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GRAPHENE OXIDE-MODIFIED HYDROXYAPATITE NANOCOMPOSITES IN BIOMEDICAL APPLICATIONS: A REVIEW

NORSURIANI CHE HASHIM*, DANIEL FRANKEL**, #DARMAN NORDIN*

*Research Centre for Sustainable Process Technology, Faculty of Engineering & Built Environment, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
**School of Engineering, Newcastle University, NE1 7RU, United Kingdom

#E-mail: darman@ukm.edu.my

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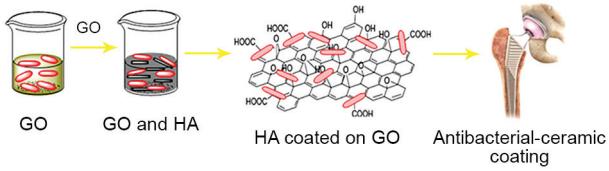
Bacterial infection and cytotoxicity associated with implant materials used in medical devices are the major cause of implant failures. This review focusses upon the development of graphene oxide (GO) – hydroxyapatite (HA) nanocomposites as potential coating materials that can provide a solution to the rejection of implants. These nanocomposites combine the unique antibacterial properties of graphene oxide with the natural mineral composition of hydroxyapatite, as found in human bones and tooth enamel. They have potential antibacterial applications in the fields of orthopaedics, orthodontics and cardiovascular medicine. The methods used for preparation of HA and GO in addition to the combining of GO–HA nanocomposite are discussed. Cytotoxicity and antibacterial affects of GO and GO–HA to biological systems are examined as well as future applications in related fields.



INTRODUCTION

The continuing evolution of biomedical nanotechnologies has enabled clinicians to recognise, prevent, and treat human diseases [1]. There is currently great interest in developing nanotechnologies that interface with biological materials such as cells and tissues. The interfacing of nano and biological materials increases the demand for in-depth understanding that enables vital information to be gained concerning relevant energetic and biological processes [2]. An understanding of the propensity for nanoparticles to attach to and disrupt cell membranes remains an open question due to the heterogeneous and dynamic nature of cell membrane. Systematic investigations of nanoparticle-cell membrane interactions can be performed using model biological membranes [3]. The surface nanoparticles like surface features and modifications are important in order to tune their response in contact towards biological environments [4].

Composite materials offer many advantages over single component systems, combining properties that meet the requirements of biomedical applications, for example orthopaedic and dentistry applications. A wide variety of composites have been constructed and tested in medical applications as a result of the seminal idea to design man-made composites that mimic the properties of natural composites [5].

HA is a bioceramic material that is often used in clinical bone grafting and implantation. It is able to chemically bond with living bone tissue due to its biological and chemical composition, and its chemical structure which is identical to native apatite in the human skeleton. HA can also encourage osteoblast adhesion and proliferation because of its bioactivity [6]. Bone tissue engineering presents a viable approach to repair and reconstruction of bone tissue imperfections. The use of biocomposite materials that mimic natural bone is well established [7]. Accordingly, HA is a widely-accepted biomaterial for the repair and regeneration of damaged bone tissue [8].

Nevertheless, the mechanical properties of HA still present a major hindrance because its low toughness and low flexural strength limit its use in bone system regeneration [5, 8-10]. Thus, second phase reinforcement of HA with materials such as metals, polymers and other organics has been developed and investigated [5, 8, 9].

Importantly, HA is not able to inhibit bacterial adhesion, which might disturb the bone healing process and cause infections that lead to implant failure. About half of the million hospital-acquired infections per year in the US are associated with implanted devices.

An awareness of surgical site infections that are related to implanted orthopaedic devices is necessary for public health due to an increasing number of aged and disabled patients. Bacterial infections causing biofilms can form on implant surfaces and are difficult to treat with antibiotics. Consequently, new strategies to reduce bacterial activity and device-related infections have been developed [11]. Implantation of composites in living organisms can lead to biofilm formation and bacterial infection. Hence there is a need for implant surfaces that not only have excellent biocompatibility but also antibacterial properties [12].

Multidrug resistant (MDR) pathogens have become resistant to certain antibiotics and contribute to problematic infections worldwide. In both the developed and the developing world, there are six pathogens ('ESKAPE' pathogens) that are most frequently found in hospitals and are hardest to treat. They comprise two Gram positive bacteria (*Enterococcus faecium* and *Staphylococcus aureus*) and four Gram negative bacteria (*Klebsiella pneumonia*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.*). These nosocomial pathogens cannot be treated by typical antibacterials and are resistant to the most used and known antibiotics. Accordingly, there is a need to find other mechanisms for killing pathogens in biomedical applications [13]. Thus the antibacterial properties of agents such as carbon nanotubes, metal nanoparticles and metal oxide nanoparticles have been explored. Recently, graphene has been proposed as an effective antibacterial material that has severe cytotoxic effects on bacteria, fungi and other pathogens [14].

Graphene has generated substantial attention from researchers since 2004, due to its fascinating physical and chemical properties, which are widely applicable in biomedicine. It is considered as a strong candidate for an antibacterial material due to its bacterial toxicity. Although graphene has been successfully applied in biomedicine, there has been growing debate in recent years about the potential toxicity of graphene and its derivatives in biological systems at different levels of bacteria. In addition, the growing usage of graphene and its derivatives means that there is a demand for greater understanding of their potential adverse impacts on human health [15]. Coating releasing antibacterial agent have shown potential to decreases nosocomial infections [16].

This review aims to offer a basic background of hydroxyapatite and graphene oxide and discuss some existing research regarding the use of graphene oxide to reinforce hydroxyapatite. The use of graphene oxide-hydroxyapatite composites as nanocoatings in biomedical implants could improve their antibacterial properties by influencing interactions with biological systems. Herein, GO and GO coated HA are discussed through in vitro and in vivo cytotoxicity as well as antibacterial activity towards bacterial cell membranes.

THEORETICAL

Implant Infections

Implant techniques have found utilization in many areas of medicine (orthopaedics, cardiovascular, dentistry, traumatology and neurosurgery). Surgical intervention with subsequent implantation influence post-operative recovery, preventing inflammatory responses and of the implant rejection processes [17]. The crucial characteristics of well-designed implants include porosity, elastic biocompatibility, appropriate mechanical strength, low surface friction, corrosion resistance, improved tribology, ability to reduce inflammation and reduced bacterial adhesion to the surface. Global annual market for orthopaedic implant devices reach $30 billion by 2010 [18], $46.5 billion by 2017, $50 billion by 2018 and expected market of more than $62.6 billion by 2022.

Millions of people worldwide, especially those over 50 years of age, suffer from bone and joint degenerative and inflammatory problems. Forecasts show that the number of bone disease sufferers will double by 2020. Worldwide millions of medical devices are used annually. Orthopaedic implant devices including prostheses for hip, knee, ankle, shoulder and elbow joints and fracture fixation such as wires, pins, plates and screws are designed to regain the normal function of load-bearing joints. Metals, polymers and ceramics are three classes of material that are frequently applied in the fabrication of orthopaedic implants. As an example of a bioactive ceramic, hydroxyapatite acts as coating materials for metal implants located at femoral stems and acetabular metalbacks for hip joints, as well as tibial and femoral components for knee joints. Sources of bacterial infection from implants include surgical apparatus, the surroundings of the operating room, clothes worn by medical and paramedical staff and resident bacteria on the patient's skin and body [19].

Nosocomial infections are a major health challenge and are the sixth most common cause of death in developed countries. Implant rejection and repeated surgery are major sources of clinical problems. Improved antibacterial coatings may help to meet these challenges and reduce the incidence of resistant bacterial infections. Bacteria can be attached to all instruments in the clinical environment as well as to implants inserted into the human body. The surfaces of biomedical devices should deter bacterial attachment to sustain stability and maintain antibacterial action in vivo. Accordingly, coating materials for medical devices and new mechanisms of antibacterial resistance have been developed in order to avoid microorganism adhesion and biofilm formation. The addition of antibacterial coatings can prevent bacterial colonisation as well as avoiding surgical complications [20].

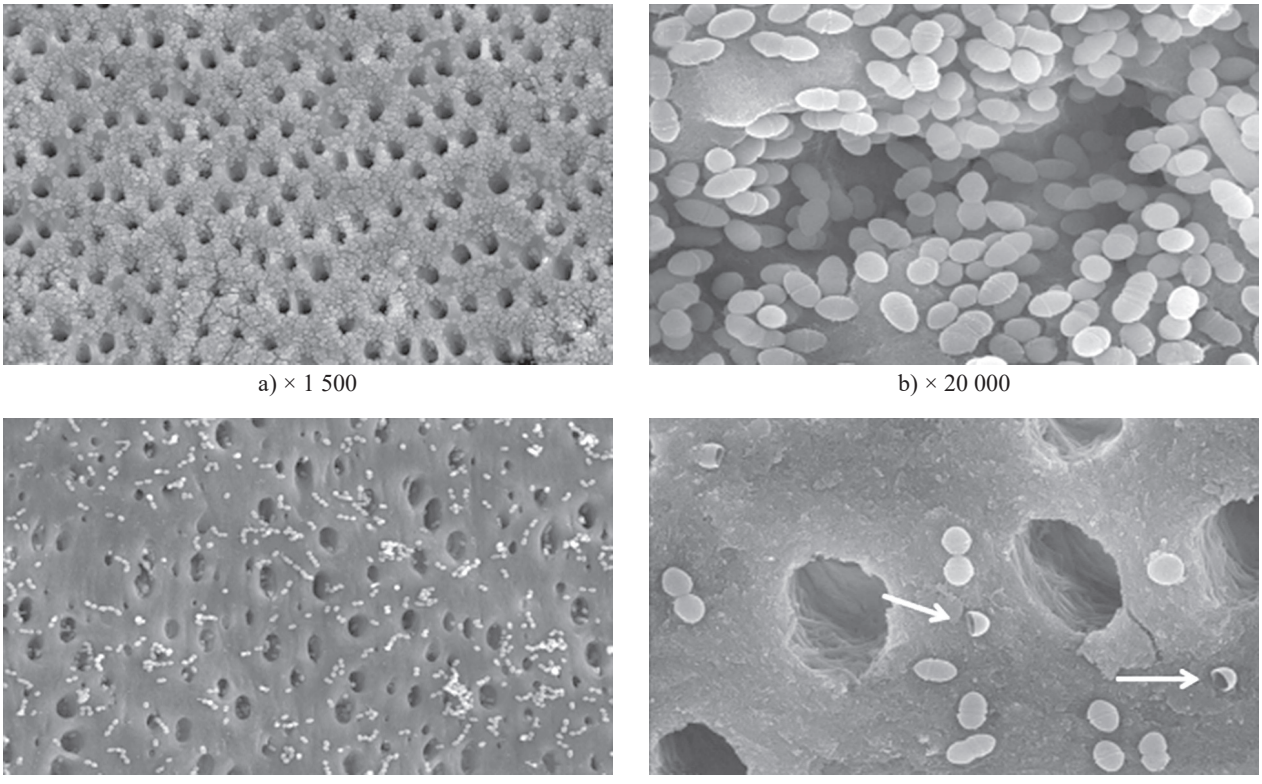
Orthopaedic implant-associated bacteria are challenging complications which can cause delayed healing,

implant loosening, implant removal, amputation or even death [21]. Infection in orthopaedic implants is one of the most common infections encountered in medicine. Surgical site infection is a major problem causing increase in non-union, osteomyelitis, implant failure, sepsis, multiorgan dysfunction and even death. Antibiotics cannot reach the bacteria in biofilms on the implant surface causing infections. 7 in every 132 patients developed infection, which make up 5.30 % cases of orthopaedic implant infection.

