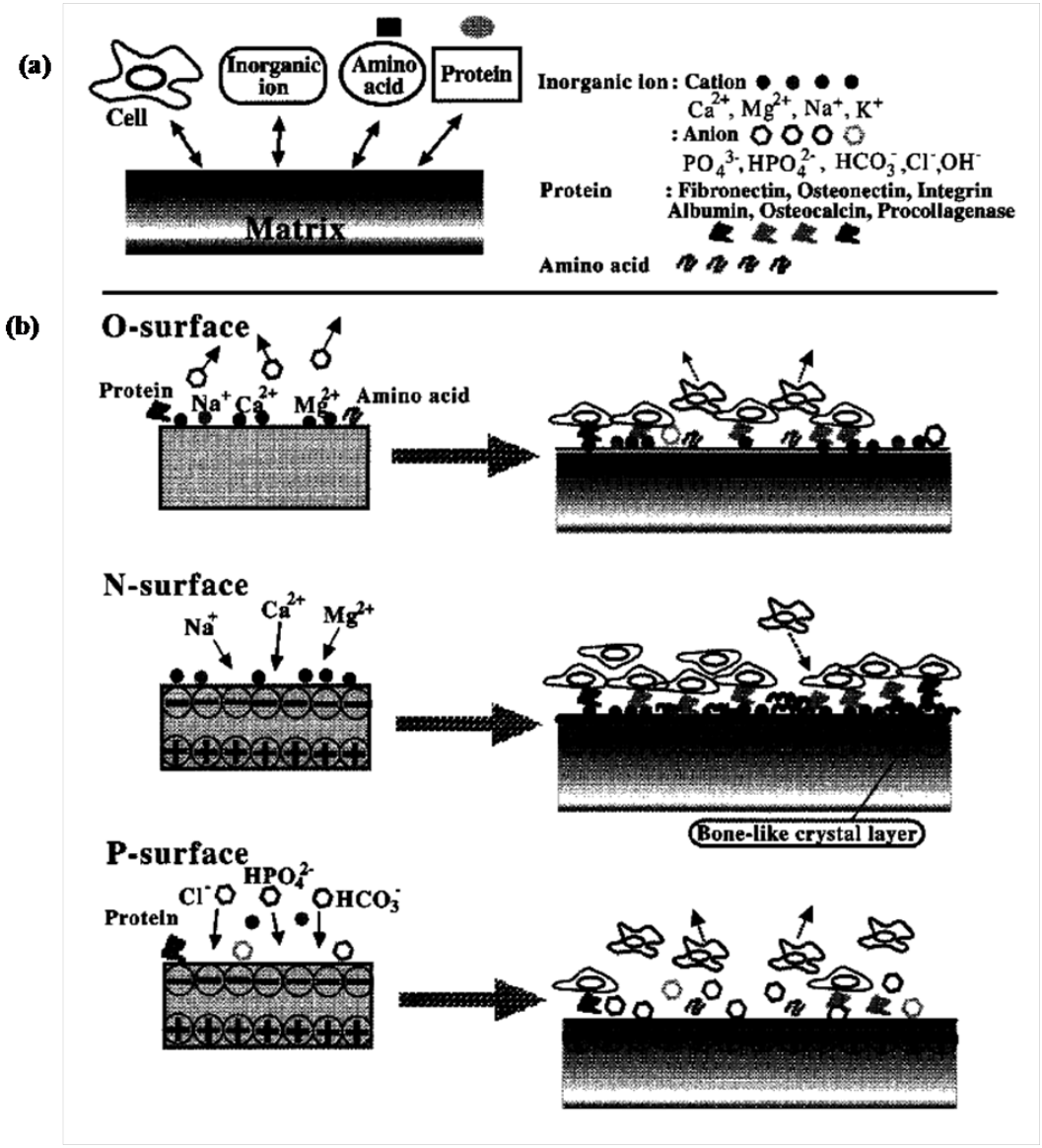

Fig. 2.13. Schematic diagram, representing the effect of surface polarization on adhesion of MC3T3-E1 cells (a) The inorganic ions, amino acids, and proteins are attached on HA surfaces, (b) normal cell adhesion on uncharged surfaces, positively charged ionic groups such as Ca^{2+} *are attracted towards in negatively charged surface and promote the formation of the bone-like layer on N-polarized HA surface. The positively charged surface actively adsorbs the anions which is not favorable for apatite layer formation and consequently, the cell adhesion. [Reproduce with permission from ref. (197): Copyright (2001), John Wiley & Sons, Inc].*

2.7. Closure

The effects of addition of various antibacterial elements as secondary phases in different ceramic matrices and application of external stimuli on antibacterial behavior were reviewed. The effect of incorporation of piezoelectric secondary phase on antibacterial response was also reviewed. In addition, the cellular response of tailored biomaterials was discussed. Further, the influence of addition of piezoelectric secondary phases, external stimuli as well as surface polarization on cellular response was also discussed. In addition, the possible mechanisms for antibacterial as well as cellular response were also discussed.

References

1. M. Geetha , A.K. Singh, R. Asokamani, A.K. Gogia, *Progress in Materials Science,* **54** (2009) 397–425.

2. Ray,N.B.; Ranjit, K.T.; Manna, A.C.Antibacterial Activity of ZnO Nanoparticle Suspensions on a Broad Spectrum of Microorganisms. *FEMS Microbiol. Lett.* **279(1) (**2008) 71–76.

3. Puckett, S.D.; Taylor, E.; Raimondo, T.; Webster, T.J. The Relationship Between The Nanostructure of Titanium Surfaces and Bacterial Attachment *Biomaterials*. **31(4), (**2010) 706–713.

4. Berbari, E.F; Hanssen, A.D.; Duffy, M.C. Risk Factors for Prosthetic Joint Infection: Case-Control Study. *Clinical Infection Disease.* **27 (1998**)-1247-54.

5. L.L. Hench, Bioceramics. *Journal of the American Ceram Society,* **81** (1998) 1705-1728.

6. S.C. Ciobanu, , L.S. Iconaru, P. Coustumer, , L. C. Violeta and P. Daniela, Antibacterial activity of silver-doped hydroxyapatite nanoparticles against gram-positive and gram-negative bacteria, *Nanoscale Research Letters,***7** (2012) 324-334.

7. J.R. Morones, J.L. Elechiguerra, , A. Camacho, J.T. Ramirez,The bactericidal effect of silver nanoparticles. *Nanotechnology,* **16** (2005) 2346–2353.

8. S.J. Kalita, H.A. Bhatt, Nanocrystalline hydroxyapatite doped with magnesium and zinc: Synthesis and characterization. *Material science and engineering C*, **27** (2007)837–848.

9.A. H. Rajabi, M. Jaffe, T. L. Arinzeh, Piezoelectric materials for tissue regeneration: A review, *Acta Biomaterialia* **24** (2015) 12-23.

10. A.S. Verma, A. Sharma., A. Kumar, A. Mukhopadhyay, D. Kumar, and A. K. Dubey, Multifunctional response of piezoelectric sodium potassium niobate (NKN) toughened hydroxyapatite based biocomposites, *ACS Applied Biomaterials,* **3 (8)** (2020) 5287–5299.

