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A Review on Graphene Based Metal/Metal Oxide Composites and Enhanced Properties along with Biomedical Applications

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Abstract

Graphene based metal/metal oxide composites are attracted much interest and attention as promising entrant to available biomedical devices owing to their good stability and unique properties. The fame of Graphene oxide (GO) has been on the increase due to their several applications in the fields of super capacitor and storage devices, ionic conductors and Nano sized membranes. GO is used as light emitting material, which makes it particularly suitable for various medical applications like protein bio-sensing and disease discovery, targeted drug delivery and antimicrobial materials are just some of the possibilities GO holds for the biomedical field. GO can easily be mixed with different polymers and other materials, and enhance properties of composite materials like tensile strength, elasticity, conductivity and more. In this present review, discussed thoroughly with graphene based metal/metal oxide composites are used for different applications like antibacterial, antimicrobial, and biomedical etc. In future we expect fine control of the quantity of RGOhybrids is an important challenge to control toxicity.

Keywords: Ag-RGO/ZnO; Hybrid; Anticancer; Ag release

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Introduction

Graphene is posed by Boehm et al. and elucidated graphene sheet from bulk graphite [1]. Graphene is a single carbon layer of the bulk graphite hexagonal nanostructure [2]. Andre et al. exfoliated graphene from graphite by simple scotch tape method [3,4]. Carbon nanomaterial such as graphene and CNTs possess amazing optical, electrical and thermal properties [5,6]. Graphene is robust highly flexible, and attractive for producing thin and flexible materials based on their structure into Fullerene (C 60), CNTs and graphene sheets densely packed graphitic bulk structure [7,8]. Graphene is a two-dimensional (2D) honeycomb, with an atomic thickness of 0.345 nm, possessing zero band gap, large specific surface area (2630 m^2g^{-1}), high charge carrier mobility (>200000 $cm^2 V^{-1} s^{-1}$) [9-11]. It conducts electricity rapidly than copper (Cu), 100 times stronger than steel and highly stronger than diamond [12].

In 1958, Hummer's initially developed graphite oxide by chemical oxidation [13]. Graphene oxide (GO) has more oxygen functionalities like hydroxyl (–OH), carboxyl (–COOH) and epoxide functional groups [14,15]. The graphene Nano sheets possess outstanding chemical and physical properties including antibacterial activity [16,17]. It is found to be a potential candidate for the applications ranging from fuel cells, super capacitors optics, gene therapy, bio-devices and biomedical fields [18-20]. In early days, three preparation methods were employed for the synthesis of GO; they are (i) Brodie's (ii) Staudenmaier's and (iii) Hummer's. Among these methods, the Hummer method is employed in large scale due to its low production cost, aqueous stability, and scalability. It uses strong oxidizing species such as potassium permanganate ($KMnO_4$) and sulphuric acid (H_2SO_4) to oxidize the bulk graphite. The synthesized GO sheets consist of hydroxyl (OH), epoxy (=O) groups at the surface and carboxylic acid (–COOH) groups at the edges **(Figure 1).**

Graphene oxide (GO) has many oxygen functionalities such as hydroxyl, epoxy and carboxyl groups present in the basal planes and at the edges of the sheets. It is highly hydrophilic in nature, less thermal stable, and less in electrical conductivity, in order to enhance the basic properties of graphene oxide. The oxygen functional groups are reduced by chemical reduction using reductants such as hydrazine 1, 1-dimethylhydrazine, hydroquinone, and sodium borohydride. The reduction of graphene oxide (GO) evolves reduced graphene oxide (RGO). The GO and RGO have significant physical and chemical properties with enhanced applications. We also note reviews of the chemistry of GO namely 'graphene oxide', and of the chemistry of graphene with some metal/metal oxides will be discussed for biomedical applications like antibacterial activity, anti-oxidant, free radical scavenging and cytotoxicity.

Metal oxide nanoparticles

Metal oxide nanoparticles play a vital role in various fields of physics, chemistry and in material science. They possess high mechanical, optical and physiochemical properties and finds wide application in all fields. The most important metal oxide nanoparticles such as Zinc oxide (ZnO), Copper oxide (CuO), Palladium oxide (PdO), Titanium dioxide (TiO_2), Aluminum oxide, (Al_2O_3) Manganese dioxide (MnO_2), Silver oxide (AgO) are used as pigments in paints, antimicrobial agents, sunscreens, cosmetic, and in industrial applications. The afore mentioned chemical properties make the Nano composites to spread its applications in anti-bacterial, anti-fungal, anti-oxidant, disinfection, and toxicity properties.