Staphylococcus aureus is the most common bacteria responsible for surgical site infection in orthopaedic implants [22] 70 % of orthopaedic implants infection is contribute by *S. aureus* and for another 8 % of infections by *Pseudomonas Aeruginosa* [23]. Gram negative *E. coli*, *P. mirabilis* and *P. vulgaris* have also been involved in implant-associated infection [24]. It was estimated that *S. aureus* and *S. epidermidis* caused about 40 - 50 % prosthetic heart valve infections and 50 - 70 % catheter biofilm infections [25]. Microbial infections are related to the colonization of pathogens on the surface in almost all medical devices or implants like orthopaedic devices, catheter (central venous, urinary), respirators, prosthetic heart valves and dental implant (Tab. 1). Research conducted by Universiti Kebangsaan Malaysia Medical Centre (UKMMC), *S. epidermidis* was the prevalent *Coagulase- negative staphylococci* identified as agent of clinical infections in UKMMC [26]. *E. faecalis* attached to dentinal surface was damaged (ruptured) membranes were often

Table 1. Biofilms in medical implants [27, 28].

Medical devices	Microorganisms
Contact lens	*P. aeruginosa*, Gram positive cocci
Denture	*Candida spp*
Urinary catheter	*E. coli*, *K. pneumoniae*, *Candida spp*, *P. mirabilis*, *E. feacilis*
Central venous catheter	*Coagulase-negative staphylococci*, *S. aureus*
Mechanical heart valve	*Coagulase- negative staphylococci*, *S. aureus*
Artificial hip prosthesis	*Coagulase- negative staphylococci*, *S. aureus*
Voice protheses	*C. albicans*, *Coagulase- negative staphylococci*
Endotracheal tubes	Enteric Gram – negative species
Orthopeadic implant	*Enterococci*, *P. mirabilis*, *P. aeruginosa*, *E. coli*, *Bacteroides spp*, *Hemolytic streptococci*
Replacement joints	*S. aureus* and *S. epidermidis*
Breast implant	*S. aureus*, *Enterococci* and *S. epidermidis*



a) × 1 500 b) × 20 000

c) × 1 500 d) × 10 000

Figure 1. SEM images of E. faecalis- infected dentine blocks treated with saline and chlorhexidine. Blocks treated with saline solution for 5 min demonstrate many adhering *E. faecalis* (a) with intact bacterial membranes (b). The group soaked with 2 % chlorhexidine show fewer adhering bacteria (c) and many lysed *E. faecalis* (d – white arrows) [29].

found in 5 min-soaked chlorhexidine (antimicrobial agent) (Figure 1).

Factors like differences in implant surface hydrophilicity, surface charge, surface energy, biomaterials composition play a role in enhance the rate of infection in implants [30]. The primary bacterial adhesion to implant surfaces mediated by reversible interactions are van der Waals forces and steric- electrostatic interactions. Afterwards, the bacterial cells surfaces (lipopolysaccharides and exopolysaccharides) adhere irreversibly to the substrates through hydrogen bonds, ionic bonding and dipole-hydrophobic interactions [27].

Antibacterial coating strategies for medical devices include the release of antibacterial agent, contact-killing (CK) and anti-adhesion (AA). In antibacterial agent release techniques, the coating is loaded with a drug which leaches out over time via diffusion, degradation or hydrolysis. This release is temporary due to the limited reservoir of antibacterial agents. In the contact-killing technique, antibacterial drugs directly kill adhered bacteria on the surface due to their ability to disrupt the bacterial cell membrane. The anti-adhesion (AA) technique is the earliest prevention step for the colonization of bacteria and biofilms using non-cytotoxic mechanisms such as PEG and zwitterions [16]. All of these strategies can be achieved by GO containing materials.

Hydroxyapatite ceramic coatings

Human bone comprises 30 % organic matter (mainly collagen) and 70 % inorganic matter (mainly hydroxyapatite, HA) [9]. It can be defined as a bioceramic composite, and has long drawn the attention of materials researchers aiming to duplicate its mechanical features such as high strength and fracture toughness [31].

Hydroxyapatite, (HA) [$Ca_{10}(PO_4)_6(OH)_2$] comprises the major part of natural bone tissue. It shows good bioactivity and has excellent biocompatibility with human tissues [32], good osteoconductivity, biodegradability, and is extensively applied in biomedical applications [33]. The ability ceramic to interact with bone tissue is a unique property of bioactive ceramics [34].

HA has a plate like structure 20 to 80 nm long, and 2 to 5 nm thick, with a hexagonal crystal system [5]. In natural bone HA is present in the form of nano-size crystals. Calcium and phosphorus are the fundamental elements in HA, with a stoichiometric ratio of 1.667 for calcium and phosphate [8]. The packing structure of hydroxyapatite crystals with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$, and a digital photograph of a hydroxyapatite implant are shown in Figure 2. HA is the most stable form of phosphate salts under both acid and alkaline conditions. The high specific surface area and unique properties of HA allow it to be widely applied in orthopaedic or bone implants, as a pharmaceutical carrier, in anti-cancer, and anti-bacterial agents [35].

It is dispersed in the human body in the form of its solid or ionic state, through bone resorption and reconstruction and metabolic processes involving calcium and phosphorus ions in bone tissue. Hence, HA is extensively applied as artificial bones in the orthopaedic field for the repair of bone defects. Previous studies have shown that the implantation of HA in bone defects can improve the division and differentiation of stem cells due to its osteoconductive ability [9]. Osteoconductive interactions with HA lead to bone bonding and regeneration [10].

Currently, many HA composites with organic materials are being developed, for example synthetic and natural polymers with good biocompatibility, antibacterial activity and will induced osteogenesis [9], and bio-active, non-inflammatory and non-immunogenic properties [8]. The high specific surface area of the rod-like HA structure can provide better protein absorbability and thus good biocompatibility and bioactivity [10]. Biocompatibility is described as chemical stability, resistance to corrosion, noncarcinogenicity and nontoxicity when a material is implanted in the human body [5].

Synthetic HA nano powder has been demonstrated as a coating material in orthopaedic and dental implants. HA coatings have good potential due to their biocompatible and bone-like ceramic properties. Orthopaedic implants have been successful due to a focus on improving the mechanical and biological properties of HA coatings with bionanotechnology. The quality of the coating is influenced by the synthesis process of the HA powder, which influences important factors such as the phase composition, purity, crystallinity, particle size, particle-size distribution, specific surface area, density and surface morphology. The HA Ca/P ratio of 1.52 - 2.0 makes it a superior choice in dental and orthopaedic bioceramic coating applications [36]. Figure 2 shows the structure and implant of HA.

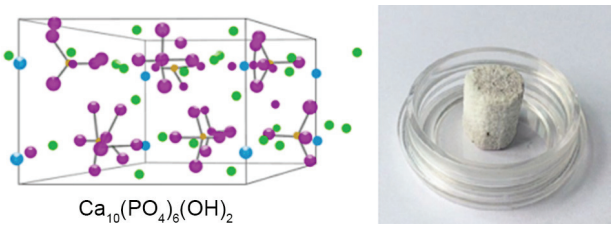


Figure 2. Packaging structure of hydroxyapatite crystal with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ (a) and digital photo of hydroxyapatite implant (b) [17].

DISCUSSION

In situ synthesis of HA

To date, many diverse methods have been published for the preparation of HA nanoparticles, while growing interest in the manufacture of HA means that

new preparation methods continue to be developed [37]. In recent years, methods applied to HA synthesis have included precipitation techniques [38], sol-gel approaches, and hydrothermal, multiple emulsion, biomimetic deposition and electrodeposition techniques [39] and polymeric sponge template [40].

Wet chemical synthesis

Wet chemical methods are aqueous methods that involve the synthesis of HA where the final products gained are characterised by a high degree of crystallisation and high purity. These properties depend on the conditions of synthesis such as the temperature, the starting concentration of the reactants, the pH environment, the acid addition rate, the stirring speed and the thermal treatment conditions applied to the dried HA powders (Figure 3) [8].

This technique is widely used and is the most popular technique for the practical and economical synthesis of HA in large amounts at low reaction temperatures, with microstructure properties in the final product and no harmful by-products [39, 41]. Moreover, this technique is one of the easiest ways to prepare HA powders. It was first proposed by Yagai and Aoki, using calcium hydroxide ($Ca(OH)_2$) and orthophosphoric acid (H_3PO_4) as the starting materials [42]. The precipitation reaction is shown in chemical Equations 1 and 2.

$$CaO + H_2O \rightarrow Ca(OH)_2 \quad (1)\ [43]$$

$$10\ Ca(OH)_2 + 6\ H_3PO_4 \rightarrow \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18\ H_2O \quad (2)\ [43]$$

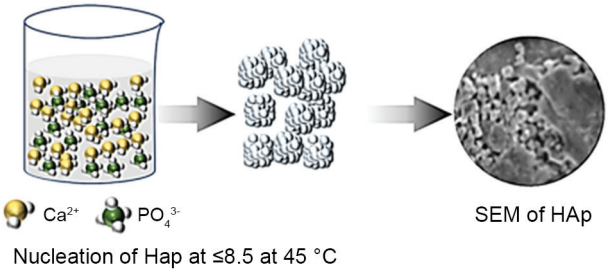


Figure 3. Graphical representation of the wet chemical synthesis of HA [8].

Sol-gel technique

A sol-gel process is a process in which a sol (colloidal suspension of solid particles) is transformed into a 3D network in the solid phase. In this technique precursors are mixed, then aged, gelated, dried and calcinated to remove remaining organic material [12]. The sol-gel is preferred because of its low synthesis temperature, high product purity, homogenous molecular mixing and ability to produce nano-sized particles [44] It also has the flexibility to produce nanocrystalline powders, bulk amorphous monolithic solids and thin films. Additionally, it is easily applied to surface coating and can be used in the preparation of high quality HA films on metal substrates [45].

Furthermore, the method does not require pH control, high temperature or vigorous agitation. The crystallinity of HA decreases with decreasing temperature. As a result, using the technique at lower temperatures might be time consuming, expensive and complicated [46]. The sol-gel HA reaction is shown in Equation 3 and Figure 4.

$$6Ca(NO_3)_2 + 6(C_2H_5O)_3P(O) \rightarrow \rightarrow Ca_{10}(PO_4)_6(OH)_2 + \text{by-products} \quad (3)\ [37]$$

Hydrothermal technique

The hydrothermal process is a chemical reaction process in aqueous solution that takes place at higher temperatures and pressures. It can also be classified as a chemical precipitation in which the aging step is operated at temperature above the boiling point of water, inside an autoclave or pressure vessel. Increasing the hydrothermal temperature can improve the phase purity and Ca/P ratio of HA. However, the hydrothermal process is more expensive than other wet methods due to the expensive equipment required for the high temperature and pressure reaction [37]. The preparation of rod like HA via the hydrothermal technique is shown in Figure 5.

The different wet methods for the preparation of HA are compared and summarised in Table 2.

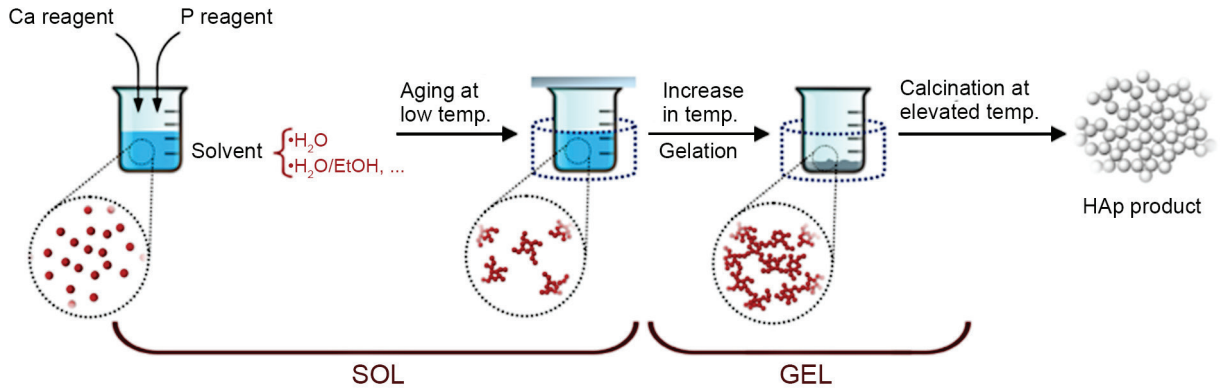


Figure 4. Synthesis of HA by the sol-gel technique [37].