11. S. Swain, R.N. Padhy, T. R. Rautray, Polarized piezoelectric bioceramic composites exhibit antibacterial activity. *Materials Chemistry and Physics*, **239** (2020) 122002.

12.W. Chen, Z. Yu, J. Pang, P. Yu, G. Tan, C. Ning, Fabrication of biocompatible potassium sodium niobate piezoelectric ceramic as an electroactive implant, *Materials (Basel)* **10** (2017)

18-21.

13. A. K. Dubey a , B. Basu a , K. Balani a , R. Guo b and A. S. Bhalla,Multifunctionality of Perovskites $BaTiO_3$ and $CaTiO_3$ in a Composite with Hydroxyapatite as Orthopedic Implant Materials, *Integrated Ferroelectrics*, **131** (2011) 119–126.

14. M.A. Ansari, H.M Khan, A.A. Khan, A. Malik, A. Sultan, M. Shahid, Evaluation of antibacterial activity of silver nanoparticles against MSSA and MSRA on isolates from skin infections, *Biology and Medicine* **3** (2011)141–146.

15. D. C. Tien, K. H. Tseng, C. Y. Liao. and T. T. Tsung, Colloidal silver fabrication using the spark discharge system and its antimicrobial effect on Staphylococcus aureus, *Medical Engineering and Physics*, **30 (8) (**2008) 948–952.

16. M. Dıaz F. Barba, M. Miranda, G. Francisco, T. Ramon, and M. Jose, Synthesis and Antimicrobial Activity of a Silver-Hydroxyapatite Nanocomposite, *Journal of Nanomaterials* , 2009,(2009), 6.

17.A.Pandey□, S. Midha□, R. K.Sharma□, R. Maurya□, V.K. Nigam□, S.Ghosh□□, K. Balani□, Antioxidant and antibacterial hydroxyapatite-based biocomposite for orthopedic applications, *Materials Science and Engineering: C* **88** (2018) 13-24.

18. M. Aurora, G. Furtosa, R. Sorin, H. Ossi, F. Chirila, G. Corina, D. Ancuta, R. Gheorghe, P.Cristina, M. Tomoaia-Cotisel, Synthesis characterization and antimicrobial effects of composites based on multi-substituted hydroxyapatite and silver nanoparticles, *Applied Surface Science,* 2**98** (2014) 225–235.

19. M. Aurora, G. Furtosa, R. Sorin, H. Ossi, F. Chirila, G. Corina, D. Ancuta, R. Gheorghe, P. Cristina, M. Tomoaia-Cotisel, Synthesis; Characterization and Antimicrobial Effects of Com composites based on multi-substituted hydroxyapatite and silver nanoparticles, *Applied Surface Science,* **298** (2014) 225-235.

20. W. Lee, K. Kim, J. Lee, A novel mechanism for the antibacterial effect of silver nanoparticles on Escherichia coli, *BioMetals,* **27 (**2014) 1191–1201.

21. A. Singh and A.K. Dubey, Various Biomaterials and Techniques for Improving Antibacterial Response. *ACS Applied Biomaterials***, 1 (**2018) 3–20.

22. A. Fujishima, and A. Donald Titanium dioxide photocatalysis, *Journal of Photochemistry and Photobiology C*, **1** (2000) 1–21.

23. A. J. Nathanael, D. Mangalaraj, S.I. Hong, Biocompatibility and Antimicrobial activity of Hydroxyapatite/titania bio-nanocomposite, *18th international conference on composite materials*, (2011)

24. M. Azimzadehirani, M. R Elahifard, S. Haghighi, M. R. Gholami, Highly Efficient Hydroxyapatite/TiO_2 Composites Covered by Silver Halides as E. Coli Disinfectant Under Visible Light and Dark Media. *Photochemical and Photobiological Sciences* **12** (2013) 1787−1794.

25. B. Durairaj, S. Muthu, T. Xavier, Antimicrobial activity of Aspergillus niger synthesized titanium dioxide nanoparticles, *Advances in Applied Science Research*, **6(1) (**2015) 45-48.

26. S. Tojo, T. Tachikawa, M. Fujitsuka, and T. Majima, The Photo-Absorption and Surface Feature of Nano-Structured TiO_2 Coatings, *The Journal of Physical Chemistry C*, **112** (2008) 14948.

27. H. Lin, W. Deng, T. Zhou, S. Ning, J. Long, X. Wang, Iodine-modified nanocrystalline titania for photo-catalytic antibacterial application under visible light illumination, *Appled Catalysis B Environmental*, **176 (**2015) 36–43.

28. M. Yadav, H. Jung, H. S. Pawar, Developments in photocatalytic antibacterial activity of nano TiO_2 A review, *Korean Journal of Chemical Engineering*, **33(7) (**2016**)** 1989-1998.

29. A. B. Djurisic, Y. H . Leung, A. M Ching, Strategies for Improving the Efficiency of Semiconductor Metal Oxide Photocatalysis. *Materials Horizons*, **1** (2014) 400−410.

30. N. Padmavathy, and R. Vijayaraghavan, Enhanced Bioactivity of ZnO Nanoparticles, an Antimicrobial Study. Sci. Technol. *Advanced Materials*, **9 (**2008) 035004.

31. S.W. Zhao, C. R Guo, Y. Z. Hu, Y. R Guo, Q. J. Pan, The Preparation and Antibacterial Activity of Cellulose/ZnO Composite: A Review. *Open Chemistry*, **16** (2018) 9-20.

32. L.V. Trandafilovic, D.K. Bozanic, S. Dimitrijevic-Brankovic, A.S. Luytc, V. Djokovica, Fabrication and antibacterial properties of ZnO alginate nanocomposites, *Carbohydrate Polymer*, **88** (2012) 263-269.

33. N. Saha, K. Keskinbora, E. Suvaci, B. Basu, Sintering microstructure mechanical and antimicrobial properties of HA-ZnO biocomposites. *Journal of Biomedical Materials Research.* **95B (2)** (2010) 430-440.

34. L. Grenho, F. Jorge, M. Maria, P. Ferraz, In vitro analysis of the antibacterial effect of nano-hydroxyapatite–ZnO composites, *Journal of Biomedical Materials Research A*, **102(10) (**2014) 3726-3733.

35. L. Zhang, J. Yunhong D. Yulong, D. N. J. Lars, P. Malcolm, A. J. Neill, Y. W. David, Mechanistic investigation into antibacterial behaviour of suspensions of ZnO nanoparticles against E. coli, *Journal of Nanoparticle Research*, **12** (2010)1625–1636.

36. G. Applerot, A. Lipovsky, R. Dror, N. Perkas, Y. Nitzan, R. Lubart, A. Gedanken, Enhanced antibacterial activity of nanocrystalline ZnO due to increased ROS-mediated cell injury. *Advanced Functional Materials*, **19** (2009) 842–852.

37. M. Premanathan, K. Karthikeyan, , K. Jeyasubramanian G. Manivannan, Selective toxicity of ZnO nanoparticles toward Gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation. *Nanomedicine,* **7(2)** (2011) 184–192.