The hydrothermal method has been employed for the preparation of fine metal nanoparticles ormetal oxide Nano particles in unusual shapes with Nano to submicron size. It is the most popular technique for the preparation of nanoparticles. The crystalline phase and particle size of the composite are highly dependent on, pressure, temperature and precursor concentration. One-step hydrothermal approach has more advantages over conventional and nonconventional methods; it produces a variety of nanostructures.

During the hydrothermal treatment, three types of processes occur:

1. Hydrothermal synthesis: used to prepare mixed oxides with controlled shape and size.
2. Hydrothermal oxidation: employed to prepare fine metal oxide particles under high temperature and pressure
3. Hydrothermal crystallization: the aqueous solution is placed in Teflon-lined autoclaves. The size of the particle is dependent on starting material and reaction temperature.

The hydrothermal method was adapted to synthesize graphene oxide/ZnO Nano composites in a different manner with simple, low cost, and easy way. It allows many advantages over other conventional and nonconventional synthesis; one-step approach can produce a wide variety of nanostructures.

Zinc oxide (ZnO) has attracted much attention among the scientific community as a 'future material'. It has been widely studied since 1935. Lattice parameter of ZnO was compared to compounds ofII-VI group materials; the bond nature of ZnO is more ionic with the radii of 0.74 Å for Zn^{2+} and 140 Å for O^{2-} and space group of ZnO is P63mc. The crystal structures for Zinc oxide exist in wurtzite (B4), Zinc-blende (B3) and rock salt (B1) in nature **(Figure 2).**

ZnO is a semiconductor with a band gap of about 3.37 eV, with high binding energy (60 meV) at room temperature. ZnO is recognized as safe food packaging material (21-CFRI-82.8991) by the Food and Drug Administration of United States. Synthesis of ZnO forms a different variety of morphologies ranging from nanostructures such as Nano rods, nanowires, Nano ribbons, Nanocombs, Nano belts, Nano fibers, Nano spheres, Nano helixes, and Nano-tetra pods **(Figure 3).** Among all, ZnO based Nano structural dimensions like (0D, 1D, 2D-thin films/ sheets) are extensively used for the formation of nanomaterial on various matrices for device applications.

ZnO has distinct optical and electrical properties; this property leads for wide applications, such as high transmittance, gas sensors, solar cells, and UV photo detectors. The morphology of the nanoparticles depends on the concentration of the precursors/ various methods followed for ZnO nanostructures formation. The catalytic behavior depends on the size and shape of the nanoparticles. ZnO has good biocompatibility, biodegradability and low toxicity, which made it as a material for biomedicine and pro-ecological systems. ZnO exhibit better antibacterial activities against virus, bacteria, and fungus. It penetrate through the bacterial membrane and causes cell damage. The catalytic, antimicrobial properties along with biocompatibility make ZnO as a potential candidate for tissue regeneration, wound dressing and bacterial resistance.

Antibacterial mechanism of metal oxide

ZnO nanostructures are good antimicrobial agents, durable and stable at high temperature, appropriate disinfectant, then organic agents. The antibacterial mechanism of ZnO nanoparticles involves the generation of reactive oxygen species (ROS). The ROS such as superoxide radical's singlet oxygen, and hydroxyl radicals damage cellular constituents like DNA, lipids, and proteins. The

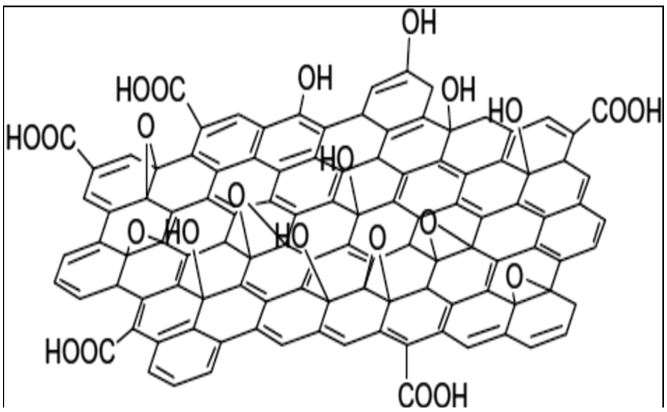

Figure 1 Graphene oxide with various functional groups.