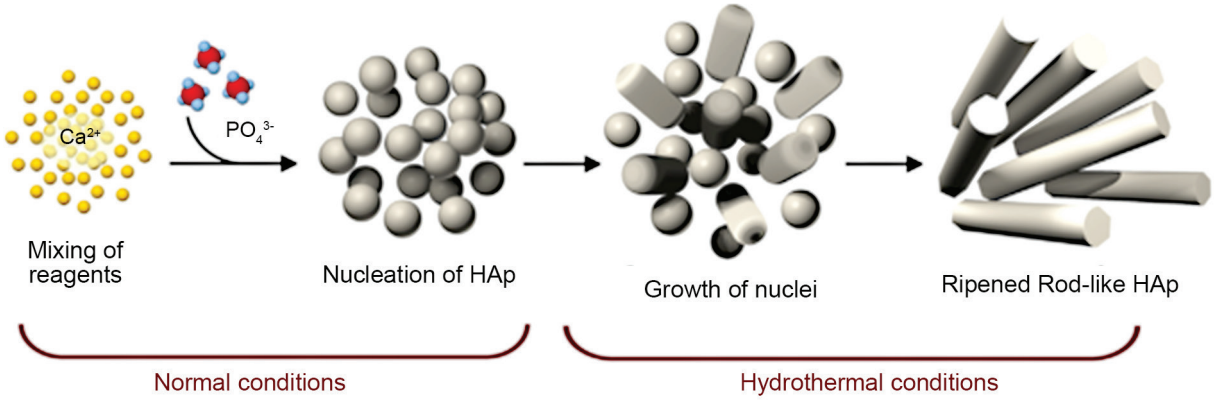


Figure 5. Preparation of rod like HA via the hydrothermal technique [37].

Table 2. Comparison of different wet methods for HA preparation. Modified from [46].

Characteristics/ Method	Chemical precipitation	Sol-gel	Hydrothermal
Cost	Low	Variable	Generally high
Morphology	Diverse	Diverse	Frequently needle-like
Crystallinity	Frequently low	Generally low	Very high
Phase Purity	Variable	Variable	Generally high
Ca/P ratio	Non-stoichiometric	Stoichiometric	Stoichiometric
Size	Generally nano	Nano	Nano or micro

Graphene oxide antibacterial coatings

One of the approaches to biomaterials surface resistant to biofilm is bactericidal or bacteriostatic coatings to the surface [25]. Two main characteristics needing of these coatings. Firstly, necessary to avoid bacterial attachment and resistance to bacteria development. Secondly, coatings should guarantee long-term applications [20].

Graphene is a two-dimensional (2D) nanomaterial comprising a monolayer of carbon atoms [47] arranged in a flat honeycomb lattice [48]. It is pristine and arranged in a sp2 bonded aromatic structure [49]. Graphene is one of the allotropes (carbon nanotube, fullerene, diamond) of carbon, with a carbon-carbon bond length of 0.142 nm, its electrons behave like massless relativistic particles [50].

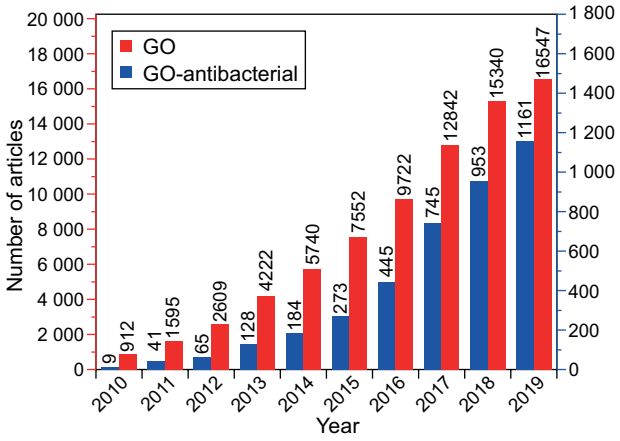


Figure 6. Publications on GO and its antibacterial interactions based on the number of articles in the "Sciencedirect" database from the year 2010 to August 2019.

These single-atom-thick, hexagonally arranged carbon atoms in two dimensional sheets, first discovered in 2004, have been the subject of an explosion of new research, and led to the Nobel Prize in 2010. In 2010, the number of published scientific papers on graphene exceeded 3000 in a single year and worldwide, there are a growing the number of companies that manufacture graphene products although most are primarily doing research and development [51]. Quantitative analysis of scientific data (Figure 6) shows that the used of graphene materials as antibacterial compound became interest in the last few years.

Reports indicate that in 2020 the market projection of graphene based products will reach $675 million [52]. Scientists have eagerly embraced graphene as it is a lighter and cheaper alternative to existing metal conductors [50]. Benefits of GO include low cost, accessibility and the ability to alter graphene [17].

Graphene has extraordinary physical, chemical, mechanical [32] and optical properties [47]. The unique properties of graphene are shown in Table 3.

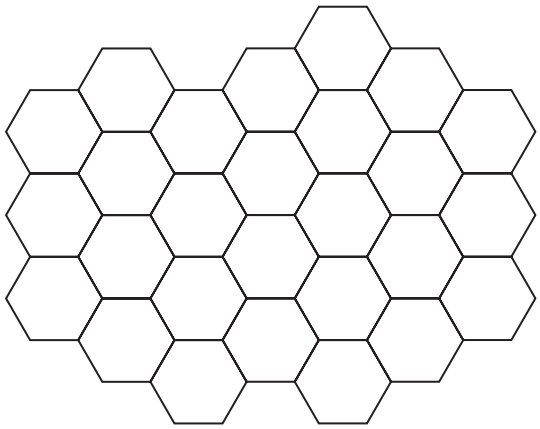
Its high specific surface area is a major benefit in the high-density bio-functionalisation that is fundamental to drug delivery [54]. The theoretical specific surface area of graphene sheets is 2630 $m^2 \cdot g^{-1}$, much larger than the latest reported carbon black, at 900 $m^2 \cdot g^{-1}$, while

carbon nanotubes (CNTs) have a surface area of about 100 to 1000 $m^2 \cdot g^{-1}$. Its larger surface area combined with other high performance properties has contributed to its use in energy applications, for instance in transparent conductive electrodes for solar cells, high capacity electrodes in lithium-ion batteries and supercapacitors, and in hydrogen storage [55].

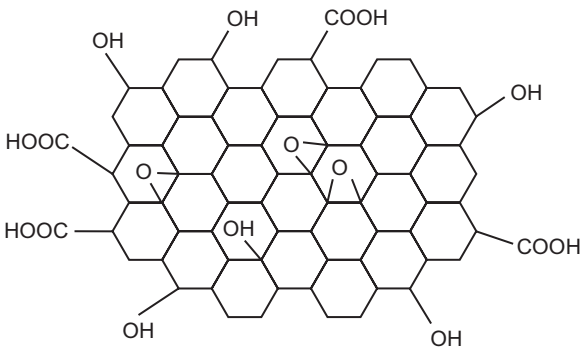
The layered structure of GO is similar to graphene, but GO carbon atom planes contain oxygen groups, resulting in expansion of the interlayer distance and making the hydrophilic atomic layer thicker. The GO sheet structure is known to contain functional oxygen groups including C–O, C=O and –OH, which act as a support on the surface of a graphene monolayer (Figure 7). Existence of oxygen-containing groups brings about the stronger hydrophilic properties of GO, as well as allowing dispersion in some solvents, such as water [56]. In other words, it has hydrophilic character, and water molecules can easily intercalate between the graphite layers. GO acts as a 2D amphiphile with hydrophilic carboxyl groups at the edges and hydrophobic epoxy, hydroxyl and graphitic domains on its basal plane [57]. GO surfaces are capable of hydrogen bonding and metal ion complexation due to partially hydrophobic and hydrophilic regions, and negatively charged carboxylate groups at edges or defect sites.

Table 3. The unique properties of graphene [53].

	Properties of graphene
i.	the thinnest material
ii.	the strongest and stiffest material
iii.	nearly transparent
iv.	the most stretchable crystal
v.	high thermal conductivity
vi.	the highest current density at room temperature
vii.	being entirely impermeable
viii.	the highest intrinsic mobility (100 times more than Si)
ix.	conducting electricity with no electron limit
x.	having a large surface area
xi.	the longest mean free path at room temperature



a) single graphene sheet



b) graphene oxide (GO)

Figure 7. Schematic structure of: a) a single graphene sheet; b) graphene oxide (GO) [56].

The presence of plentiful carbonyl, epoxide and hydroxyl groups in GO provides large numbers of chemically reactive sites. GO also offers advantageous mechanical properties, high hydrophilicity and good biocompatibility that could make it a promising nanoscale reinforcement filler in biocomposites that can increase interfacial bonding among the components [32].

The main function for GO hybrid is to enhance the composite mechanically. GO is frequently used in HA biomimetic synthesis due to its lack of toxicity to cells and the high mechanical strength of graphene-based composites [35]. Improved mechanical function could be observed in Table 4.

GO has been used in a wide range of applications such as cell imaging, drug delivery and gas separation membranes [47], antibacterial treatments [67], adsorbent materials [68], tissue engineering [69] and filler in polymer membrane [70]. Other engineering applications have emerged due to research into graphene properties including uses in structural composites, conducting polymers, antibacterial papers and biomedical technologies [51]. Figure 8 presents the overall application graphene-based have been investigated for biomedical applications. non-medical and medical applications [71].

Table 4. Improved mechanical function by GO hybrid.

GO hybrid	References
GO/HA/PVA	[58]
GO/HA/PLA	[59]
GO/HA/chitosan (CS)	[9]
GO/polycaprolactone (PCL)	[60]
GO/HA	[39]
GO/HA/ sodium alginate (SA)	[61]
GO/HA	[62]
GO/HA/CS	[63]
GO/HA/PLA	[41]
GO/PLGA	[64]
GO/starch	[65]
GO/epoxy	[66]

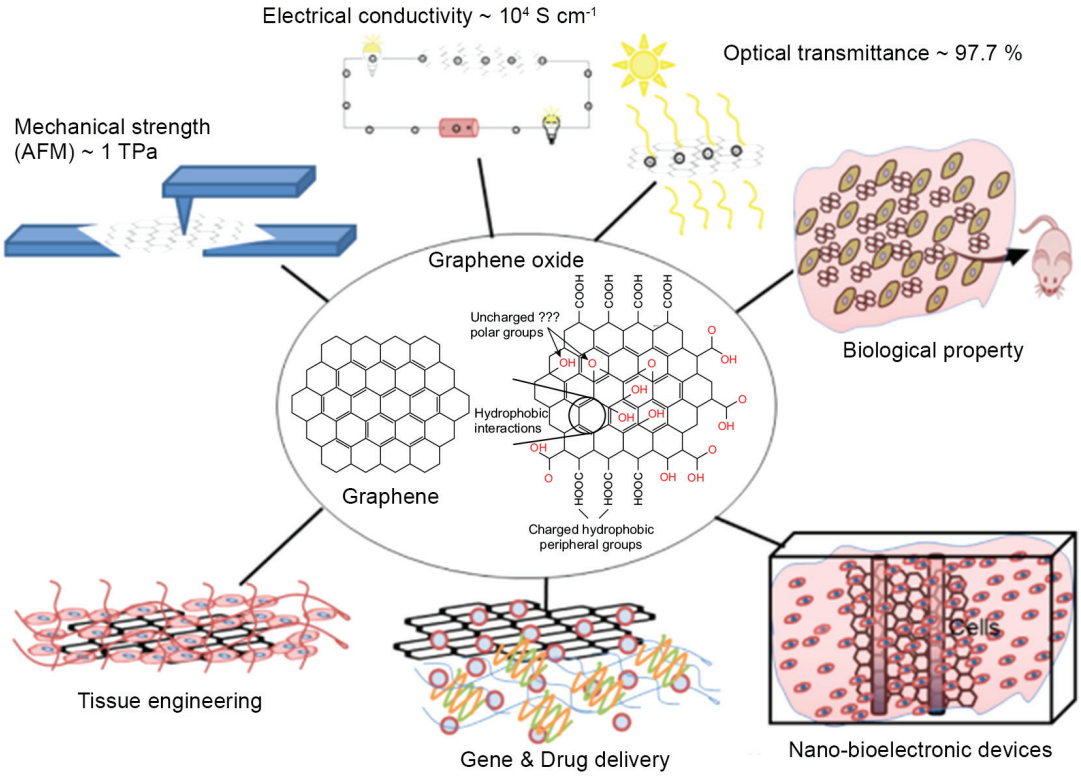


Figure 8. Graphene and graphene oxide (GO): Various non-medical and medical applications [71].