38. M. Li, L. Zhu, D. Lin, Toxicity of ZnO nanoparticles to Escherichia coli: mechanism and the influence of medium components. *Environmental Science & Technology***, 45(5)** (2011) 1977–1983.

39. W. Song, J. Zhang, J. Guo, J. Zhang, Ding, F.; Li, L.; Sun, Z.; Role of the dissolved zinc ion and reactive oxygen species in cytotoxicity of ZnO nanoparticles. *Toxicology Letters*, **199(3), (**2010) 389–397.

40. M. Heinlaan, A. Ivask, I. Blinova, H.C. Dubourguier, A. Kahru, Toxicity of nanosized and bulk ZnO, CuO and TiO_2 to bacteria Vibrio fischeri and crustaceans Daphnia Magna and Thamnocephalus platyurus. *Chemosphere.* **71(7) (**2008) 1308–1316.

41. Y. Zheng, X. Zhang, M. Huan, Photoluminescent ZnO Nanoparticles and Their

Biological Applications, *Materials.* **8** (2015), 3101-3127.

42. H. S Ragab, F. A. Ibrahim, , F. Abdallah, A. A. Ghamd, E. F. Tantawy, Synthesis and In Vitro Antibacterial Properties of Hydroxyapatite Nanoparticles. *IOSR Journal of Pharmacy and Biological Science*, **9** (2014) 77−85.

43. X. Lian, H. Liu, X. Wang, S.Xu, F. Cui, X. Bai, Antibacterial and Biocompatible Properties of Vancomycin-Loaded Nano-Hydroxyapatite/Collagen/Poly (Lactic Acid) Bone Substitute. *Progress in Natural Science*, **23 (6)** (2013) 549−556.

44. K. Vijayalakshmi, D. Sivaraj, Enhanced Antibacterial Activity of Cr Doped ZnO Nanorods Synthesized Using Microwave Processing. *RSC Adv*, **5** (2015) 68461−68469.

45. H. Gholap, R. Patil, P. Yadav, A. Banpurkar, S. Ogale, W. Gade, CdTe−TiO_2 Nanocomposite: An Impeder of Bacterial Growth and Biofilm. *Nanotechnology* **24 (13)** (2013) 195101.

46. V. Sanyal, C. R Raja, Structural and Antibacterial Activity of Hydroxyapatite and Fluorohydroxyapatite and Co-Substituted with Zirconium-Cerium Ions. *Applied. Physics A: Materials Science Process*, (2016) 122-132.

47. A. Mocanu, G. Furtos, S. Rapuntean, O. Horovitz, C. Flore, C. Garbo, A. Danisteanu, G. Rapuntean, C. Prejmerean, M. Tomoaia- Cotisel, Synthesis Characterization and Antimicrobial Effects of Composites Based on Multi-Substituted Hydroxyapatite and Silver Nanoparticles. *Applied Surface Science*, **298** (2014) 225−235.

48. A.K. Gupta, and M. Gupta, Synthesis and Surface Engineering of Iron Oxide Nanoparticles for Biomedical Applications. *Biomaterials.* **26(18)** 2005, 3995-4021.

49. E. Taylor, T. J. Webster, Reducing Infections Through Nanotechnology and Nanoparticles. *International Journal of Nanomedicine*, **6** (2011) 1463–1473.

50. S. S. Behera, J. K. PatraPramanik, N. Panda, H. Thatoi, Characterization and Evaluation of Antibacterial Activities of Chemically Synthesized Iron Oxide Nanoparticles. *World Journal of* . *Nanoscience and Engneering*, **2** (2012) 196-200.

51. P. Espitia, N.D. Soares, J.D. Coimbra, De. Andrade, N. Cruz, R. Medeiros, Zinc Oxide Nanoparticles: Synthesis, Antimicrobial Activity and Food Packaging Applications. *Food Bioprocess. Technology*, **5** (2012) 1447–1464.

52. H. Hu, W. Zhang, Y. Qiao, X. Jiang, X. Liu, C. Ding, Antibacterial Activity and Increased Bone Marrow Stem Cell Functions of Zn-Incorporated TiO_2 Coatings on Titanium. *Acta. Biomaterialia,* **8(2) (**2012) 904–915.

53. L.S. Reddy, M. N. Mary, J. Mary, and P. N. Shilpa, Antimicrobial Activity of Zinc Oxide (ZnO) Nanoparticles Against Klebsiella Pneumonia. *Pharmaceutical Biology,* **52(11) (**2014) 1388–1397.

54. C. Wang, L. L. Liu, A. T. Zhang, P. Xie, J. J. Lu, and Z. X. Ting, Antibacterial Effects of Zinc Oxide Nanoparticles on Escherichia Coli K88. *African Journal of Biotechnol*ogy, **11(44) (**2012) 10248-10254.

55. R.V. Rai, J. A Bai, Nanoparticles and their Potential Application as Antimicrobials. *Formatex Research Center*, **12** (2011) 197–209.

56. J. Li, L. Tan, X. Liu, Z. Cui, X. Yang, K. W. K. Yeung, K. P. Chu, and S. Wu, Balancing Bacteria−Osteoblast Competition through Selective Physical Puncture and Biofunctionalization of ZnO/Polydopamine/ Arginine-Glycine-Aspartic Acid-Cysteine Nanorods. *ACS Nano*, **11 (**2017) 11250−11263.

57. S. Stankic, S. Suman, F. Haque, and J. Vidic, Pure and Multimetal Oxide Nanoparticles: Synthesis, Antibacterial and Cytotoxic Properties. *Journal of Nano biotechnology*, **14** (2016) 73-81.

58. E. Taylor, T. J. Webster, Reducing Infections Through Nanotechnology and Nanoparticles, *International Journal of Nanomedicine*, **6** (2011) 1463–1473.

59. S. Stankic, S. Suman, F. Haque, and J. Vidic, Pure and Multimetal Oxide Nanoparticles: Synthesis, Antibacterial and Cytotoxic Properties. *Journal of Nanobiotechnology*, **14 (**2016) 73.

60. X. Wang, F. Yang, W. Yang, X. Yang, A Study on the Antibacterial Activity of One-Dimensional ZnO Nanowire Arrays: Effects of the Orientation and Plane Surface. *Chemical Communication*, **42** (2007) 4419–21.

61 Li, M.; Zhu, L.; Lin, D. Toxicity of ZnO Nanoparticles to Escherichia Coli: Mechanism and the Influence of Medium Components. *Environmental Science and Technology*, **45(5),** (2011) 1977–1983.

62. E. Taylor, T. J. Webster, Reducing Infections Through Nanotechnology and Nanoparticles. *International Journal of Nano medicine*, **6** (2011)1463–1473.

63. A. Sirelkhatim, S. Mahmud, A. Seeni, K. N. H. Mohamad, A.L. Chuo, B. Mohamad, K. H. D. Siti, Mohamad, Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. *Nano Micro Letters*, **7(3) (**2015) 219–242.

