GRAPHENE-BASED MATERIALS FOR IMPLANTS

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Abstract

Over the years, many different materials, e.g., titanium (Ti) and its alloys, have been used in biomedicine for several purposes. A common application of such materials is seen in their usage as implants. However, quite a number of graphene-based materials have emerged and developed from a two dimensional single atomic thick block of a carbon allotrope, known as graphene. Since the discovery and isolation of graphene from graphite in the year 2004, there has been tremendous positive improvement in health conditions which require treatments that involves the use of implants. World-wide, this has led to significant attention and appreciation of this versatile material, in biomedicine and obviously, in all fields of science and engineering. Examples of some of the graphene-based materials to be discussed, include: reduced graphene oxide (rGO) and graphite oxide (GO). Although, graphene-based materials are distinguishable by their individual and unique properties, nevertheless, they still have certain characteristics in common. Owing to these properties, possessed by different graphene-based materials, they are able to serve in the biomedical field as implants in order to combat a wide range of diseases that have been a challenge, previously. This chapter elaborates on some different graphene-based materials, in respect to their: structures, synthesis, properties, advantages and disadvantages and the applications of these materials as implants in biomedicine.

Key words: Implants, reduced graphene oxides (rGO), graphene oxide (GO), carbon allotrope

1.0 Introduction

In the biomedical field, research for implant materials have been ongoing for years. This has led to the discovering of some few suitable materials, one of which is graphene and graphene based materials (GBM). The study of the properties if graphene and its derivatives have received a lot of attention and interest from researchers since its discovery [1-10]. The goal is to investigate properties that can potentially make them serve as better implants in several biomedical applications [11]. In the year 2004, graphene which is the youngest allotrope of carbon was discovered by scientists. Although, there were theoretical evidences of the existences of graphene in graphite as far back as more than 50 years ago [12]. However, the ability to separate individual two dimensional sheet was seen to be impossible from principle. Two scientists namely: Andre Geim and Kostya Novoselov from the University of Manchester were able to successfully separate the two dimensional sheets of graphene from the multilayered graphite. After overcoming the strong forces (van der Waals) that bonded the stacks of graphene sheets in the graphite crystal, the sheets were proven to be free standing and highly stable [13, 14]. This success and the outstanding properties of the material led to a Novel prize award in 2010 [15]. Despite the discovery of graphene in 2002, it ability of becoming a universal material is just recently discovered and explored [16].

Graphene exists in the form of a honeycomb which consists of hexagonal rings formed by the atoms of a one-thick layer of graphite [8, 14, 15, 17, 18]. The bonds that exists between the structure of graphene is difficult to break however, it accounts for its durability and ability to stretch (20-25% of its initial length) [19]. The properties of graphene, which makes it an interesting material is attributed to the configuration of its bonds and the uniqueness of its two-dimensional structure [11, 20, 21]. Some of these awesome properties which make graphene and graphene based materials excellent candidate for implants include impermeability [6] , great strength, low weight [22], almost transparent (as it absorbs about 2.3% of white light hence makes it slightly visible to the naked eye [4, 5], high chemical reactivity, biocompatibility and unparalleled thermal, electrical [5, 20, 23-25], surface properties [26-29]. These properties accounts for the advantages associated with the use of graphene and graphene based materials. The biomedical application of graphene has been greatly explored due to its ability to physically interact with other biomolecules such as DNA, enzymes, proteins, or peptides [30-32]. The main advantages of using graphene based materials for implant are that it is more durable in the body and less harmful compared to materials that have been in use over the years [11, 33]. In addition, some antimicrobial properties capable of boosting