Production of GO

Modified Hummers method

The synthesis of graphene involves exfoliation and cleavage, chemical vapour deposition (CVD), thermal decomposition and electrochemical reduction. Attractive methods of preparation that have been used in recent years are graphite oxidation, GO aqueous dispersion and GO reduction [72]. However, the above methods are less effective in large scale manufacture because they produce very low yields and cannot achieve high-quality on the industrial scale [73]. Hummer's method is the most popular technique used by researchers [72].

GO is graphite that has been oxidised together with oxygen molecules in carbon layers. The interplanar spacing between the layers of graphite is increased when oxidising agents react with graphite [74]. The first synthesis of GO was demonstrated by Brodie in 1859, adding a sample of potassium chlorate to a slurry of graphite in fuming nitric acid. The improvement by Staudenmaier in 1898 used a mixture of concentrated sulphuric acid and fuming nitric acid and continued with the dual addition of chlorate to the reaction mixture, resulting in highly oxidised GO. In 1958, Hummer reported an alternative method to synthesise graphene oxide by using $KMnO_4$ and $NaNO_3$ in concentrated H_2SO_4 [56, 75].

GO-HA nanocomposite coating

Like other composite system, homogenization of second phase reinforcement is crucial to the performance of GO-HA. There are two main goals during composite preparation when combining HA and GO. First is to create homogenous dispersion of HA coated on GO, ensuring uniform properties throughout the composite. Second to encourage interaction between the HA and GO. The well mixing and blend allow composite can disperse easily and can improve interfacial bonding. Figure 9 present the classification of different powder or precursor processing technique reported for GO-HA system.

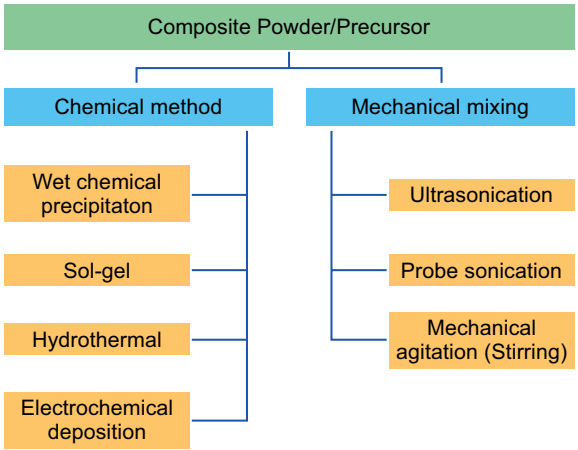


Figure 9. Classification of techniques adopted for composite powder or precursor of GO–HA.

Composite interaction

The interaction of GO and HA is an electrostatic interaction. Abundant amounts of oxygen functional groups such as carboxylic acids as well as phenolic hydroxyls on GO surface and edges make GO highly negatively charged. Electrostatic interactions between GO-HA occurred when the negatively charged GO can absorb the positively charged calcium atoms (C sites) in (100) crystal plane of HA. After compositing, the positively charged calcium cations would be adsorbed the negatively charged of GO through electrostatic charged, as shown in Figure 10. Previous studies proved that GO could form strong interface bonding with positively charged bioceramic. Recently GO has been used to absorb some organic chemicals containing benzene rings or pyridine such as polystyrene, polyaniline, DNA, porphyrin through π-π stacking interaction and absorbing some cations inorganic particles such as Ag_3PO_4, ZnO and Al_2O_3 through electrostatic interaction [76].

HA nanoparticles can be prepared with graphene nanosheets through spark plasma sintering (SPS) process, bioinspired mineralization, biomimetic mineralization, in situ chemical precipitation reaction, simple precipitation and hydrothermal method [77], radio frequency chemical vapour deposition (RF-CVD) technique. All these techniques were promising in-vitro bioactivity, however all these fabricating methods are complicated and time consuming comparing with in-situ synthesis. In-situ synthesis employed more facile, economical and effective process in producing GO-based HA nanocomposite, yet better distribution and strong interfacial bonding of HA towards GO nanosheets [63].

To date, there are methods on the formation of GO-HA composites that have been reported. There are a number of approaches to synthesis of GO-HA such as mechanical mixing method, wet chemical precipitation method, sol-gel method, hydrothermal method electrochemical deposition method / electrophoretic (EPD) method and ultrasonic-assisted method.

The mechanical method involves physical mixing of HA dispersed onto a GO surface using a magnetic stirrer and electrospinning devices by applying mechanical forces. Mechanical mixing takes a long duration and can be augmented by chemical mixing to enhance HA dispersion on GO. Several methods can be used to disperse HA in the matrix for example physical blending and mixing. GO-HA was prepared by the wet chemical precipitation method [7, 59, 78]. Composite GO-HA has the potential to act as a osteoconductive composite to support adhesion of osteoblast cells with good viability. The GO-HA composite functions well with a high viability of osteoblast cells compared to uncoated GO nanoparticles. Surface functionalization of GO by HA increases cell attachment and prolifera-tion in order to enhance bone formation [7].

HA filled on the GO surface, indicating a strong interaction between HA nanoparticles and GO films. In

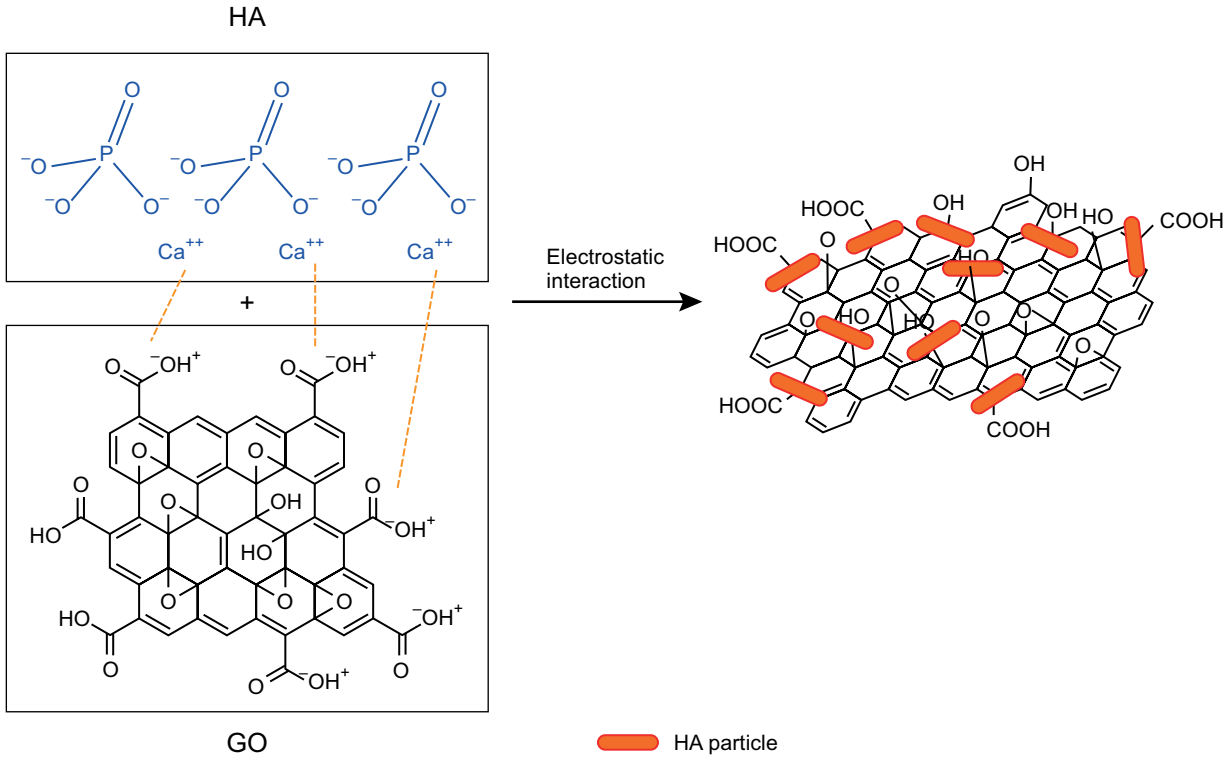


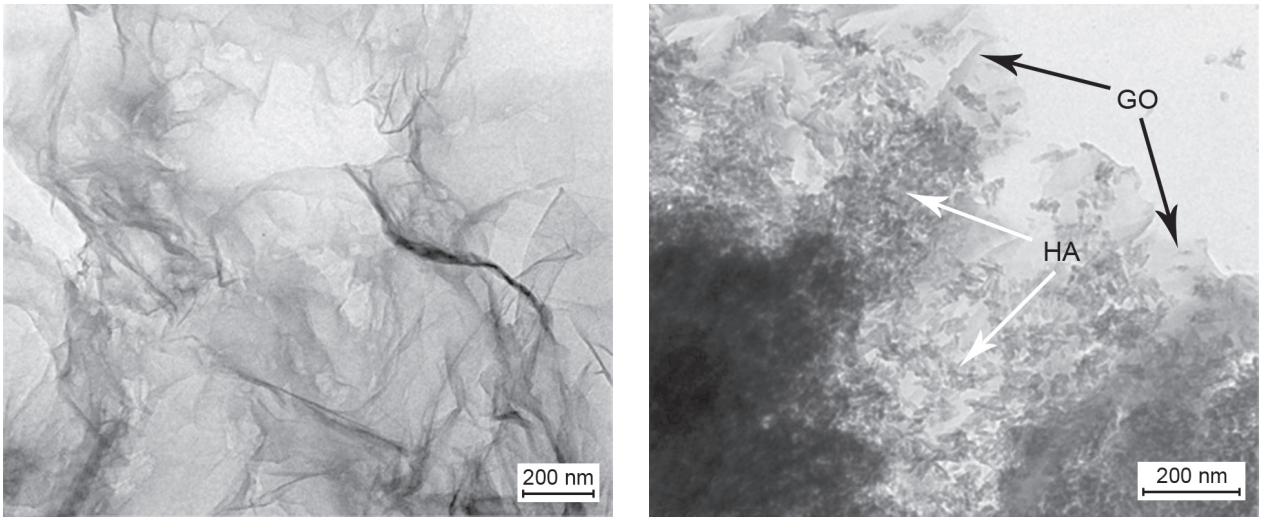
Figure 10. Schematic illustration interaction of GO–HA composite.

addition, nanoparticles of HA prevent GO films from agglomeration and enhances electricity properties of GO films. GO-HA nanocomposite can be potential candidate for biosensor and catalytic application [78].

While preparing GO-HA using research methods activity by Raucci, M et al. 2016 [32] were in-situ sol gel and biomimetic method. Sol-gel technology which is a simple, economical and effective approaches formation of hybrid material based on nano-HA embedded in GO sheet. Sol-gel method was produced in gel water due to hydrophilicity and the electrostatic repulsion of GO [79]. This material is a promising biomedical coating for bone implant other surgery applications [58].