64. R. Brayner, R. Ferrari-Iliou, N. Brivois, Djediat, M.F. Benedetti, F. Fievet, Toxicological Impact Studies Based on Escherichia Coli Bacteria in Ultrafine ZnO Nanoparticles Colloidal Medium. *Nano Letters*, **6** (2006) 866–868.

65. A. Kumar, Z. Ansari, H. Fouad, A. Umar, S. Ansari, Oxidative Stress Control in E. coli and S. aureus Cells Using Amines Adsorbed ZnO. *Science of Advance materials*, **6** (2014) 1236–1243.

66. D.J. Barillo, D.E. Marx, Silver in Medicine: A Brief History BC 335 to Present. *Burns*. **40 (suppl 1) (**2014) S8.

67 G. V. Vimbela, S. M. Ngo, C. Fraze, L. Yang, D. A. Stout, Antibacterial Properties and Toxicity of Metallic Nanomaterials. *International Journal of Nanomedicine*, **12** (2017) 3941−3965.

68. Y. Zhou, Y. Kong, S. Kundu, J.D. Cirillo, H. Liang, Antibacterial Activities of Gold and Silver Nanoparticles Against Escherichia Coli and Bacillus Calmette-Guérin. *Journal of Nano biotechnology*, **10(1)** (2012) 1-9.

69. C.N. Lok, C.M. Ho, R. Chen, Q.Y. Yu, W.Y. Sun, H.Z. Tam, P.K.H. Chiu, J.F. Che, Proteomic Analysis of the Mode of Antibacterial Action of Silver Nanoparticles. *Journal of Proteome Research*, **5** (2006) 916–924.

70. W.R. Li, X.B. Xie, Q.S. Shi S.S. Duan, Y.S. Ouyang, Y.B. Chen, Antibacterial Effect of Silver Nanoparticles on Staphylococcus Aureus. *Biometals.* **24 (**2011) 135–141.

71. D. Wu, F. Wei, A. Kishen, S.D. Fan, W. Kishen, L. James, B. Gutmann, Evaluation of the Antibacterial Efficacy of Silver Nanoparticles Against Enterococcus Faecalis Biofilm. *Journal of Endododontics*, **40(2)** (2014) 285–290.

72. I. Sonhi, S. Sondi, Silver Nanoparticles as Antimicrobial Agent: A Case Study on E. coli as A Model for Gram-Negative Bacteria. *Journal of Colloid and Interface Science*, **275 (1)** (2004) 177-182.

73. S. H. Kim, H.S. Lee, D. S. Ryu, S.J. Choi, and D.S. Lee, Antibacterial Activity of Silver-Nanoparticles Against Staphylococcus Aureus and Escherichia Coli. *Korean Journal of Microbiology and Biotechnology*, **39(1)** (2011) 77–85.

74. X. Xianzhou, M. Congyang, L. Xiangmei, Z. Z. Yanzhe, X.Y. Cui, W. K. Kelvin, H. Yeung, P. Paul, and W. Shuilin, Synergistic Bacteria Killing through Photodynamic and Physical Actions of Graphene Oxide/Ag/Collagen Coating. *ACS Applied Materials & Interfaces*, **9** (2017) 26417−26428.

75. C. M. Jones, M. Eric, V. Hoek, A Review of the Antibacterial Effects of Silver Nanomaterials and Potential Implications for Human Health and the Environment. *Journal of Nanoparticle Research,* **12** (2010) 1531–1551.

76. P. V. Asharani*,* L.K.Grace, M. P. Hande, and S. Valiyaveetti, Cytotoxicity, and Genotoxicity of Silver Nanoparticles in Human Cells. *ACS. Nano*, **3(2) (**2009) 279–290..

77. O. Choi, K. Deng. N. Kim, L. Ross, R. Surampalli, Z. Hu, The Inhibitory Effects of Silver Nanoparticles, Silver Ions, and Silver Chloride Colloids on Microbial Growth. *Water Resources*, **42 (12)** (2008) 3066–3074.

78. D. Manno, E. Filippo, G. Di, M. Serra, A Synthesis and Characterization of Starch-Stabilized Ag Nanostructures for Sensors Applications. *Journal of Non-Crystalline Solids*, **354** (2008) 5515-5520.

79. J. S. Kim, E. Kuk, K. Yu, N. Kim, S. J. Park, H. J. Lee, S. Kim, H. Park, Antimicrobial Effects of Silver Nanoparticles. *Nano medicine Nanotechnology*, **3(5)** (2007) 95-101.

80. J. P. Ruparelia, A. K. Chatterjee, S. P. uttagupta, and S. Mukherji, Strain Specificit in Antimicrobial Activity of Silver and Copper Nanoparticles. *Acta Biomaterialia*, **4(3) (**2008) 707–716.

81.G. Gregor, R. Christopher, and M. Solioz, Metallic Copper as an Antimicrobial Surface. *Applied and Environmental Microbiology,* **77(5)** (2011) 1541–154.

82. A. Azam, A.S. Ahmed, M. Oves, M.S. Khan, M. Adnan, Size-Dependent Antimicrobial Properties of CuO Nanoparticles Against Gram-Positive and -Negative Bacterial Strains, *International Journal of Nanomedicine,* **7** (2012) 3527–3535.

83. M. Valodka, S.Modi, S. Thakore, Synthesis and Antibacterial Activity of Cu, Ag and Cu–Ag Alloy Nanoparticles: A Green Approach. *Materials Research Bulletin,* **46(3)** (2011) 384-389.

84. Y. Dong, K. Wang, Y. Tan, Q. Wang, J. Li H. Mark and S. Zhang, Synthesis and Characterization of Pure Copper Nanostructures Using Wood. Inherent Architecture as a Natural Template. *Nanoscale Research Letters,* **13 (**2018) 119.

85. Y. Lu, L. Li, Y. Zhu, X. Wang, M. Li, Z. Lin, X. Hu, Y. Zhang, Q. Yin, H. Xia, and C. Mao, Multifunctional Copper-Containing Carboxymethyl Chitosan/Alginate Scaffolds for Eradicating Clinical Bacterial Infection and Promoting Bone Formation. *ACS Applied Material Interfaces,* **10 (**2018) 127−13.

86. J. Ramyadevi, K. Jeyasubramanian, A. Marikani, G. Rajakumar, A.A. Rahuman, Synthesis and Antimicrobial Activity of Copper Nanoparticles. *Materials Letters*, **71** (2012) 114–116.

87. M. Din, I. F. Arshad, Z. Hussain and M. M. Catalytic, Antibacterial, Cytotoxicity, and Antioxidant Activities. *Nanoscale Research Letters*, **12 (**2017) 638.

88. M. Li, Z. Ma, Y. Zhu, H. Xia, M. Yao, X. Chu, X. Wang, K. Yang, M. Yang, Y. Zhang, and C. Mao, Toward a Molecular Understanding of the Antibacterial Mechanism of Copper-Bearing Titanium Alloys against Staphylococcus aureus. *Advanced Healthcare Materials,* **5** (2016) 557–566.

89. V. Aruoja, H. Dubourguie, K. Kasemets, A. Kahru, Toxicity of Nanoparticles of CuO, ZnO and TiO_2 to Microalgae Pseudokirchneriella Subcapitata. *Science of the Total Environment,* **407(4)** (2009) 1461–1468.