biocompatibility of implants have been seen in graphene and its derivatives. A very exciting characteristic of graphene is the highly specific surface area it possesses [18, 20, 21, 34]. This makes it possible for every carbon atom to be exposed on both surfaces. Thus, a maximum surface area for nano-sized materials is obtainable, thereby providing a platform for bio-functionalization [18, 35, 36]. The optical properties of graphene such as saturable UV/visible absorbance and surface-enhanced Raman scattering have been reported to be useful in biological imaging and bio-sensing applications [37]. By utilizing the electrochemistry and fluorescent properties of graphene, graphene based materials can be designed with better performing abilities for biomedical applications. Also graphene has the shape, size, morphology, the thickness and degree of oxidation that is favorable for bio-molecular studies [38]. The surface area of graphene is 2630 m^2/g [39], stiffness of 1TPa and tensile strength of 130 GPa [1, 22]. The large surface area allows for the anchoring of large amount of molecules [26, 40]. The length of its carbon-carbon bond is about 0.14 nm and interplanar spacing of 0.34 nm, the distance between the carbon atoms of graphene makes it act as a quasi-solid net hence its impermeability [11, 41]. Its stiffness contributed to its applicability in bone and neural tissue engineering [42-47]. At a temperature of $350^{O}C$, graphene is liable to getting burnt and it generally has edges that are very chemically reactive [48]. For graphene to be utilized in any application, it must first be extracted from graphite however this yields only a small amount of graphene. Therefore, in order to produce a large quantity, a method known as chemical vapor deposition (CVD) is used [49-52]. This versatile method is the most widely employed method of synthesizing graphene because it yields thin films of graphene and which is flexible and hydrophobic. Other methods employed in the synthesis of graphene is seen in the Table 1 below [53, 54].

Method of synthesis	**Properties of obtained graphene and advantages of the method**
Chemical vapor deposition (CVD) method	• One-layer graphene is obtained using copper as a catalyst • Graphene with high quality • Inexpensive and realistic method to obtain multi-layered graphene • Ability to scale up production
Wet-chemistry approach	• Compared to exfoliation and epitaxial growth, it is more versatile • Ease of scaling up • Alteration of the electronic, optical, and mechanical properties of graphene may

	occur as obtained graphene is partially synthesized.
Exfoliation and cleavage method	• Graphene possesses excellent electrical and structural quality • It is the simplest method, although it leads to the formation of uneven graphene films • The simplest and earliest method.
Epitaxial growth method	• Graphene with multilayered structure is obtained • Ability to control the number of layers formed is made possible • Graphene obtained via this route has limited application in biomedicine • Graphene synthesized through this method is difficult to functionalize • _ It is difficult to functionalize graphene obtained via this route.

The different techniques used for graphene synthesis leads to the formation of graphene based materials with different number of layers and/or chemical groups [55]. Furthermore, graphene is known to be a major building block for a lot of allotropes of carbon such as carbon nanotubes, fullerenes, graphite etc [22, 28]. Several carbon modifications can be done on graphene to obtain new undiscovered materials that are carbon allotropes [56]. The chemical and/or physical modification of graphene sheets has led to the formation of graphene related materials such as single and multi-layered graphene, graphene oxide (GO), and reduced GO (rGO). Each of the graphene based material has its individual unique tunable properties [33]. Graphene and graphene based materials have become a class of nanomaterials that are very vital in the biomedical science. It has also become a model system for quantum behavior. Graphene based materials are mostly preferred, very useful and effective in biomedical applications because of the great functional groups attached to their backbone [38]. These materials have found application in areas such as imaging, tissue engineering, bioelectronics [57, 58], biomolecular analysis, discovery of biomarkers, photothermal therapy [59] and drug/gene delivery among others [33, 60-67]. This new and very versatile material has opened new research areas for scientists in several other fields [68, 69] and it has the potential of changing a lot in the biomedical field in the twenty-first century.

In this chapter, the structure, synthesis, properties and some of the applications of graphene based materials in biomedicine will be discussed. In addition, biodegradability and the risk factors associated with using graphene based materials for implants will be briefly highlighted.