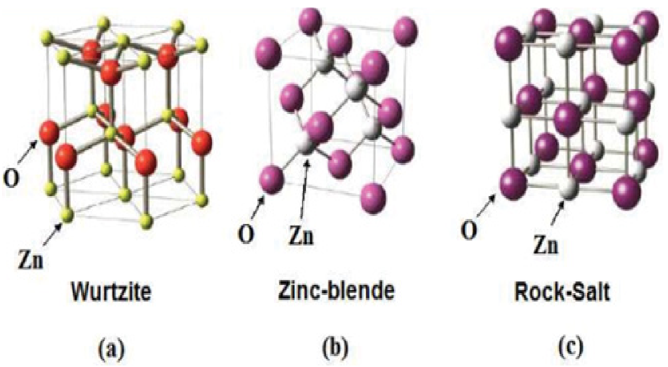

Figure 2 Stick and ball representation of ZnO: (a) hexagonal wurtzite (B4), (b) cubic zinc blende (B3), and (c) Cubic rocksalt (B1).

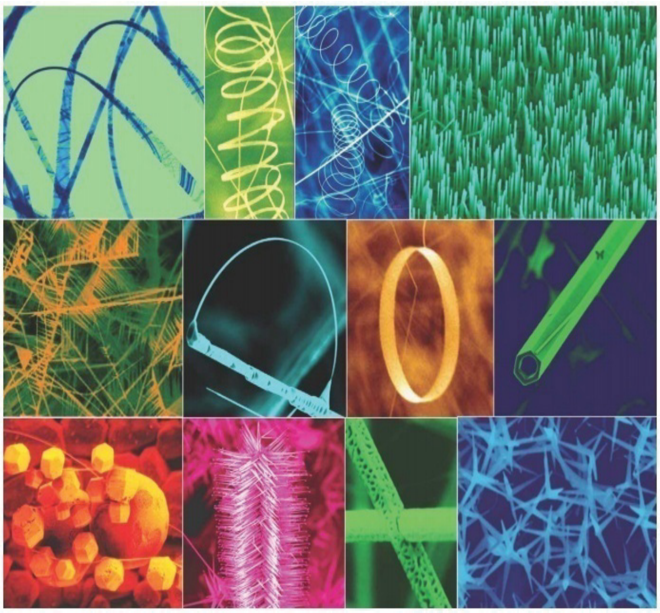

Figure 3 Collection of nanostructures of ZnO synthesized by various techniques.

principle is, when ZnO is illuminated with great photo energy more than its band gap energy, electrons present in the valence band of ZnO NPs are promoted to the conduction band, and creates a hole (h^+) in the valence band. The electron present in the conduction band and hole in the valence band produce strong oxidizing and reducing power. When the electron reacts with O^2 through a reductive process, superoxide anion radicals ($O^{2\bullet -}$) are produced. Hydroxyl radicals ($OH^{\bullet}$) are produced by the oxidative process. OH° is very reactive and reacts quickly with biomolecules such as lipids, carbohydrates, nucleic acids, DNA and amino acids. The predominant antibacterial mechanism depends on the production of H_2O_2 from ZnO, and chemical interactions between it with bacteria. The ZnO nanoparticles incorporated on reduced graphene oxide sheets provides good antibacterial activity against gram-positive (*S. auresus*) and gram-negative bacteria (*E. coli,*).

Metal nanoparticles

Silver nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) are predicted as global consumptions due to their remarkable properties compared to other nanomaterial. AgNPs have high demand in electronics and electrical fields and it has significant growth. AgNPs are materials with sizes 1-100 nm. They possess unique physical, chemical and biological properties which include optical, thermal, and high electrical conductivity. They have been used for wide applications such as industrial, healthcare, household, optical sensors, medical device coatings, orthopedics, food industry, drug delivery, antibacterial, and anticancer drugs. Synthesized Nano-metallic particles have a high surface-to-volume ratio, and possess considerable physical, chemical and biological properties. The biological activity of AgNPs depends on size, shape, composition, particle morphology, agglomeration, the efficiency of ion release, and dissolution rate. AgNPs could be synthesized from several approaches such as physical, chemical and biological methods; among all process chemical one-pot route system provides controlled nanostructures of AgNPs with uniform size, and well-defined morphology. AgNPs synthesized from chemical reduction with hydrazine hydrate produces AgNPs with the size of 10-20 nm. Certain properties of AgNPs are highly dependent on their morphology such as shape and size. The spherical shaped AgNPs has good antibacterial activity. Smaller particles of AgNPs having huge surface area are most effective antibacterial compared to larger size. They are widely applicable for biomedical applications.