90. M.S. Hassan, T. Amna, O. Yang, M.H. El-Newehy, S.S. Al-Deyab, M.Khil, Smart Copper Oxide Nanocrystals: Synthesis, Characterization, Electrochemical and Potent

Antibacterial Activity. *Colloids and Surfaces B: Biointerfaces,* **97** (2012) 201–206.

91. V. Aruoja, H. Dubourguie, K. Kasemets, A. Kahru, Toxicity of Nanoparticles of CuO, ZnO and TiO2 to Microalgae Pseudokirchneriella Subcapitata. *Science of the Total Environment,* **407(4)** (2009) 1461–1468.

92. E.N. Taylor, T.J. Webster, The Use of Superparamagnetic Nanoparticles for Prosthetic Biofilm. *International Journal of Nanomedicine,* **4(1)** (2009) 145–152.

93. M. Mahmoudi S. Sant B. Wang S. Laurent and T. Sen, Superparamagnetic Iron Oxide Nanoparticles (Spions): Development, Surface Modification and Applications in Chemotherapy. *Advanced Drug Delivery Reviews*, **63(1-2) (**2011) 24–46.

94. T. Monica, S. Sitaram, S. K. Kannaiyan, and G. Subbiahdoss, Antibacterial Efficacy of Iron-Oxide Nanoparticles Against Biofilms on Different Biomaterial Surfaces. *International Journal of Biomaterials*, (2014) 1- 6.

95. S. Lin, X. Liu, L. Tan, Z. Cui, X. Yang, K. Yeung, H. Pan, and S. Wu, Porous Iron-Carboxylate Metal−Organic Framework: A Novel Bioplatform with Sustained Antibacterial Efficacy and Nontoxicity.*ACS Applied Materials and Interfaces,* **9 (**2017) 19248−19257.

96. N. Tran, A. Mir, D. Mallik, A. Sinha, S. Nayar, T.J. Webster, Bactericidal Effect of Iron Oxide Nanoparticles on Staphylococcus Aureus. *International Journal of Nanomedicine*, **5** (2010) 277–283.

97. J. A. Imlay, Pathways of Oxidative Damage. *Annual Review of Microbiology,* **57** (2003) 395–418.

98. Y. T. Prabhu, K. Rao, K. B. Venkateswara, K. V. Siva, S. Sai, P. Tambur, Synthesis of Fe_3O_4 Nanoparticles and its Antibacterial Application. *International Nano Letters,* **5** (2015) 85–92.

99. N.J. Sushma, D. Prathyusha, G. Swathi, Facile Approach to Synthesized Magnesium Oxide Nanoparticles by Using Clitoria Ternatea Characterization and In vitro Antioxidant Studies. *Applied Nano science,* **6(3)** (2016) 437- 444.

100. N. Takahashi, Simple and Rapid Synthesis of MgO with Nano-Cube Shape by Means of A Domestic Microwave Oven. *Solid State Sciences*, **9** (2007) 722-724.

101. M. Mirhosseini, Evaluation of the Antibacterial Effect of Magnesium Oxide Nanoparticles with Nisin and Heat in Milk. *Journal of Nano medicine*, **3(2)** (2016) 135-142.

102. Z. X. Tang, and B.F. Lv, MgO Nanoparticles as Antibacterial Agent: Preparation and Activity. *Brazilian Journal of Chemical Engineering*, **31(3) (**2014) 591 – 60.

103. M. M. B. Reddy, S. Ashoka, G. T. Chandrappa, and M. A. Pasha, Nano-MgO: An Efficient Catalyst for the Synthesis of Formamides from Amines and Formic Acid Under MWI. *Catalysis Letters,* **138 (**2010) 82-87.

104. S. Makhluf, R. Dror, N. Yeshayahu, A.R. Yaniv, and A. Gedanken, Microwave-Assisted Synthesis of Nanocrystalline Mgoand its uses as a Bacteriocide. *Advanced Functional Materials*, **15** (2005) 1708-1715.

105. Y. Zhao, L. Shi, X. Ji, J. Li, Z. Han, S. Li, R. Zeng, F. Zhang, Z. Wang, Corrosion Resistance and Antibacterial Properties of Polysiloxane Modified Layer-By-Layer Assembled Self-Healing Coating on Magnesium Alloy. *Journal of Colloid and Interface Science*, 2018 ,doi: https://doi.org/10.1016/j.jcis.2018.04.071.

106. M. Sundrarajan, J. Suresh, R. R. Gandhi, A Comparative Study on Antibacterial Properties of MgO Nanoparticles Prepared Under Different Calcination Temperature. *Digest Journal of. Nanomaterials and Biostructures,* **7(3)** *(*2012) 983-989.

107. J. Sawai, S. Shuga, and H. Kojima, Kinetic Analysis of The Death of Bacteria in CaO Powder Slurry. *International Biodeterioration & Biodegradation*, **47(1)** (2001) 23-26.

108. L. Huang, D. Q. Li, Y. J. Lin, M. Wei, D. G. Evans, X. Duan, Controllable Preparation of Nano MgO and Investigation of its Bactericidal Properties. *Journal of Inorganic Biochemistry*. **99(5) (**2005) 986-993.

109. K. S. Peter, L. Rosalyn, L. M. George, and J. K. Klabunde, Metal Oxide Nanoparticles as Bactericidal Agents. *Langmuir*, **18(17)** (2002) 6679-6686.

110. J. Sawai, H. Kojima, H. Igarashi, A. Hashimoto, S. Shoji, A. Takehara, T. Sawaki, T. Koku gan, and M. Shimizu, Escherichia Coli Damage by Ceramic Powder Slurries. *Journal of Chemical Engineering,* **30 (**1997) 1034-1039.

111. J. Sawai, H. Kojima, H. Igarashi, A. Hashimoto, S. Shoji, T. Sawaki, A. Hakoda, E. Kawada, T. Kokugan, and M. Shimizu, Antibacterial Characteristics of Magnesium Oxide Powder. *World Journal of Microbiology and Biotechnology,* **16(2) (**2000) 187-194.

112. E. Alpaslan, M. Benjamin, H. Y. Geilich, J. W. Thomas, pH Controlled Cerium Oxide Nanoparticle Inhibition of Both Gram- Positive and Gram-Negative Bacteria Growth. *Scientific Reports,* **7** (2017) 45859.

113. P.K. Jain, X. Huang, I.H. El-Sayed, M.A. El-Sayed, Noble Metals on the Nanoscale: Optical and Photothermal Properties and Some Applications in Imaging, Sensing, Biology, and Medicine. *Accounts of Chemical Research*, **41(12) (**2008) 1578–1586.

114. A.U. Khan, Q. Yuana, Y. Weia, G.M. Khanb, Z. Khan, H. Ul, K. Shafiullah, F. Ali, Kamran, A. Ahmad, F. Khan, Photocatalytic and Antibacterial Response of Bio

Synthesized Gold Nanoparticles. *Journal of Photochemistry and Photobiology B,* **162** (2016*)* 273-277.