2.0 Graphene based materials

Materials that are related to graphene are generally referred to as graphene based material (GBM). They can be classified based on the number of graphene layers they possess (single or multilayered graphene) or their chemical modification (reduced graphene oxide or graphene oxide) [55]. Recently, graphene based materials have generated increasing interest because of the uniqueness of their two-dimensional carbon geometry. This offers excellent physicochemical properties that are promising in diverse fields including biomedicine [70]. Over the years, researchers have developed various graphene based constructs by employing methods such as coating, hydrogel blending, wet/dry-spinning procedures, and 3D printing to make 2D or 3D. They have also been able to enhance the properties of graphene based materials by tethering them with other biological materials [33].

2.1 Synthesis and properties

Various methods are available for the synthesis of graphene in different forms: these are "topdown" and "bottom-up" methods. The "topdown" method involves mechanical exfoliation of graphite and it is otherwise known as "Scotch tape" or peel-off method [55]. In this technique, graphene flakes which are micrometer in size are detached from a crystal of graphite by using adhesive tape [9, 22, 50]. Another form of "topdown" method is the chemical exfoliation of graphite. This involves the oxidation of graphite with the use of strong acids such as sulphuric or nitric acid, oxygen atoms are inserted in between the individual graphene sheet, thus, causing a separation [55]. The other technique of synthesizing graphene based materials is the "bottom-up" method. The different methods used in synthesizing and preparing graphene based materials are controllable thus, specific and desired properties for various applications can be conferred in them [71].

2.1.1 Graphene oxide (GO)

Graphene oxide is a monolayer graphene based material that has high oxygen content. It is the highly oxidized form of graphene and it is obtained by oxidizing and exfoliating graphite, accompanied by an extensive basal plane modification via oxidation [55, 72]. The chemical exfoliation of graphite results in suspension of GO sheets that are further filtered and isolated to obtain GO flakes [8, 73]. GO is an amphiphilic compound that permits functionalization of

the surface and it can easily spread in solutions that are aqueous, physiological media, and other organic solvents [74]. Upon dispersing GO in water, it becomes negatively charged and this was proven by measuring the surface charge of GO by using zeta potential measurements. The stability of GO in suspension is attributed to the electrostatic repulsion between the negative charges and the environment. Furthermore, GO consists of covalently bonded oxygen-containing functional groups, majorly hydroxyls and epoxides. In addition to these groups found at the basal plan and the edges of GO, carbonyl carboxylic groups are also thought to populate the edges [51]. Therefore, GO is a combination of both sp2/sp3 hybridized carbon atoms [35, 75]. As a result of a defective surface and the energy gap created by oxidation, GO has a compromised electric property as seen in the ability to conduct electricity [35, 76]. This surface defect also creates sites that are chemically reactive, which allows the breakdown of GO into smaller pieces. This leads to the formation of nanosized sheets with properties that are different from the original material [35, 77]. However, the presence of several oxidation groups on the edges and plane helps its physiological solubility and stability. Hence, it permits GO to be more biocompatible and does not induce oxidative stress since no catalyst is involved in the process of synthesis [24, 35]. In addition, there is a great possibility of a wide range of organic and inorganic molecules to interact with GO due to the oxidative groups present on the surface. The molecules are bonded to GO by either covalent, non-covalent (π-π or hydrophobic) and/or ionic interactions [18, 24, 35, 78]. This ability to interact with several molecules opens GO up for diverse biological applications [24]. Thus, they have found great relevance in areas of biomedicine such as gene/drug delivery and substrate modification [3, 30, 34, 64, 79-81]. Although thin membranes produced by using GO permits the flow of water across the membrane but not harmful gases.