Antimicrobial activity of AgNPs

The interesting behavior of AgNPs with unique properties explored it in wide applications. The toxic properties of AgNPs inhibit bacterial growth, lethal to cell-based *in-vitro* systems. The toxicity properties include antimicrobial, cytotoxicity and genotoxicity. These unique properties of AgNPs evolve antimicrobial agents, bio-detectors, and dental biomaterials demonstrated and reported antimicrobial activity of AgNPs against *Escherichia coli*, smaller the particle size of AgNPs showed more efficient antibacterial activity than larger particles. It reveals that antibacterial activity is size and shape dependent.

One-pot hydrothermal synthesis of chitosan–AgNPs composite has shown higher antimicrobial activity because one-pot synthesis favors the quick formation of small AgNPs. AgNPs forms many pits and gaps indicating that it could damage the bacterial cell membrane. AgNPs synthesized by *Cryphonectria* sp. has shown dose-dependent higher antibacterial activity against *S. Typhi* and *C. albicans*. Silver-deposited reduced graphene oxide-Zinc oxide has shown enhanced antibacterial activity against *E. coli* and *S. aureus* using the disk diffusion method.

Ag-ZnO/RGO Nano composite is a promising antibacterial agent against common nosocomial bacteria, particularly antibiotic-resistant MRSA. AgNPs incorporated on reduced graphene-oxide Zinc oxide Nano composites are used in several technologies and take advantage in conductive, optical, and antibacterial applications **(Figure 4).**

Palladium nanoparticles (PdNPs)

Palladium was discovered by William Hyde Wollaston in 1803. It is named as Pallas after the discovery of asteroid. It belongs to group 10 in the periodic table; its configuration is [Kr] $4d^{10}$.

Palladium nanoparticles are typically in the range of 20-100nm with surface area 1-3 m^2/g range. Their increased surface area, make it an effective catalyst in all chemical reactions. PdNPs are identified as a strong catalyst; its catalytic activity is size-dependent, the shapes of PdNPs include cube, octahedron, and tetrahedron. It slowly dissolves in conc. sulphuric acid. The common oxidation states of palladium are 0, +1, +2, and +4. Palladium is a versatile catalyst; it speeds up petroleum cracking, alkane oxidation, and hydrogenation of alkynes to alkenes. Alloy clusters of Nano-palladium was investigated for number of carbon-carbon bond forming reactions (Heck reaction and Suzuki coupling), carbon–fluoride bond formation. It is an excellent electro catalyst for oxidation of primary alcohols. Palladium is used as a catalyst for pharmaceuticals sensors for detection of analytes, destroy harmful environmental pollutants. In addition, Pd, Pd^{2+} ion plays a vital role in biotechnological processes. According to the baseline toxicity, it is examined for their antimicrobial applications. The interesting behavior of PdNPs with unique properties explored it in various biomedical applications.

Antimicrobial activity of PdNPs

Palladium nanostructures emerged as self-therapeutics; its anti-microbial and cytotoxic activities were reported. Adams et al. reported size dependent anti-microbial activity of PdNPs. It exhibited higher growth inhibition against gram-positive bacteria *S. aureus* that gram-negative bacteria *E. coli,* this results reveal that PdNPs are useful antimicrobial agents for gram-positive bacteria. Palladium supported mesoporous silica materials displayed high cytotoxic activities against human cancer cell lines and it was reported by Balbin et al. Metallic palladium is used in the form of radioactive in the clinic. Pd needles and Pd alloys are used in cancer treatment and dental appliances. Bio distribution and accumulation patterns of palladium lead to toxicities, small changes in shape, size surface functionalization significantly block the pharmacological activity. Innovation tends to prepare controlled size and shape of Pd nanostructures to meet therapeutic and safety requirements. The unique properties and low toxicity of Pd nanostructures provide the simple system with combined effects to plays a vital role in the Nano medical field.