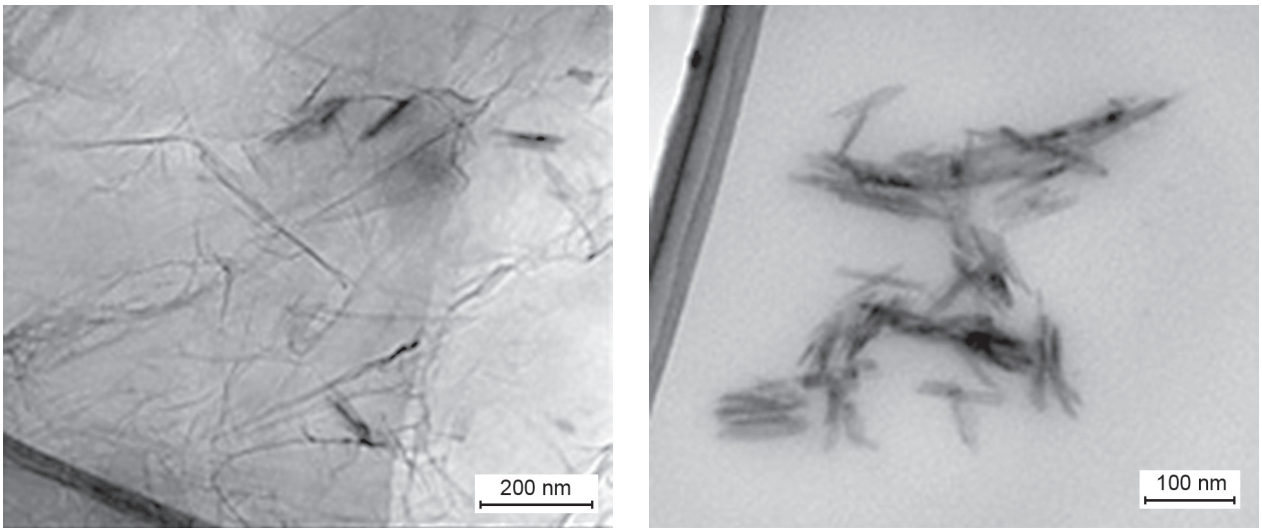
Zhou et al. 2017 [80] and Ramadas et al. 2017 [77] has succeeded in preparing GO-HA composites via a hydrothermal method. Similarly work carried out by Yao et al. 2016 [35] which hydrothermally treated served as drug carriers. GO/HA nanocomposites treated by hydrothermal not only highly biocompatible but also mechanical improved features and applying in implant biomaterials [81].

Electrochemical deposition or electrophoretic deposition (EPD) is a ceramic production by colloidal process that suitable for preparing cost-effective coatings on substrates with complex geometry. This technique used by powder particles suspended in a liquid medium which attracted and deposited onto a conductive substrate of opposite charge. It is a widely used coating technique for ceramics as well as biomaterials [82]. Work performed by Zeng et al. 2016 [39] and Li et al.



a) b)

Figure 11A. TEM micrographs of GO, HA, GO-HA composite suggesting good dispersion of HA in GO by wet chemical precipitation [59].



a) GO b) HA

Figure 11B. TEM micrographs of GO, HA, GO-HA composite suggesting good dispersion of HA in GO by sol-gel method [32].
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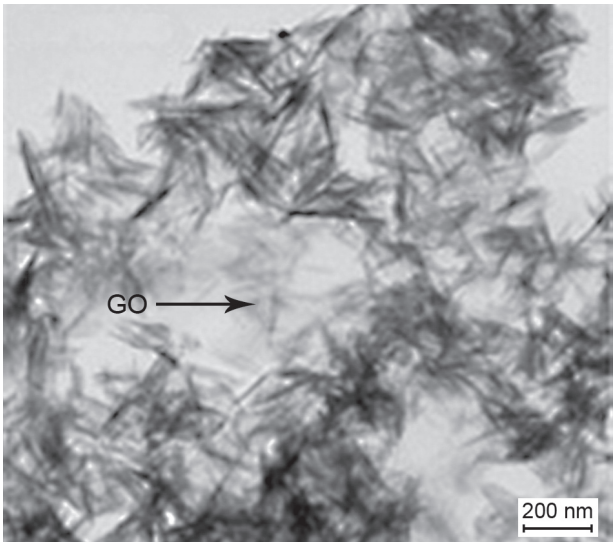
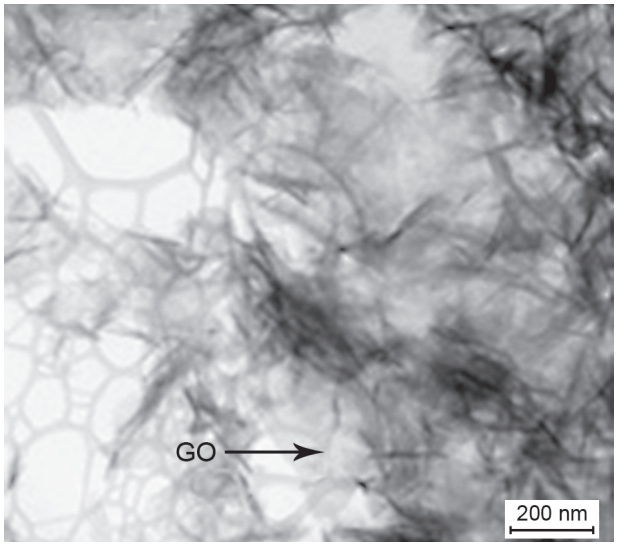
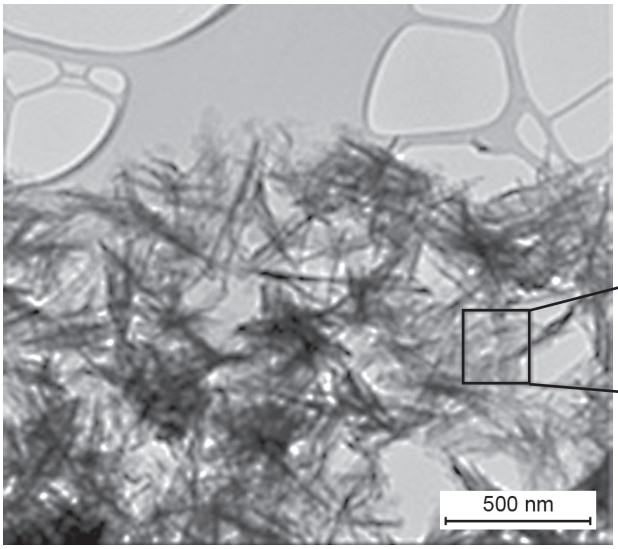


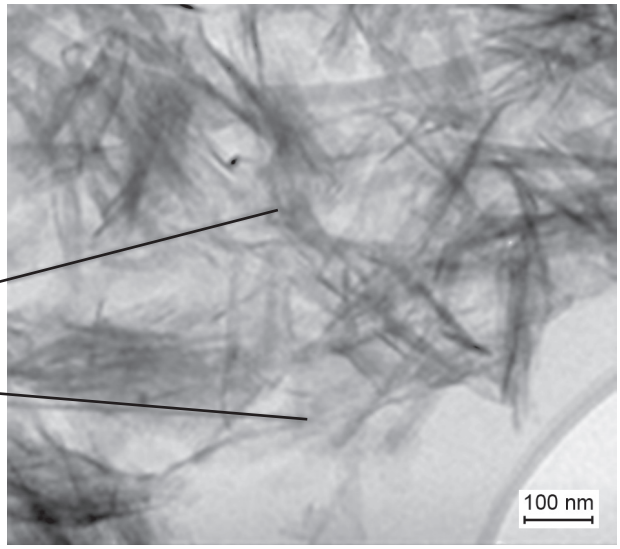
c) GO-HA 1 %



d) GO-HA 1.5 %

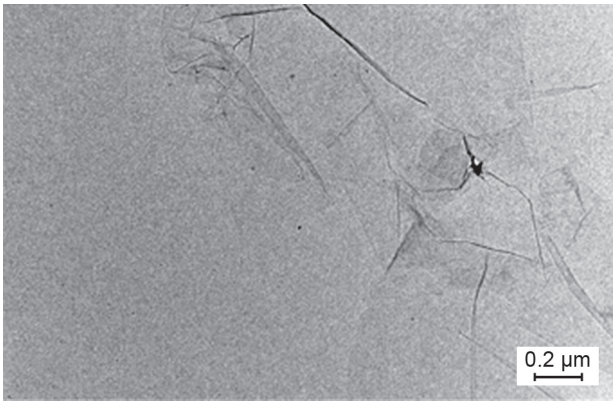


e) GO-HA 2 %



f) GO-HA 2 %

Figure 11B. TEM micrographs of GO, HA, GO-HA composite suggesting good dispersion of HA in GO by sol-gel method [32].



a)

b)

Figure 11C. TEM micrographs of GO, HA, GO-HA composite suggesting good dispersion of HA in GO by hydrothermal [80].
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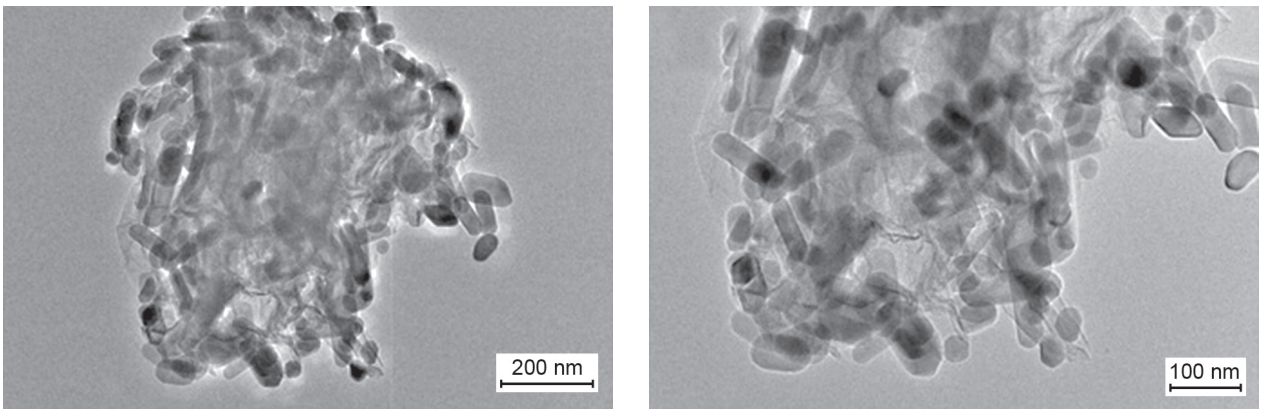
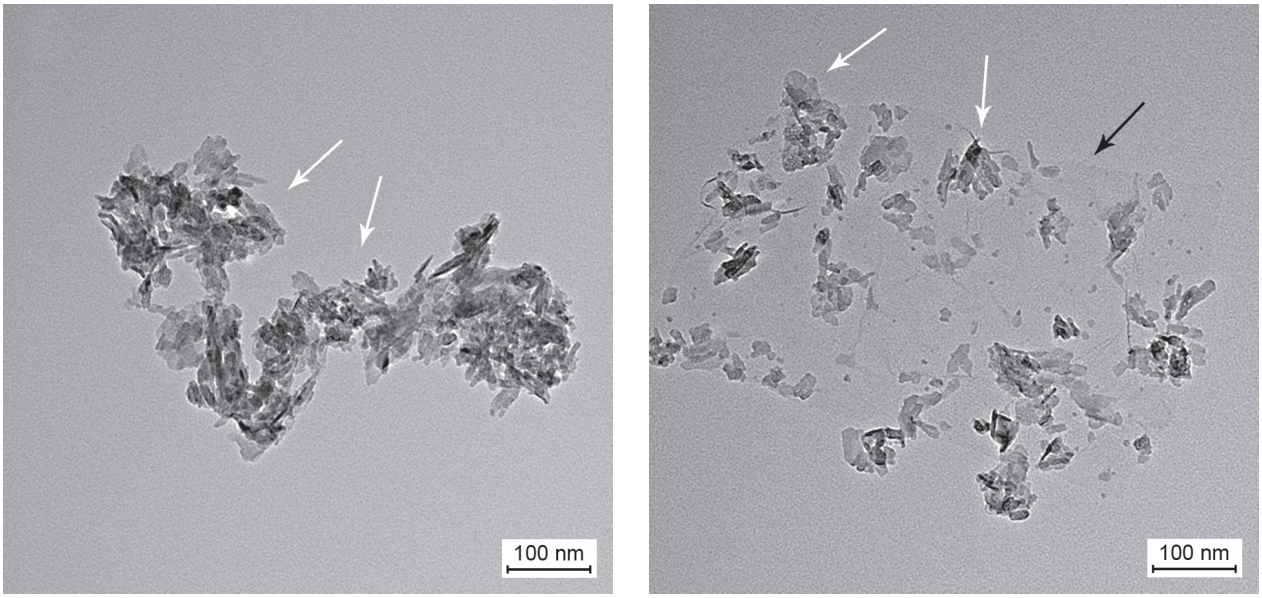


c) d)

Figure 11C. TEM micrographs of GO, HA, GO-HA composite suggesting good dispersion of HA in GO by hydrothermal [80].



a) GO b) HA

Figure 11D. TEM micrographs of GO, HA, GO-HA composite suggesting good dispersion of HA in GO by electrochemical deposition [39].