2.1.2 Reduced graphene oxide (rGO)

This form of GBM is obtained by reducing the oxygen content of graphene oxide by using different methods. The reduction of GO involves the conversion of sp3 carbon to sp2 carbon [82, 83]. This can be chemical, photo-chemical, thermal, photo-thermal, microwave or microbial/bacterial methods [72, 84-87]. The reduction of GO to obtain rGO is a very crucial process as this largely affects the quality of the reduced graphene oxide produced and the structural closeness to pristine graphene [88]. Chemical method of synthesizing reduced graphene oxide is the most scalable method however, poor yields of rGO is often produced with respect to the surface area and electronic conductivity. The use of hydrazine hydrate ($N_2H_4 \cdot H_2O$) as a reducing agent is the most commonly used chemically used method. However,

other reducing agents such as dimethyl-hydrazine [89], hydroquinone [90] and $NaBH_4$ [82, 91, 92], have also been employed to prepare rGO have also been reported. Thermally reduced graphene oxide at temperatures of 1000^{o}C or above on the other hand yields rGO with very high surface area similar to pristine graphene. However, this has a detrimental effect on the produced rGO. The mechanical strength and the mass are potentially affected as a result of the damage caused to the structure of the graphene platelet by heating. Although it is generally easier to obtain rGO when compared to other graphene based materials. However, it does not have a wide range of application [84]. The synthesized rGO can further be reduced to graphene-like sheets by the removal of the groups containing oxygen [3, 10]. In a study, rGO was generated by treating GO with hydrazine at 100°C for 24 hours [93]. The obtained rGO had less surface oxygen thus, causing it to be less stable in water (hydrophobic). In another study, ascorbic acid was used as a reducing agent instead of hydrazine. It was discovered that rGO obtained using ascorbic acid was more biocompatible when compared to hydrazine derived rGO [85, 94, 95]. It can therefore be said that rGO obtained by using ascorbic acid as the reducing agent is more suitable for biomedical applications. One of such application is in tissue engineering which requires good electrical properties that enables cell to cell signaling [33]. Other methods of synthesizing rGO in the past includes the following;

- Heating graphene oxide in a furnace to very high levels
- Exposing graphene oxide to strong pulse light. For example, light produced by xenon flashtubes
- Linear sweep voltammetry
- Exposing graphene oxide to hydrogen plasma for few seconds
- Heating a solution containing graphene oxide and a reducing agent such as urea

Apart from the above listed methods of rGO synthesis, there are several other ways which reduced graphene oxide can be obtained. Some of the other novel reduction methods that have been proposed for reducing GO include photo-catalytic method [87, 96, 97], biomolecule-assisted methods [98, 99], plant extract method [100], supercritical fluid method [101] and electrochemical method [102]. However, they are all based on chemical, thermal or electrochemical means. Some of these methods have the ability to produce reduced graphene oxide with very high quality that is comparable to the pristine graphene but they may be time consuming and complex. A large scale production of reduced graphene oxide has been done by using electrochemical method and a high quality of reduced graphene oxide was produced. In this method, graphene oxide was used to coat different substrates (tin oxide and glass

respectively) and electrodes were placed at the ends of the substrates in order to create a circuit through GO. Linear sweep voltammetry technique was employed on the GO in a sodium phosphate buffer. It was observed that GO reduction started at 0.6 volts and at 0.87, maximum reduction was observed [103]. Other experiments that have employed the electrochemical technique have reported that the carbon to oxygen ratio and the electronic conductivity of the obtained rGO is higher than other materials such as silver. Another advantage of this method is that it does not involve the use of harmful chemicals hence, no need to dispose any toxic waste [104]. The down side however is the scalability of this technique as it is difficult to deposit GO onto the electrodes in bulk. It is interesting to know that once reduced graphene oxide has been synthesized successfully via any of these methods, it can be functionalized for different applications.