Copper nanoparticles (CuNPs)

Copper belongs to IV period element. It has high thermal and electrical conductivity. It appears as brown or black powder. The copper atom is coordinated by four oxygen atoms in an approximately square planar configuration. The work function of bulk CuO is 5.3 eV. Copper (II) oxide is a p-type semiconductor, with a narrow band gap of 1.2 eV. Cupric oxide can be used to produce dry cell batteries. It combines with other elements and forms alloys such as brass (Cu-Zn). They are graded as highly flammable solids. It easily agglomerates and forms a cluster. The non-agglomerated spherical, copper nanoparticles are used as catalysts, lubricants, and as Nano-fluids, etc. CuNPs are synthesized by three different methods: physical, chemical, and biological. Micro emulsion, reverse micelles, gamma irradiation, ultraviolet irradiation, polyol process, sonochemical methods, laser irradiation, thermal decomposition, chemical reduction, microwave-assisted and biological methods are employed.

CuNPs possess electrical, optical, magnetic, properties. It has high surface-to-volume ratio and yields high antibacterial and antiviral properties and used as disinfectants. Researchers proved that antibacterial activity of CuNPs on *Escherichia coli* and *Bacillus subtilis* are higher than those of AgNPs, the catalytic property of the CuNPs is highly shape and size dependent. Spherical shaped uniform CuNPs are used as lubricants. CuNPs serves as a thermally conductive material, antimicrobial agent, used in displays, printed electronics, and Trans missive conductive thin film applications.

Graphene holds up metallic Nano composites

Materials that incorporated Nano sized particles ($1nm=10^{-9}m$) into a standard matrix material are known as Nano composites. Composite materials are composed of two phases; one is continuous phase (matrix) and another is discontinuous phase (reinforcing material); they are the new budding materials with the average properties between these two phases; they display better properties such as thermal stability, catalytic, adsorption properties than that of the individual materials. The addition of nanoparticles gives tremendous improvement in properties such as strength, mechanical electrical and thermal conductivity. Nano composite materials possess (i) large surface area (ii) enable good adhesion with surface, (iii) transparency due to small size, and (iv) Improved mechanical strength. These unique properties make the Nano composite to be the potent candidate in all fields. The improved mechanical properties such as thermal stability, chemical resistance, surface appearance, flame retardancy and optical clarity made the Nano composite to explore its applications in different fields. Nano composites are divided into two broad categories; (i) functional materials (based on electrical, magnetic and optical properties) and (ii) structural materials (based on mechanical properties). The applications of Nano composites are growing at a faster rate. Nano composites are used in engineering, medicine, drug delivery, anti-corrosion, barrier protection and UV protection gels, etc.

Carbon based Nano composites specifically graphene (G), graphene oxide (GO), reduced graphene oxide (RGO) are potent candidates due to their excellent thermal, mechanical, and optical properties. Graphene based metal Nano composites possess low cytotoxicity to human cells, excellent bacterial activity, and highly explored for biomedical applications. The study of graphene based polymer Nano composites are focused on its antibacterial property and cytotoxicity to mammalian cells.

Metal based Nano composites are synthesized by constructive methods; the commonly used metals are Aluminum (Al), copper (Cu), zinc (Zn), silver (Ag), and cobalt (Co) etc.; the synthesized

Nano composites have distinct properties with respect to the metal present.

Graphene is a two-dimensional carbon structure; graphene-based metallic composites possess excellent chemical, thermal and catalytic properties. The Vander Waals forces, π-π stacking, make graphene sheets to agglomerate and decrease the surface area. This can be prevented by incorporation of metal oxide nanoparticles on graphene sheets. Graphene supported metal oxide Nano composites has increased surface area with good mechanical stability. Introduction of metal Nano particles in the host matrix along with graphene has attracted many researchers for facilitating the composite with improved properties.

Pt/Au-GO synthesized by chemical reduction method, acts as a sensor and shows high sensitivity to detect hydrogen peroxide; Pd/Co-RGO Nano composite prepared by rapid reduction method is an agent for electro catalytic oxidation of alcohol; Pd/Yt-RGO Nano composite is used in the oxygen and ethanol oxidation reactions ; Fe/Ag-RGO Nano composite prepared by one-pot method served as a potential antibacterial agent against *Bacillus subtilis, Escherichia coli* etc. ; Pt/Ru-RGO Nano composite can be used as electrochemical biosensors for hydrogen peroxide detection; Thus, it is noticed that graphene-based bimetallic Nano composites have a wide range of applications in sensors, catalyst, health care.