2014 [63] involved the synthesis of GO-HA composite coatings using electrochemical deposition methods suggested that GO-HA composites could be promising candidates for implants coatings.

Regarding to works by Radha et al. 2017, facilitates the stable interfacial nanocomposites between GO-HA and promotes cell proliferation. GO-HA nanocomposite was prepared through probe sonication process by functionalizing the HA onto the GO surface which would increases the mechanical, biostability and compatibility of GO-HA nanocomposite [83]. The amount of spherical HA in composites was decreased as increase weight percentage of GO, and the folds on GO surface were multiplied. An irregular wrinkles surface facilitates mechanical lock and increase stress transfer of GO-HA. This non-toxic and biocompatible composite demonstrated the osseointegration ability and good proliferation for bone replacement or repair materials [84]. Nanorod HA/GO composite synthesis by ultrasonic and biomimetic mineralization is a GO-based, free template, non-toxic and bioactive composite that may be applied as bone replacement materials [85]. Figure 11 shows TEM images of the successful preparation of GO-HA composite by different technique approaches and Table 5 presents the summary of preparation GO-HA and their related applications.

Antibacterial and cytotoxicity effects

GO

The antibacterial activity of graphene has both physical and chemical effects. Physical damage can be induced by direct contact of sharp graphene edges with

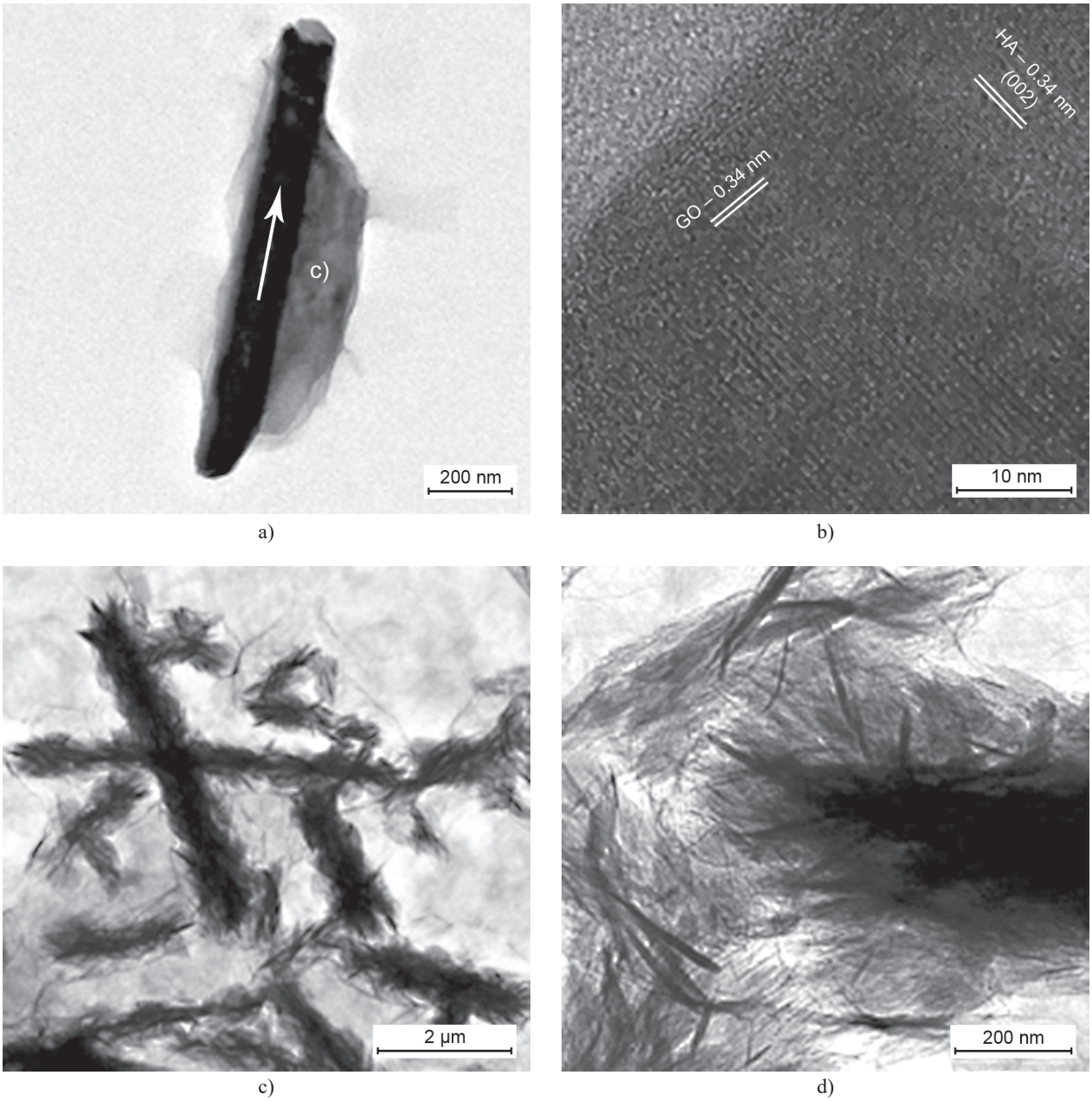


Figure 11E. TEM micrographs of GO, HA, GO-HA composite suggesting good dispersion of HA in GO by ultrasonic-assisted [85].

bacterial cell membranes and destructive extraction of lipid molecules. Physical damage by sharp edges of graphene is highlighted among the mechanisms of the antibacterial activity of graphene. The density of these edges is considered to be one of the key factors in antibacterial activity, because the sharp edges can puncture the bacterial cell membrane forming pores and leading to osmotic imbalance and bacterial death. The chemical effects primarily involve oxidative stress created by reactive oxygen species (ROS) or charge transfer [86].

Mechanisms of cytotoxicity are still under discussion and investigation due to the finding that GO exhibits toxic effects on gram-positive and negative bacteria and on human cells. Further clarification of GO cytotoxicity is required to ensure the safe design of GO-based materials. Previous studies suggest that GO cytotoxicity mechanisms include the destruction lipid bilayers, oxidative stress, inhibition of cell proliferation and penetration of lipid membranes by edges and corners of GO nanosheets [47].

Studies have shown strong antibacterial activity for graphene and GO, and severe cytotoxicity to bacteria, for example in *Escherichia coli*. The direct interaction of graphene and bacterial cell membranes arising from graphene induced cytotoxicity results in serious physical damage to cell membranes. However, toxicity is

Table 5. Techniques for the preparation of GO-HA composite and their related properties.

Technique	Composite	Application	Biocompatibility	Ref.
Wet chemical precipitation	GO/HA/Cellulose	Scaffolds of bone defects	MG-63 and NIH-3T3 cells	[7]
	PLA/HA/GO	Load-bearing orthopedic implants	–	[59]
	GO/HA	Biosensor and catalytic	–	[78]
Sol-gel	GO/HA	Bone tissue engineering	Human mesenchymal stem cell (hMSC)	[32]
	HA/GO/PVA	Coating of bone implants	–	[58]
	GO/HA	Bone regeneration	–	[79]
Hydrothermal	PCL/HA/Graphene	Biomedical	–	[80]
	GO/HA	Drug delivery, orthopedic and dentistry	Human skin cancer cells (A431)	[77]
	GO/HA	Implant biomaterials	NIH-3T3 cells	[81]
	GO/HA	Drug delivery	–	[35]
Electrochemical deposition	GO/HA	Coating of implants	MG-63	[39]
	Chitosan/GO/HA	Bone repair, bone augmentation, coating of biomedical implants	Murine fibroblast (L-929 cell line) and human osteoblast (MG-63 cell line)	[63]
Ultrasonic- assisted	GO/HA	Cell proliferation	Erythrocytes	[83]
	GO/HA	Bone replacement or repair materials	MC3T3-E1 cells	[84]
	GO/HA	Bone substitution	Mouse embryonic osteoblast (MC3T3-E1)	[85]

reduced when nanosheets are surrounded by proteins. According to Bianco, recent studies concerning the toxicity of graphene and its derivatives, including in vitro and in vivo studies, clearly show that there are no specific risks, while others argue that there might be health hazards. Other reviews state that the toxicity of graphene towards different microorganisms, cells and animals, is influenced by its physicochemical properties including surface functional groups, size, charge, and coatings. Structural deficiencies may alter its behaviour and also its toxicity in biological systems [54].

Accordingly, larger sized GO sheets showed stronger antibacterial activity due to their ability to completely cover bacteria, block their active sites and decrease their viability. In comparison, smaller sized GO had weaker antibacterial activity due to adhesion of GO only at the bacterial surface, without efficiently isolation from the environment. In addition, the antibacterial activity of graphene is influenced by the time of exposure and the concentration. Higher GO concentrations led increased antibacterial activity [86].

An exploration of the antibacterial properties of GO interacting with Gram-negative bacteria (*E. coli*) by Huang and co-workers in 2010 stated that GO damaged cell membranes and restrain the growth of *E. coli*. Further studies using Gram-negative *E. coli* and Gram-positive *S. aureus* as model bacteria have demonstrated the bacterial toxicity of GO and rGO nanowalls. GO and rGO deposited on stainless steel substrates damaged cell membranes through direct contact with the very sharp edges of the nanowalls [15]

GO had the strongest antibacterial activity followed by rGO, graphite and graphite oxide [15, 48, 52] at the same concentrations and under the same incubation conditions. However, some controversial studies suggest GO may lack antibacterial activity. There is some evidence suggesting that GO has neither antibacterial activity nor cytotoxicity properties. Hence, careful studies to understanding the toxicity of graphene-based materials are still required [15]. Table 6 presents the mechanisms and effects of GO interacting with Gram positive and Gram-negative bacterial cells.

Nguyen et al. 2015 [87] investigated the antibacterial activity of GO against human intestinal bacteria and in vitro cytotoxicity of GO using the Caco-2 cell line derived from a colon carcinoma. The results exhibited no toxicity against *E. coli*, *L. acidophilus* and *B. animalis* at different concentrations (10-500 μg ml^{-1}) and mild cytotoxicity towards Caco-2 cell line after 24 h exposure suggest it biocompatibility. Besides, in view of GO oxidation degree related to their toxicity. According to Zhang et al. 2015 [88], the cytotoxicity of three GO samples with different oxidation degrees on mouse embryo fibroblasts (MEFs), found that the decreased

in oxidation degree GO exhibited a higher degree of cytotoxicity and apoptosis.

The studies on GO dose-dependent toxicity to cells and animals, Wang et al. 2011 [89] evaluated the toxicity of GO against human fibroblast cells and mice. The result showed that the low dose of GO less than 20 $\mu g \cdot ml^{-1}$ did not exhibit toxicity towards human fibroblast cells, while the high dose of GO more that 50 $\mu g \cdot ml^{-1}$ exhibit clearly cytotoxicity which cause decreasing cell adhesion, inducing cell apoptosis, entering into lysosomes, mitochondrion, endoplasm and nucleus. Similarly, in vivo cytotoxicity towards mice indicated no cytotoxicity with low dose and middle dose, 0.1 and 0.25 mg respectively, while high dose of GO, chronic toxicity for high doses of GO, 0.4 mg affecting mice death, lung granuloma formation mainly located in lung, liver, spleen and kidney.

Similarly, Chang et al. 2011 [90] concluded that no obvious cytotoxicity of GO towards A549 cells because GO do not enter A549 cells, but cause a dose-dependent oxidative stress in cell and high concentration slightly loss cell viability. Other studies of GO cytotoxicity by various sizes and oxygen content have been investigated in human erythrocytes and skin fibroblasts through in vitro hemolysis and WST-8 viability assays. The results demonstrated that all GO was dose-dependent hemolytic activity on RBCs. Sonicated (smaller), individual GO sheets showed higher hemolytic activity despite of non-sonicated (larger) and aggregated sheets [91].