2.1.3 Graphene nanomaterials

Graphene nanomaterials are generally defined as graphene based materials with two dimensional structure and a thickness or lateral dimension of less than 100 nanometers. Examples include graphene nanoflakes, graphene nanosheets, graphene nanoribbons [72]. Graphene nanoribbons are one dimensional carbon crystals, thin strips of graphene. They can exist as Zigzag GNR or Armchair GNR depending on the structure of the edge. Their different electronic state which is either metallic or semiconducting depends on the width of the strip. Therefore, they can be particularly suitable in different applications. Graphene nanomaterials are ideal materials for composites that requires good electrical conductivity. Although they are not primary part of a carbon material, they can be suspended freely and can also bind to substrate [72]. Apart from these above discussed graphene based materials, other examples of graphene based materials include few layer graphene (FLG) or multilayer graphene (MLG). They contain between two to ten layers of graphene, they can be counted, well defined and are stacked graphene layers with lateral dimensions that are extended [53]. They can exist as sheets, films that are free standing or as substrate bound to coatings [72]. Initially, few layer graphene was considered a by-product during the synthesis of monolayer graphene, however, it later gained recognition as an interesting material with commercial value [53] . In recent years, it has attained a high level of biomedical application. In addition, graphene quantum dots (GQDs) are another set of functionalized graphene structures that are nanometers sized with quantum phenomena. Like other graphene based materials, they have received significant interest among researchers due to their optical properties in the presence of photoluminescence. These GBM has the ability to bind specifically to a broad range of biological molecules. For

example, their morphological and intrinsic characteristics enables them serve in the analytical transduction of biosensors on the limit of detection, sensitivity, selectivity, repeatability and biocompatibility [71].

2.2 Applications of graphene based materials

Graphene based materials are typical examples of novel materials that have recently been introduced in the biomedical field. They possess great properties/characteristics that make them very useful in biomedical applications. Due to the different methods of preparing and synthesizing these materials, the characteristics of these materials, there is a variation in their physicochemical properties. Majority of the studies that have been carried out on the application of graphene based materials is on GO and rGO therefore there is need for an increase in the scope of studies [70]. Graphene based materials have relevance in fields such as mechanical engineering, electrical engineering, electronics (micro-electronics), desalination, tissue engineering, cancer treatments, coatings, biosensors, nanocarriers for drug and gene delivery, devices for cell imaging and phototherapy for cancer [3, 18, 64, 81, 105, 106] implants, metal detection and removal, as well as nuclear waste treatment etc [72, 107]. However, only the potential application of graphene based materials in implants will be considered and discussed.

2.3 Implants

Biomedical implants are primarily considered to be any material, structure or device that are directly inserted into the human body for the purpose of improving the health condition of a patient. They help to enhance the quality or function of a biological structure or support a damaged biological structure [108]. The use of implants began in the mid of the 20th century with the aim developing materials which are biocompatible (with little or no toxic effect on the host). The main materials which used were stainless steel and cobalt alloys, they aimed to have properties which are similar to the replaced tissues [109]. As the years rolled by, researchers became concerned with developing other materials capable of interacting with the biological environment of the body [11]. They discovered new materials such as metals however, they were not bioactive so there was need to coat them before they were used for biomedical applications. Examples of materials they were used for coating are ceramics (hydroxyapatite) and bioactive glasses. At the moment, attention is given to new materials that can serve as implant at the molecular level in order to arouse specific cellular response [11, 109]. Also, there is careful investigation on the biodegradability of these materials in addition to their bioactivity.

Thus, the development of bio-absorbable materials. One of the major material that have been commonly used is titanium and its alloy [110]. However, due to its lack of bioactivity, there is need for discovery other materials that are more suitable and appropriate for implant application. Additionally, limitations such as inability of titanium alloy to match the mechanical behavior of natural bone, inferior wear resistance and fracture toughness hindered its long term clinical application [11]. Therefore, the quest to develop other materials for such application of which graphene based materials have shown to be promising. Graphene and its derivatives have indeed proven to be excellent candidates for a wide range of implant applications [111].