Graphene composites in biomedical applications

Graphene Nano composite has wider applications encompassing engineering, medicine, electronics, energy, industrial, biosensors, biomolecules carrier, batteries, solar cells, household design etc. Majority of the applications dealt with electronics and sensor. Shen et al. reviewed the biomedical applications of graphene Nano composites in drug delivery, cancer therapy, gene delivery, bio imaging, bio sensing, antibacterial, and cell culturing. The synergetic effect of graphene sheets along with metal oxide nanoparticles exhibits outstanding properties; it helps in diagnosis of disease in the human body and determines the nutrient level in it. Magnetic bimetallic nanoparticles or Nano composites have been used for the controlled drug delivery. Pankhurst et al. reported that magnetic bimetallic Nano composites eliminate infected cells from the body by the influence of external magnetic field. Graphene supported metal oxides Nano composites have been synthesized that includes ZnO, TiO_2, SnO_2, MnO_2, Co_3O_4 and Cu_2O finds wide range of bio medical applications etc.

Antibacterial activity

Antibacterial substances are anti-infection agents that slaughter micro-organisms. The utilization of antibacterial drugs for contamination is known as antibacterial chemotherapy. Antibacterial agents are mainly utilized in industries like textiles, construction, disinfection, water, medicine and food. Microorganisms have great resistance towards antibiotics; it leads to investigate Nano composites with renewed antibacterial properties. Graphene, one of the two dimensional carbon nanostructures is used as antibacterial agent due to its excellent surface to volume ratio property. Organic compounds are toxic to human body. Inorganic metal oxides possess strong antibacterial property even at low concentrations, and serves as disinfectants.

ZnO exhibited potential bacterial growth inhibition. ZnO nanoparticles synthesized by chemical reduction method have shown maximum inhibition at a concentration of 100 µl against *P. aueroginosa* and *E. coli*. ZnO- Fe/Mg composite prepared by simple method with aqueous phase showed good antibacterial activity against *S. aureus* than *E. coli.* Reduced graphene oxide along with Zinc Oxide (RGO-ZnO) Nano composite rendered more effective antibacterial activities against bacterial pathogens. It is used in food packaging and coating of biomedical devices. Valodkar reported the inhibitory activity of Cu-Ag bimetallic Nano composite against gram-positive and gram-negative bacteria at micro molar concentrations. Inorganic metals such as Silver (Ag), Copper (Cu), Palladium (Pd) have potential antibacterial property against *S. aureus, E. coli, K. pneumonia, P. aureginosa*. Zinc oxide-reduced graphene oxide Nano composites have exhibited good antibacterial activity against gram-positive, gram-negative bacterial and fungal pathogens.

Antioxidants

Antioxidant agent plays a vital role in health protecting factor. Many diseases and degenerative process in ageing happens due to the presence of oxygen-centered free radicals or reactive oxygen species (ROS). Free radicals formation is inevitable during metabolism. ROS includes Superoxide anion, H_2O_2, peroxyl (ROO-) radicals. Reactive Nitrogen Species (RNS) includes nitric oxide and peroxynitrite anion (ONOO-). These free radical species causes oxidative stress diseases such as cardiovascular disorders, rheumatoid arthritis, and neurological diseases. To prevent the diseases, the body's antioxidant defense system should be enhanced. Naturally, several enzyme systems are within the body to scavenge free radicals, Vitamin E (α-tocopherol), Vitamin C (Ascorbic acid) that are present in our regular food system, interacts with free radical, terminates the chain reaction, and prevents the vital molecules.

Numerous studies have demonstrated for supplementing dietary antioxidants. Plenty of methods are available for determining free radical scavenging activity of the composite.

DPPH radical scavenging method

DPPH (1, 1- Diphenyl-2-picrylhydrazyl) is a stable free radical has the absorbance (λmax 515-517 nm) accepts hydrogen from donor; decolourization takes place with respect to the number of electron captured. This is the most conventional low cost method for evaluating free radical of any new drug;higher the discolouration-higher the reducing property. The sample is tested by the method described by Bondet. The reduction in absorbance is measured with a spectrophotometer. The radical scavenging activity is calculated by % inhibition. Sharma reported that DPPH radical scavenging activity of ZnO/rGO has shown its absorbance at 520 nm. The observed antioxidant activity is due to the neutralization of DPPH by charge transfer of an electron. Sharma reported that rGO is potent as inhibitor for spinach assisted green reduction by DPPH free radical scavenging activity.