In addition, Hu et al. 2011 [92] demonstrated the cytotoxicity studies of GO on A549 cells affecting fetal bovine serum (FBS) and formation of a protein corona . The concentration-dependent cytotoxicity showed that the sensitivity of human cells at low concentrations of FBS (1 %) and the presence of 10 % FBS in cell media reduced the GO cytotoxicity. The cytotoxicity of GO arises from the direct physical contact GO towards plasma membranes. The observations suggested that FBS-mitigate GO cytotoxicity as an alternative and convenient route for safe biomedical and environmental applications.

J. Wu et al. 2015 [93] investigated the potential in vitro cytotoxicity of GO against model human breast cancer MDA-MB-231 cell line. The result of high concentration of GO ($\geq$ 100 $\mu g \cdot ml^{-1}$) in a significant time and dose-dependent demonstrated the suppression colony-formation capacity and cellular proliferation as well as the generation of intracellular ROS by oxidative stress. Oxidative stress is the possible anti-breast cancer mechanisms induced by cytotoxicity of GO towards MDA-MB-231 cell. In vitro toxicity studies in cell lines included generation of reactive oxygen species (ROS), inflammatory responses, DNA-damage and oxidative proteins damage [94].

The toxicity study on male rat was exposed to GO via tail vein injection with varied GO concentration for 7 days resulted to the lung, liver and spleen inflammations. This study indicated that the low concentration level of GO is nontoxic [95]. In vivo cytotoxicity study of GO towards Japanese white rabbits via intravitreally injection of GO into rabbits' eyes, indicated that few changes in eyeball appearance, intraocular pressure (IOP), eyesight and histological examination [96].

Plus, in lines of dental and periodontal diseases, He et al. 2015 [97] revealed that the antimicrobial affects of GO nanosheets against dental pathogen is predominant. GO suppress the growth and viability of dental pathogens (*Streptococcus mutans*, *Porphyromonas gingiyalis* and *Fusobacterium nucleatum*) as well as destroy their cell wall and membrane cell and leak out (Figure 12A). Thus, GO is superior candidate in dental care application. In addition, they observed that GO concentration in the range 50 - 100 $\mu g \cdot ml^{-1}$ keeps the balance between minor cytotoxic effects and major antibacterial activity [98]. Figure 12B presents the TEM images of cell morphology of E. coli bacteria that were incubated with 100 $mg \cdot ml^{-1}$ GO nanosheets at 37 ºC. Three have three stages (I, II and III) and two types (Type A and Type B) molecular mechanisms of *E. coli* morphology were observed during 2.5 h incubation process [99].

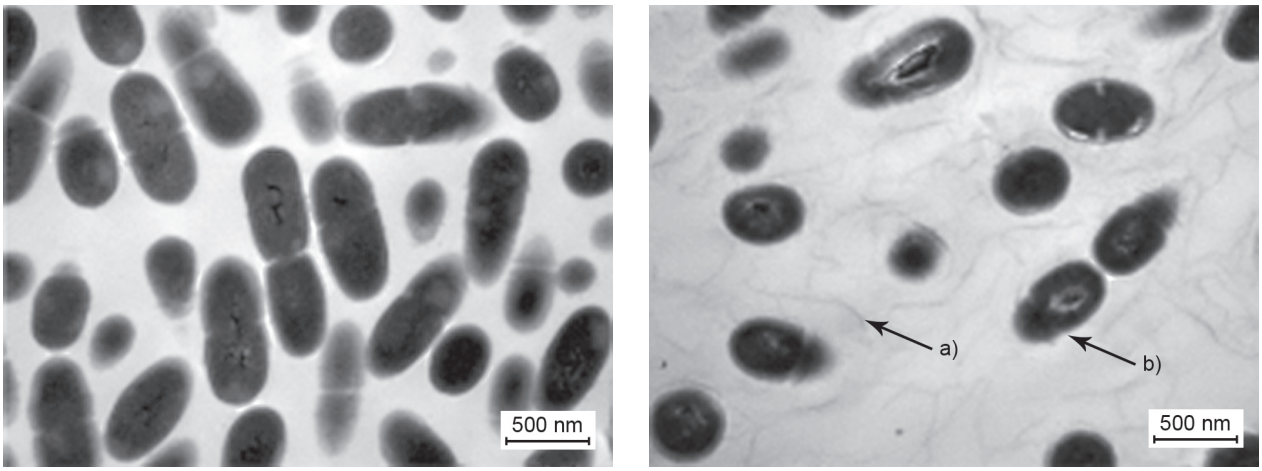
According to Shubha et al. 2016 [100], the study of antimicrobial surface against nosocomial pathogens (*Streptococcus aureus* and *Pseudomonas aeruginosa*) for surface disinfectant purposes clearly revealed that GO successfully inhibited the nosocomial pathogen and perturbed bacterial membrane. Hence, this result suggested GO successfully can be applied as coating over hard surfaces in hospital set-up. Zone of inhibition is larger for the high concentration of GO [101].

Table 6. Mechanisms and effects of GO interacting with Gram positive and Gram negative bacterial cells [13].

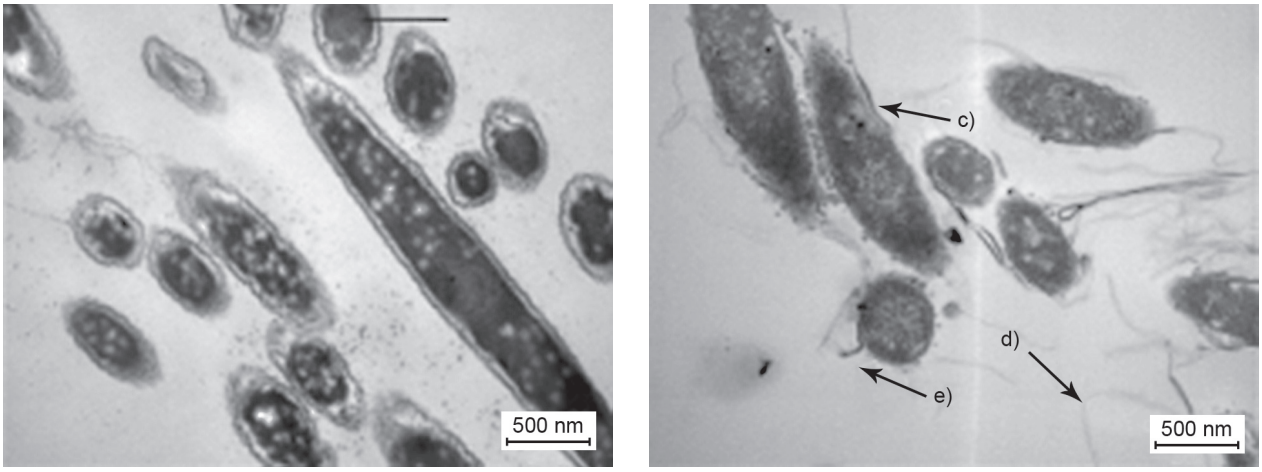
GO interaction with bacteria		Gram positive and/or Gram negative bacteria
Mechanism	Effect	
Physical contact interaction- entrapment of bacteria	Cell death	Gram negative
Cell membrane damage, efflux of cytoplasmic materials, decreasing metabolism	Cell death	Gram positive, Gram negative
ROS production, glutathione loss, oxidative stress	Cell death	Gram positive, Gram negative
Adhesion of the bacterial cell to the graphene surface, proliferation and stimulation of biofilm formation	Cell growth, GO reduction	Gram negative

Besides of antibacterial and toxicity of GO, GO also work in antiviral properties. An investigation done by Ye et al. 2015 [102] revealed that GO inactivated pseudorabies virus (PRV, a DNA virus) and porcine edidemic diarrhea virus (PEDV, an RNA virus) by structural destruction before entry into cells.

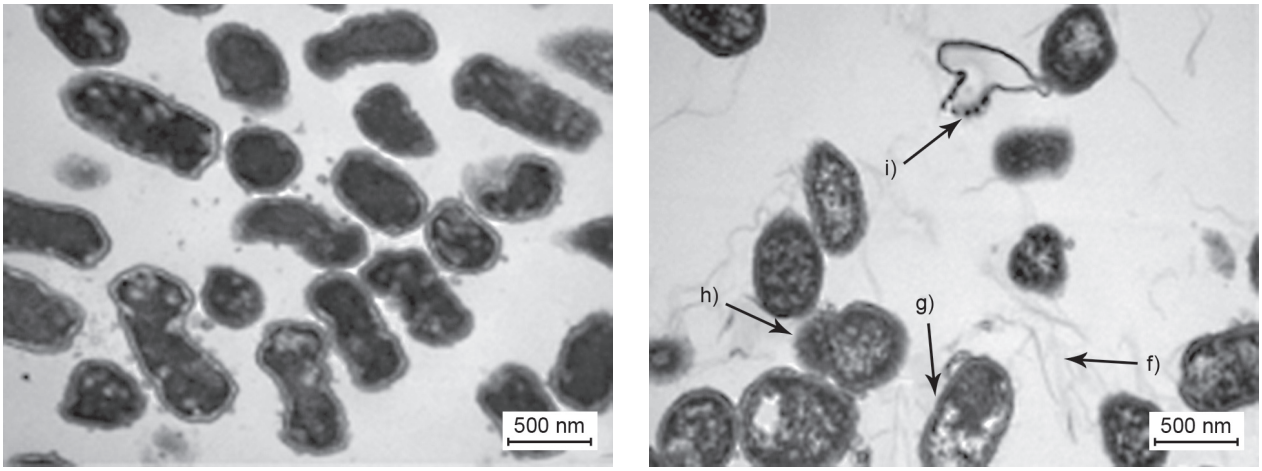
The study conducted by Ma et al. 2018 [103] investigated the interaction of GO with ubiquitin-proteasome system, found that GO-induced the inhibition activity of both purified 20S proteasome and proteasome in living cells. Computational techniques also demonstrated the blockage of central gate in the α-subunits for entry



a) *S. mutants*



b) *F. nucleatum*



c) *P. gingivalis*

Figure 12A. TEM images of *S. mutans*, *P. gingiyalis* and *F. nucleatum* cells after incubation with 80 µl GO nanosheets dispersion (right side) 2 h and after incubation with saline solution for 2 h as control (left side) [97].