115. C. Yan, Z. Yuyun, T. Yue, W. Zhang, X. Lu, X. Jiang, The Molecular Mechanism of Action of Bactericidal Gold Nanoparticles on Escherichia Coli. *Biomaterials,* **33** (2012) 2327-2333.

116. Y. Zhao, Y. Tian, Y. Cui, W. Liu, W. Ma, X. Jiang, Small Molecule-Capped Gold Nanoparticles as Potent Antibacterial Agents that Target Gram-Negative Bacteria. *Journal of the American Chemical Society,* **132(35)** (2010) 12349-56.

117. Y. Cui, Z. Yuyun, T. Yue, W. Zhang, X. Lu, X. Jiang, The Molecular Mechanism of Action of Bactericidal Gold Nanoparticles on Escherichia Coli. *Biomaterials* **33** (2012) 2327−2333

118 L. K. Adams, D. Y. Lyon, P. J.J. Alvarez, Comparative Eco- toxicity of Nanoscale TiO_2, SiO_2, and ZnO Water Suspensions. *Water Research*, **40 (19) (**2006) 3527−3532.

119 I. Matai, A. Sachdev, P. Dubey, S. Uday Kumar, B. Bhushan, P. Gopinath, Antibacterial Activity and Mechanism of Ag-ZnO Nanocomposite on S. aureus and GFP-Expressing Antibiotic Resistant. *Colloids and Surface B,* **115 (1)** (2014) 359−367.

120 D. Manyasree, P. Kiranmayi, R. V. S. S. N. R. Kumar, Synthesis, Characterization and Antibacterial Activity of Iron Oxide Nanoparticles. *Indo American Journal of Pharmaceutical Research,* **13 (9)** (2016) 2231−6876.

121. Ahmad, R.; Sardar, M. TiO_2 Nanoparticles as an Antibacterial Agents Against E. coli. *International Journal of Innovative Research in Science, Engineering and Technology*, **2(8)** (2013) 3569−3574.

122. S. Akhtar, I. Ali, S. Tauseef, F. Ahmed, S. Ahmed, and S. K. Sherwani, Synthesis, Characterization and Antibacterial Activity of Titanium Dioxide (TiO_2). *Nanoparticles,* **6 (2)** (2016) 141−147.

123. V. Vellora, T. Padil, M. Cernik, Green Synthesis of Copper Oxide Nanoparticles Using Gum Karaya as a Biotemplate and their Antibacterial Application. *International Journal of Nanomedicine*, **8** (2013) 889−898.

124. E. J. Ibrahem, K. M. Thalij, A. S. Badawy, Antibacterial Potential of Magnesium Oxide Nanoparticles Synthesized by Aspergillus Niger. *International Journal of biotechnology*, **18 (1) (**2017) 1−7.

125. Q. He, J. Liu, J. Liang, C. Huang, W. Li, Synthesis and Antibacterial Activity of Magnetic $MnFe_2O_4$/Ag Composite Particles. *Nanoscience Nanotechnology Letters*, **6** (2014) 1−7.

126. R. M. Hussain, F. Bhatti, T. Akhter, J. Hameed, A. Hasan, Antibacterial Characterization of Silver Nanoparticles Against E. coli. ATCC-15224. *Journal of Materials Science & Technology* **24 (2)** (2008) 192−196.

127. K. Y. Yoon, J. H. Byeon, C. W. Park, J. Hwang, Antimicrobial Effect of Silver Particles on Bacterial Contamination of Activated Carbon Fibers. *Environmental Science & Technology*. **42 (4)** (2008) 1251−1255.

128. M. Ahamed, H. A. Alhadlaq, M. A. Khan, P. Karuppiah, N. Al- Dhabi, Synthesis, Characterization, and Antimicrobial Activity of Copper Oxide Nanoparticles. *Journal of Nanomaterererials, (*2014) 1-4.

129. M. Thukkaram, S. Sitaram, S. K. Kannaiyan, G. Subbiahdoss, Antibacterial Efficacy of Iron-Oxide Nanoparticles Against Biofilms on Different Biomaterial Surfaces. *International Journal of Biomaterials*. **6** (2014).

130. A.T. Poortinga, R. Bos, H.J. Bussche, Controlled electrophoretic deposition of bacteria to surfaces for the design of biofilms. *Biotechnology and Bioengineering*. **67(1) (**2000) 117–120.

131. P. Wang, J.K. Bang, H.J. Kim, J.K. Kim, Y. Kim, S.Y. Shin, Antimicrobial specificity and mechanism of action of disulfide-removed linear analogs of the plant-derived Cys-rich antimicrobial peptide Ib-AMP1, *Peptides.* **30(12)** (2009) 2144–2149.

132. Y. Ali, J. Mostafa, and S. Rashidi, Antimicrobial Effects of Electromagnetic Fields: A Review of Current Techniques and Mechanisms of Action, *Journal of Pure Applied. Microbiology,* **8(5)** (2014) 4031-4043.

133. D. Faten, Why Magnetic Fields are Used to Enhance A Plant's Growth and Productivity, *Annual Research & Review in Biology,* **4(6)** (2014) 886-896.

134. S. Ludek, V. Vladim, S. Jan, Effects of low-frequency magnetic fields on bacteria Escherichia coli, *Bioelectrochemistry*, **55(1-2) (**2002) 161– 164.

135. Younis, H.Y.A.; Jamee, W.; Omar, B.; and Salih, J.M.; Bio-impedance detector for Staphylococcus aureus exposed to magnetic fields, *J. Phys., Conference Series* **2012**,407,012020.

136. A. Khaled, T. Abdullah, The Effect of Static Magnetic Field on E. coli, S. aureus and B. subtilis Viability, *Journal of Natural Science Research,* **5(24) (**2015) 2224-3186

137. T. Koji, N.K.O. Kazuhiro, A. Takashi, and S. Makoto, Effect of homogeneous and inhomogeneous high magnetic field on the growth of Escherichia Coli, *Journal of Fermentation Bioengineering*, **81(4)** (1996) 343- 336.

138. S.K. Boda, K. Ravikumar, D.K Saini, B. Basu, Differential viability response of prokaryotes and eukaryotes to high strength pulsed magnetic stimuli, *Bioelectrochemistry,* **106(B)** (2015) 276-89.

139. I. Bajpai, K. Balani, B. Basu, Synergistic effect of static magnetic field and HA-Fe_3O_4 magnetic composites on viability of S. aureus and E. coli bacteria, *Journal of Biomedical Materials Research***, 102B (**2014) 524–532.

140. L. Strasak, V. Vetterl, J. Smarda, Effects of low-frequency magnetic fields on bacteria Eschrichia coli. *Bioelectrochemistry*. **55** (2002) 161–164.

141. W. Gao, Y. Liu, J. Zhou, and H. Pan, Effects of a Strong Static Magnetic Field on Bacterium Shewanella oneidensis: An Assessment by using whole Genome Microarray, *Bioelectromagnetics*, **26(7)** (2005) 558-63.

142. F. Jasmina, B. Kraigher, B. Tepus, V. Kokol, M.M. Ines, Effects of low-density static magnetic fields on the growth and activities of wastewater bacteria Escherichia coli and Pseudomonas putida, *Bioresource Technology*. **120 (**2012) 225–232.