Nitric oxide scavenging assay

Nitric oxide scavenging activity is measured by Green et al. The compound sodium nitroprusside decompose at pH (7.2) and produces NO, which reacts with oxygen under aerobic conditions to produce stable products (nitrate and nitrite). The absorbance of the chromophore formed is immediately read at 550nm. The scavenging activity of NO by RGO-ZnO composite is highly concentration dependent. Tushar reported that nitric oxide scavenging activity of *chlorophytumtuberosum* against ascorbic acid is measured at 550 nm.

Hydrogen peroxide scavenging assay

The ability of the composite to scavenge hydrogen peroxide was determined by Ruch et al. H_2O_2 rapidly cross the cell membranes inside the cell reacts with metal ions such as Cu^{2+}, Pd^{2+} to form hydroxyl radical which may be the cause of toxic effects. Christabel reported that hydrogen peroxide radical scavenging activity of *M. aundinacea* extract showed maximum activity against standard ascorbic acid. The scavenging activity is determined by % inhibition. OH radical scavenging effect (%) = $(A_o – A_1)/(A_o \times 100)$, where A_o is the absorbance of the control and A_1 is the absorbance in the presence of the sample**.**

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the composite is evaluated according to Halliwell. The compositeof different concentration is taken along the standard ascorbic acid; the hydroxyl radical scavenging activity of *P. florida* mushroom against ascorbic acid is found to be dose dependent. The effect of graphene-based materials on decolouration rate in the presence of OH° is used as a measure of antioxidant activity. Yakimovich reported that antioxidant activity of (gold) AuNPs with hydroxyl radicals significantly depends on the specific surface of the particles.

Cytotoxicity

The ability of some chemical compounds or substances to destroy living cell is termed as cytotoxicity. It is a helpful tool to identify whether the synthesized composite pose any health threats in humans. The cytotoxicity assays measure the cell viability of the compounds; it classifies the methods assessing loss of membrane metabolic activity, membrane integrity, and destroys of cells in cell cycle. Commercial test kits assess the membrane integrity by Lactate dehydrogenase (LDH) and Trypan blue assay; these assays afford the extent of cell damage administered by the nanoparticles. MTT assay is generally chosen over other methods, it is a reliable indicator of cellular metabolic activity. MTT assay is employed on the synthesized Nano composite dissoluted in DMSO and analyzed spectrometric ally (500nm), reduction of MTT results in purple colored formazan crystals, the spectra of nanoparticle evaluates the extent of cytotoxicity. Kidney and liver are the target organs for assessing *in vitro* organ toxicity of nanoparticles. Sharma reported that ZnO NPs induced cytotoxicity is highly concentration-and time dependent. Karlsson reported that CuNPs cause cytotoxicity and DNA damage in human epithelial cell line A549. Compared to bulk formulations, small ZnONPs and CuNPs are more toxic towards aquatic organisms.

Reduced Graphene oxide-Zinc oxide (RGO-ZnO) Nano composites have exalted many applications as antibacterial and cytotoxicity. Systematic study of RGO-ZnO is examined towards MCF-7 cells, and it is reported about the cell destruction. The size of the Nano particles present in the (rGO-Ag) Nano composite induced cell death and decreases cell viability through the generation of ROS. Cytotoxicity analysis for Au-rGO Nano composite is performed on HeLa cell line, MCF cells, using the MTT assay; dose-dependent cytotoxicity is observed for both cells. These reports confirmed that RGO-ZnO/metal Nano composites have good biocompatibility towards MCF-7 cells, HEK 293 and HeLa cell line.

Discussion and Conclusion

Here we reviewed sequel of graphene oxide formation from its raw carbon material coupled with their antimicrobial applications. Further improvement of the quality along with development of a composites for such hybrid will help to realize many applications including graphene-based biomedical devices, for Ag releasing, for anticancer, and others health care industrial application. In future we expect fine control of the quantity of RGOhybrids is an important challenge to control toxicity. If the hybrid satisfies the above statement RGOhybrids are expected to become good biomedical devices.

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