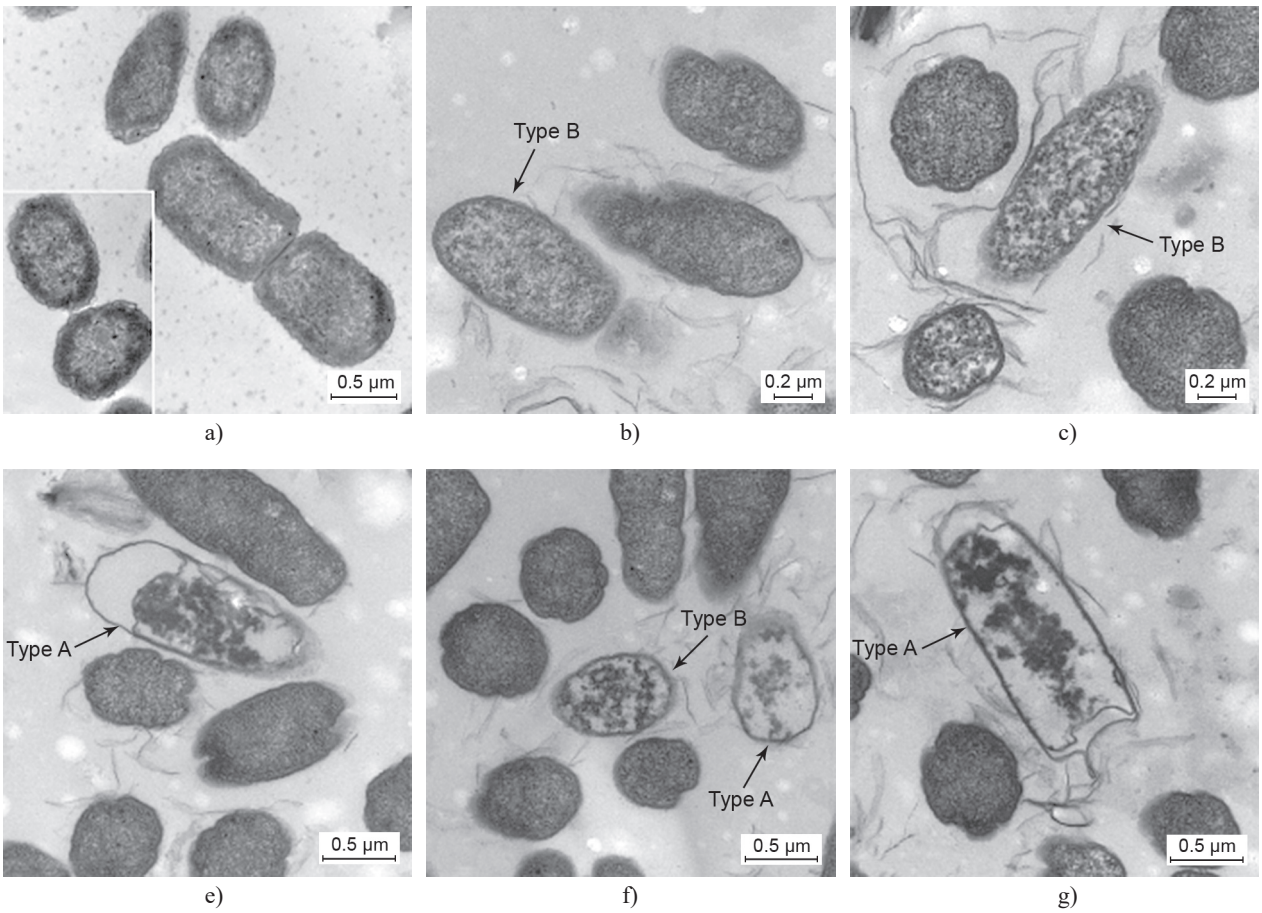


Figure 12B. TEM images of *E. coli* exposed to GO nanosheets; a) – f) showing *E. coli* undergoing changes in morphology after incubation with 100 µg·ml^{-1} GO nanosheets at 37 ºC for 2.5 h. Three stages of destruction can be seen: a) Initial morphology of *E. coli* (control or Stage 1, two individual TEM images (inset and main page) are shown; b) and c) Partial damage of cell membranes with some bacteria showing lower density of surface phospholipids (Stage II). Arrows indicate Type B mechanisms, where graphene nanosheets extract phospholipids from the cell membrane: d) – f) Three representative images showing the complete loss of membrane integrity with some demonstrated 'empty nests' and missing cytoplasm (Stage III); d), f) Representative images showing Type A mechanisms, where graphene nanosheets cut off large areas of membrane surfaces [99].

and exit of proteasome to active site of protease. The findings promote GO as future oncologic therapeutics applications.

In contrarily, S. Wu et al. 2014 [104] proved that low cytotoxicity of GO and did not affect the antitumor activity of doxorubicin (DOX) against human multiple myeloma cells (RPMI-8226). GO did not induce cell apoptosis and inhibited cell proliferation. The results suggest that GO is suitable for anticancer drug nanocarrier and hematological malignancies treatment.

GO could induce in vitro apoptosis of erythroid cells through oxidative stress in E14.5 fetal liver erythroid and in vivo GO-declined erythroid population in spleen led to erythropoiesis disordered in mice [105].

GO-HA nanocomposite

Toxicity and biocompatibility are major concerns in biomedical materials. Cytotoxicity assays are methods to analyze the biocompatibility of prepared nanocomposite to determine whether it can be toxic or harmful towards living organisms. According to Ramadas et al. 2017 [77] GO-HA nanocomposite showed no toxicity effects on the human skin cancer cell line (A431) using MTT assays. This confirmed GO-HA to have excellent biocompatibility and to be a potential biomaterial for orthopedic, drug delivery and dentistry applications.

Antibacterial tests of electrodeposited GO on HA-P-ATi revealed toxicity towards both Gram-positive and Gram-negative models. It was found to be more toxic towards Gram-positive bacteria (*S. aureus* bacteria) compared to Gram-negative bacteria (*E. coli* bacteria). Membrane damage of bacteria was implemented by exposing bacteria to phosphate buffer solution. Membrane damage was caused by increasing GO concentrations through direct contact interaction. GO edges (good acceptors) causes membrane damage of bacteria. *S. aureus* less resistant to the GO damaging due to thicker peptidoglycan layer of Gram-positive bacteria

(20 - 80 nm) compared to *E. coli*, Gram-negative bacteria (7 - 8 nm). Nanocomposite GO-HA can reduce bacteria susceptibility for *S. aureus* and *E. coli*, hence it is potential antibacterial nanocomposite to restrain bacteria for orthopedic implants [31].

The preparation nanocomposite by in vitro cytotoxicity was investigated by using CCK-8 assay on the murine fibroblast L-929 cell line and human osteoblast-like MG-63 cell line. The result after incubation for 5 days was shown that the prepared nanocomposite induced no in-vitro cytotoxicity towards L-929 cell and MG-63 and had no time for cytotoxicity. GO-based HA provide new prospects to bone repair, bone augmentation, and biomedical implant coating [63].

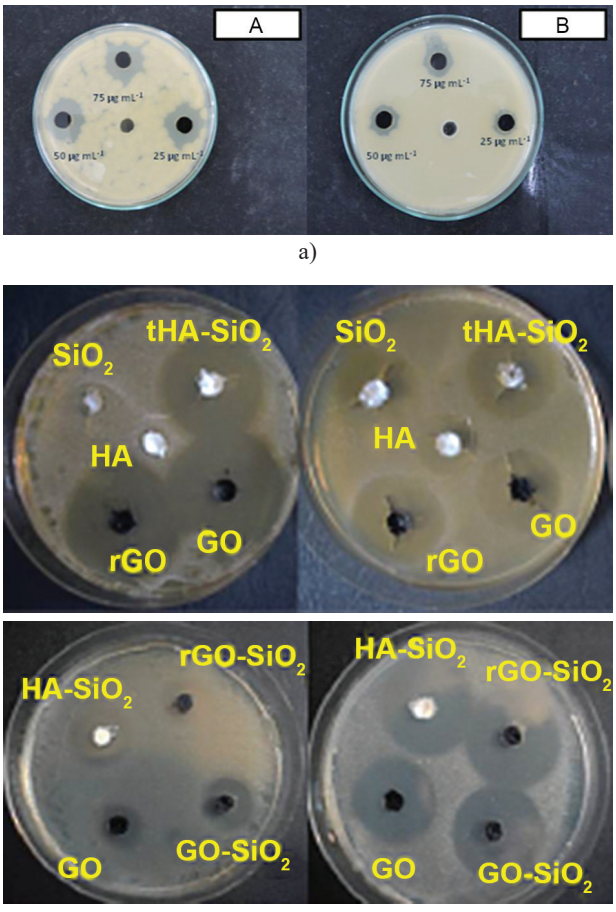
According to Surendran et al. 2017, in-vitro antibacterial assay of GO-HA nanocomposite was carried out by agar well diffusion method against Gram-positive *Enterococcus faecalis* and Gram-negative *Pseudomonas aeruginosa* bacteria. As the result show that the antibacterial revealed GO-HA nanocomposite inhibited *Enterococcus faecalis* for 6, 10 and 12 mm and Pseudomonas aeruginosa for 2, 4 and 8 mm diameter inhibition zones for concentration 25, 50 and 75 $\mu g \cdot ml^{-1}$ respectively. Thus, it is can be good candidate for biomedical application especially in orthopedics [106].

The inhibitory zone by agar disc diffusion method (at 37 °C for 24 h) of 10 wt. % PCL/HA/GO composite at different doses (25, 50, 75 and 100 µl) against *S. aureus* was 10, 11, 14, 16 mm and *E. coli* was 10.5, 12, 14.5, 16.5 and 20.5 mm, respectively [107]. A good in vitro antibacterial ability by SiO_2-GO-HA composite against *E. coli* and *S. aureus* using agar well diffusion method [108]. The results of in vitro antibacterial activity were shown in Figure 13.

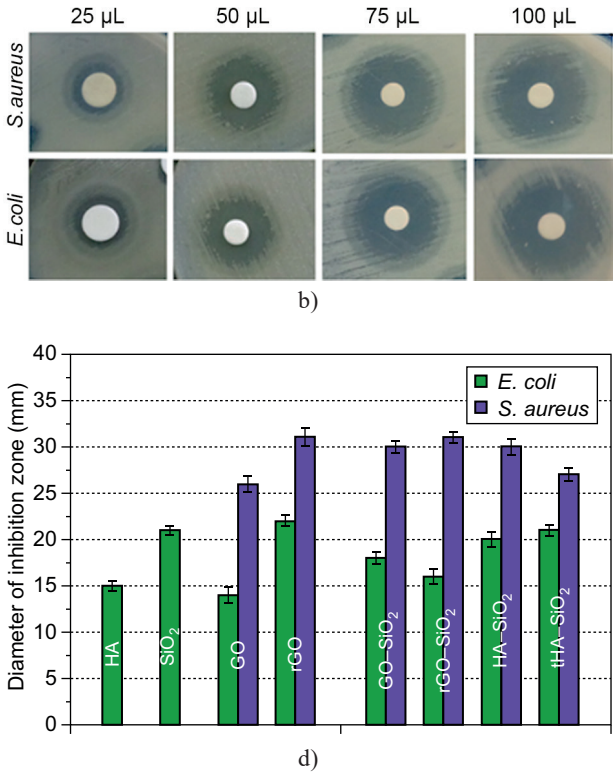
CONCLUSION

Research highlights

- The GO-HA combination was successfully carried out by different approach including wet chemical precipitation method, sol-gel method, hydrothermal method electrochemical deposition method/ electrophoretic (EPD) method and ultrasonic-assisted method.
- GO-HA nanocomposite induced the antibacterial and cytotoxicity towards bacterial cells.
- GO is often utilized to reinforce the coating mechanical properties.



a) c)



b) d)

Figure 13. Zone of inhibition of: a) GO-HA against *Enterococcus faecalis* (A) and *Pseudomonas aeruginosa* (B) [106]; b) 10 wt. % PCL/HA/GO composite against *S. aureus* and *E. coli* [107]; c) silicate-based composites against *E. coli* and *S. aureus* by agar well diffusion method (after 48 h) and d) bar chart of antibacterial activity of silicate-based composites against *E. coli* and *S. aureus* [108].

- GO-HA nanocomposite could be a promising candidate for antibacterial coating in biomedical devices.
- Future GO-HA studies of antibacterial activities towards bacterial cell membranes and inclusion of third element in nanocomposite might improve antibacterial properties in nanocomposite.

Future Perspectives

In this review, we have discussed the properties of HA and GO nanoparticles, the combining approach of GO-HA nanocomposite as well as its antibacterial and toxicity towards biological system. The uniqueness properties of GO itself contribute to antibacterial activity and at the same time reinforced HA to increase their mechanical properties. The successful combining GO-HA nanocomposite was proven by many ways including wet chemical precipitation method, sol-gel method, hydrothermal method electrochemical deposition method/ electrophoretic (EPD) method and ultrasonic-assisted method. The different the method used the different the result of application will achieved.

In this review, we focused on recent studies concerning the cytotoxicity and antibacterial activity of GO and GO-HA composites both *in vitro* and *in vivo*. Most of the previous studied discovered of biocompatibility of HA and GO rather than antibacterial activity and cytotoxicity of GO and HA. GO-HA nanocomposite induced antibacterial and cytotoxicity activity towards bacterial cells. Antibacterial activity and cytotoxicity of GO and GO-HA composites has been studied mostly in terms *in vitro* analysis and further *in vivo* analysis should be conducted being considered for clinical applications. Coating of implants in bone tissue and for longer period is required absolute assessment of *in vivo* antibacterial to establish the application of GO-HA composites in biomedical.

GO-HA was served as future excellent biologic coating implant. The studies on GO-HA nanocomposite in term of antibacterial coatings in biomedical field have not much been explored. Therefore, future research should be concerned on antibacterial mechanism of the composites with model bacterial cell membranes will provide in depth understanding of the interaction. The inclusion of third elements in composites, and other synthesis methods may also be exploited in order to produce anti-bacterial composites with improved properties.

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