143. M. R. Sofieh, M.K. Ali, The inhibitory effects of static magnetic field on Escherichia coli from two different sources at short exposure time, *Reports of Biochemistry and Molecular Biology*. **5(2**) (2017) 112-116.

144. R. Mittenzwey, R. Siigmuth, W. Mei, Effects of extremely low-frequency electromagnetic fields on bacteria the question of a co-stressing factor, *Bioelectrochemestry and Bioenergetics*, **40 (**1996) 21-27.

145. G. Itai, M. Herzberg, and Y. Oren, The effect of electric fields on bacterial attachment to conductive surfaces, *Soft Matter The Royal Society of Chemistry*. **9 (**2013) 2443–2452.

146. J. Nafziger, H. Desjobert, B. Benamar, J.J. Guillosson, M. Adolphe, DNA mutations and 50 Hz electromagnetic fields. *Bioelectrochemestry and Bioenergetics*, **30 (**1993) 133-41.

147. M. Mehdi, A. Alireza, A. Kasaeian, N. Pirasteh, N. Mojtaba, S.D. Darban, K. S. Sajjad, F. Mozhgan, F. Davardoost, Antibacterial effect of alternating current against Staphylococcus aureus and Pseudomonas aeroginosa, *Russian Open Medical Journal*, **4(2)** (2015) 1-5.

148. A. Malicki, M. Owski, M. Oziembl, T.T. Jerzy, and S. Bruzewicz, Effect of Pulsed Electric Field (Pef) On Escherichia Coli Within The Liquid Whole Egg, *Bulletin of the Veterinary Institute in Pulawy,* **48** (2004) 371-373.

149. S. Min, G.A. Evrendilek, and H.Q. Zhang, Pulsed electric fields: processing system, microbial and enzyme inhibition, and shelf life extension of foods, *IEEE Transaction o Plasma Science,* **35(1) (**2007) 59-73.

150. H.L. Merriman, C.A. Hegyi, C.R. Albright-Overton, J. Carlos, R.W. Putnam, and J.A. Mulcare, A comparison of four electrical stimulation types on Staphylococcus aureus growth in vitro. *Journal of Rehabilitation Research and Development,* **41(2)** (2004) 139-146.

151. R. Newton, and T. Karselis , Skin pH following high voltage pulsed galvanic stimulation, *Physical Therapy,* **63(10) (**1983) 1593-1596.

152. R. A. Mohammad, And T. Giti, Bacterial Inhibition By Electrical Stimulation *Advances in Wound Care* **3(2) (**2014) 91-97.

153. J.S. Petrofsky, E. Schwab, T Lo, M. Cuneo, J. George, J. Kim, and A. Marty, Effects of Electrical stimulation on Skin Blood Flow in Controls and in and around Stage III and IV Wounds in Hairy and Non Hairy Skin. *Medical Science Monitor,* **11** (2005) 309 -316.

154. U. Zimmermann, G. Pilwat, and F. Riemann, Dielectric breakdown of cell membranes. *Biophysical Journal,* **14(11) (**1974) 881-899.

155. A. Sale, and W. Hamilton, Effects of high electric fields on micro-organisms: III. Lysis of erythrocytes and protoplasts. *Biochimica et Biophysica Acta,* **163(1)** (1968) 37-43.

156. K. Shamsi, and F. Sherkat, Application of pulsed electric field in the non-thermal processing of milk. *Journal of Agricultural & Food Industrial Organization*, **2(3)** (2009) 216-244.

157. W.K. Liu, M.R. Brown, and T.S. Elliott, Mechanisms of the bactericidal activity of low amperage electric current (DC). *Journal of Antimicrobial Chemotherapy,* **39(6)** *(*1997) 687-695.

158. J.L. Del Pozo, M.S. Rouse, J.N. Mandrekar, S. M. Fernandez, J.M. Steckelberg, Effect of electrical current on the activities of antimicrobial agents against Pseudomonas aeruginosa, Staphylococcus aureus, and Staphylococcus epidermidis biofilms, *Antimicrobial Agents and Chemotherapy***, 53(1) (**2009) 35-40.

159. R. M. Gubina, and L. Vodovnik, Growth Inhibition in Candida albicans due to low intensity constant direct current. *Journal of Bioelectricity,* **10 (1-2) (**1991) 1-16.

160.C.P. Davis, M.E. Shirtliff, S.L. Hoskins, and M.M. Warren, Quantification, qualification, and microbial killing efficiencies of antimicrobial chlorine-based substances produced by iontophoresis. *Antimicrobial Agents and Chemotherapy,* **38(12) (**1994) 2768-2774.

161. S.A. Jackman, G. Maini, A.K. Sharman, and C.J. Knowels, The effects of direct electric current on the viability and metabolism of acidophilic bacteria. Enzyme, *Enzyme and Microbial Technology,* **24 (**1999) 316-324.

162. A. T. Poortinga, H. C. Smit, M. Van der, and H.J. Busscher, Electric field induced desorption of bacteria from a conditioning film covered substratum *Biotechnology and Bioengineering*, **76** (2001) 395-399.

163. I.; Bajpai, N. Saha, B. Basu, Moderate Intensity Static Magnetic Field has the Bactericidal Effect on E. coli and S. Epidermidis on Sintered Hydroxyapatite. *Journal of Biomedical Materials Research B,* **100B** (2012) 1206−1217.

164. I. Bajpai, K. Balani, B. Basu, Synergistic Effect of Static Magnetic Field and HA-Fe_3O_4 Magnetic Composites on Viability of S. aureus and E. coli Bacteria. *Journal of Biomedical Materials Research B* **102** (2014) 524−532.

165. A. T.; Poortinga, Bos, R.; Busscher, H. J. Lack of Effect of an Externally Applied Electric Field on Bacterial Adhesion to Glass. *Colloids and Surface B,* **20** (2001) 20189−194.

166. S. K. Boda, I. Bajpai, B. Basu, Inhibitory Effect of the Direct Electric Field and HA-Zno Composites on S. aureus Biofilm Formation. *Journal of Biomedical Materials Research B* 2016, **104 (6),** 1064−1075.

167. M. Ueshima, T. Satoshi, N. Satoshi, Yamashita, K. Manipulation of Bacterial Adhesion and Proliferation by Surface Charges of Electrically Polarized Hydroxyapatite. *Journal of Biomedical Materials Research B,* **60 (4)** 2002, 578−584.

168. K. Nozaki, H. Koizumi, N. Horiuchi, M. Nakamura, O. Toshinori, K. Yamashita, N. Akiko, Suppression Effects of Dental Glass-Ceramics with Polarization-Induced Highly Dense Surface Charges Against Bacterial Adhesion. *Dental Materials Journals*, **34 (5)** (2015) 671−678.

169. S. Swain, R.N.Padhy, T. R. Rautray, Polarized Piezoelectric Bioceramic Composites Exhibit Antibacterial Activity Mater. *Chemical Physics*. **239** (2020) 122002.

170.A. H. Rajabi, M. Jaffe, T. L. Arinzeh, Piezoelectric materials for tissue regeneration: A

review, *Acta Biomaterialia*, 24 (2015) 12-23.

171. A.S. Verma, A. Sharma., A. Kumar, A. Mukhopadhyay, D. Kumar, and A. K. Dubey, Multifunctional response of piezoelectric sodium potassium niobate (NKN) toughened hydroxyapatite based biocomposites, *ACS Applied Biomaterials*, **3** (2020) 5287-5299. 5210.

172. S. Subhasmita, R. N. Padhy, T. R. Rautray, Polarized piezoelectric bioceramic composites exhibit antibacterial activity, *Materials Chemistry and Physics* **239** (2020) 122002.

173 Harkes G, Feijen J, Dankert J. Adhesion of EWerichia COD on to a series of poly(methacrylates) differing in charge and hydrophobicity, *Biomaterials* **12** (1991) 853-860.

174 N. Saha, A. K. Dubey, B. Basu, Cellular Proliferation, Cellular Viability, and Biocompatibility of HA-ZnO Composites. *Journal of Biomedical Material Research Part B.* **100B** (2012) 256-264.

175. J. Marchia , C. Ribeirob , A. Almeida Bressianic , M. M. Marquesd, Cell Response of Calcium Phosphate Based Ceramics, a Bone Substitute Material, *Materials Research*, **16(4)** (2013) 703-712.

176. A, Rajendran, R.C. Barik D. Natarajan, M.S. Kiran, D.K. Pattanayak. Synthesis, phase stability of hydroxyapatite-silver composite with antimicrobial activity and cytocompatability. *Ceramics International*, **40 (**2014) 10831-10838.

177. F.Wu, J. Wei, H. Guo, F.Chen, H. Hong, C. Liu, Self-setting bioactive calcium–magnesium phosphate cement with high strength and degradability for bone regeneration, *Acta Biomaterialia* **4** (2008) 1873–1884.

178. P. Duan, J. Shen, G. Zou, X. Xia, B. Jin, Biomimetic mineralization and cytocompatibility of nanorod hydroxyapatite/graphene oxide composites, Front. *Chemical Engineering Science*, **12(4)** (2018) 798–805.

179. A.K. Dubey, G.Tripathi, B.Basu, Characterization of hydroxyapatite-perovskite (CaTiO3) composites: Phase evaluation and cellular response. *Journal of Biomedical Material Research partB,* **95(2) (**2010) 320-9

180. A. E. Ghannam, Bone reconstruction: from bioceramics to tissue engineering, *Expert Review of Medical Devices.* **2** (2005),87-101

181.Y. Zhang, L. Chen, J. Zeng, K. Zhou, D. Zhang, Aligned porous barium titanate/hydroxyapatite composites with highpiezoelectric coefficients for bone tissue engineering, *Materials Science and Engineering C* **39** (2014) 143–149.

182. A. K. Dubey, B. Basu, K. Balani, R. Guo, and A. S. Bhalla, Multifunctionality of Perovskites $BaTiO_3$ and $CaTiO_3$ in a Composite with Hydroxyapatite as Orthopedic Implant Materials *Integrated Ferroelectrics*, **131** (2011) 119–126.

183. A. K. Dubey, B. Basu, Pulsed Electrical Stimulation and Surface Charge Induced Cell Growth onMultistage Spark Plasma Sintered Hydroxyapatite-Barium Titanate Piezobiocomposite, *Journal of the American Ceramic Socity,* **97 (2)** (2014) 481–489.

184. F.R.Baxter, I.G. Turner, C. R.Bowen, J.P.Gittings, J.B.Chaudhuri, An In vitro Study of Electrically Active Hydroxyapatite-Barium Titanate Ceramics Using SaOS-2 Cells, *Journal of Materials Science: Materials in Medicin*e, **20** (2009) 1697-1708.

185. D. Kumar, J.P. Gittings, I.G. Turner, C.R. Bowen, Polarization of Hydroxyapatite: Influence on Osteoblast Cell Proliferation *Acta Biomaterialia*, **61**(2010) 549–1554.

186. S.L. Percival, P.G. Bowler, D. Russell, Bacterial resistance to silver in wound care. *Journal of hospital infection*, **60(1)** (2005) 1-7.

187. D. Kumar, J.P. Gittings, I.G. Turner, C.R. Bowen, L.A. Hidalgo-Bastida, S.H. Cartmell, Polarization of hydroxyapatite: Influence on osteoblast cell proliferation, *Acta Biomaterialia*. **6(4)** (2010) 1549-54.

188. A. K. Dubey, B. Basu ,Pulsed Electrical Stimulation and Surface Charge Induced Cell Growth on Multistage Spark Plasma Sintered Hydroxyapatite-Barium Titanate Piezobiocomposite, *Journal of the American Socity,* **97 (2)** (2014)481‑489.

189. A. Singh, R. Kuppili, A. K. Dubey, combined effect of surface polarization and addition of ZnO on antibacterial and cellular response of HA- ZnO composite. *Materials Science and Engineering C,* **107** (2020) 110363

190. O. Masataka, T. K. M. Katsura, K. Yamashita, Manipulation of Selective Cell Adhesion And Growth by Surface Charges of Electrically Polarized Hydroxyapatite *Journal of Biomedical Material Research,* **57(3)** (2002) 366-73.

191. S Bodhak, S. Bose, A. Bandyopadhyay, Role of Surface Charge and Wettability on Early Stage Mineralization and Bone Cell-Materials Interactions of Polarized Hydroxyapatite. *Acta Biomaterialia,* **5** (2009) 2178–2188.

192. Tan G, Wang S, et al., Surface-Selective Preferential Production of Reactive Oxygen Species on Piezoelectric Ceramics for Bacterial Killing. *ACS Appl Mater Interfaces,* 8 (2016) 24306–24309

193. L. Atanasoska, R. Radhakrishnan, S. Schewe, Medical devices employing piezoelectric

materials for delivery of therapeutic agents. *In: Google patents* (2014) US8702682B2.

194. A. S Verma, D. Kumar, A. K. Dubey, Antibacterial and cellular response of piezoelectric $Na_{0.5}K_{0.5}NbO_3$ modified1393 bioactive glass, *Material Science and Engineering C,* **116** (2020) 111138.

195. Takayuki Kobayashi, Satoshi Nakamura, Kimihiro Yamashita, Enhanced osteobonding by negative surface charges of electrically polarized hydroxyapatite, *Journal of Biomedical materials Research*, 2001, https://doi.org/10.1002/1097-4636(20011215)57:4<477.

196. F. Grinnell, M.K./ Feld, Fibronectin adsorption on hydrophilic and hydrophobic surfaces detected by antibody binding and analyzed during cell adhesion in serum containing medium, *Journal of Biological Chemistry,* **257** (1981) 4888-1982.

197. M. Ohgaki, T. Kizuki, M. Katsura, and K. Yamashita, “Manipulation of Selective Cell Adhesion and Growth by Surface Charges of Electrically Polarized Hydroxyapatite,” *Journal of Biomedical materials Research*, **57** (2001) 366–73.