2.3.1 Orthopedic implants

Over the years, there has been an increasing interest in materials and techniques that can positively improve attachment, proliferation and differentiation of cells. Such materials promote reconstruction and quick healing of major/large bone defects. Graphene and its derivatives have emerged one of such materials with remarkable properties for such application in biomedicine. It has been discovered that they have the ability to induce and sustain the growth and differentiation of stem cell into different lineages. Also, osteogenic differentiation of the human MSCs is enhanced and promoted by graphene based materials due to their mechanical strength and protein adsorption capability [55, 112, 113]. Thus, graphene based materials are excellent candidates for scaffolds and implantable devices to promote the proliferation and differentiation of cells [111, 114]. These abilities as well as their biocompatibility and low cytotoxicity have made very useful in bone tissue engineering. In addition, the intrinsic antibacterial properties of graphene oxide have been seen to prevent implant induced infection in some research [115, 116]. It is interesting to know that graphene based materials can speed up the differentiation of cells in the absence of growth factors (e.g BMP-2) which are commonly used [117]. This may be attributed to their ability to increase local dexamethasone concentration through π- π stacking between the aromatic rings in the biomolecules [40, 118]. Another benefit associated with the use of graphene based materials for bone regeneration is the ability to enhance osteoconductivity. This is achieved by biomineralization and cellular osteogenic differentiation. A typical example is the mixture of calcium carbonate (biomineral) with graphene oxide sheets and graphene in order to boost biomineralization [119]. Also, high viability and elongated shapes were seen in an experiment where osteoblasts were grown on mineralized GO or graphene calcium phosphate composites [33]. In a study, rats were implanted with graphene hydrogel film and it was observed that this

material Induced bone regeneration by osteogenic differentiation. This was attributed to the good mechanical and rough surface morphology of the graphene based material [120]. Therefore, the high elastic modulus of graphene-based material of approximately between 1 to 24 Tpa can lead to a spontaneous osteogenic differentiation [121]. The greater the disorderliness of the topography of protein based materials, the better the environment provided by them for protein adsorption and subsequently, the growth of cells. For implantation purpose, porous graphene hydrogels obtained by a non-covalent interaction are better options that the conventional hydrogel system. This is because they have greater mechanical strength and at the same time maintain mechanical flexibility [120]. In some experiments, graphene based materials have been used in combination with hydroxyapatite (HAP: $Ca_{10}(PO_4)_6(OH)_2$; which is the most abundant composition of the inorganic part of bone [119, 122, 123]. They reported that the formation of new bone and osteogenic differentiation of cells was enhanced. In addition, GO/graphene-HAP composites provided an environment that can be likened invivo as a result of the high viability of osteoblasts with elongated morphology that was observed. By modifying the surface of GO, biomimetic mineralization on GO can be enhanced. Functional groups such as a sulfate-containing moiety can stimulate the binding of Ca^{2+} hence, points of nucleation for the mineralization of HAP [124]. In an experiment, natural polysaccharides (carrageenan) consisting of highly sulfated units was functionalized on the surface of graphene oxide. The growth of MC3T3-E1 cells on Car-GO and GO was compared. A higher cell viability and proliferation in addition to elongated shapes was observed in cells grown on Car-GO compared to GO. The cellular activity of ALP on Car-GO grown cells showed significant increase when compared to GO. They also reported that HAP mineralization was greatly induced, cellular attachment was enhanced and bone mineralization activity was stimulated [33]. In another study, the MSCs of mice were cultured on graphene-HAP nanocomposite hydrogel and reduced graphene oxide. A higher cellular viability with more elongated morphology of the cell was observed in the nanocomposite hydrogel when compared to reduced graphene oxide. This suggests an enhanced cellular affinity on the graphene-HAP nanocomposite. The observations made from this study can be attributed to the ability of graphene and HAP nanoparticles to self-assemble and form a 3D nanocomposite hydrogel via colloidal chemistry synthesis technique [125]. The hydrothermal treatment undergone by the materials results in the increased thickening of GO nanosheets and the π-π interaction causes an attraction between them (graphene and HAP nanoparticles). Furthermore, the presence of citrate ion in the citrate-stabilized HAP nanoparticles leads to the reduction of graphene oxide to reduced graphene oxide forming a graphite-like shell [126, 127]. This shells

serves as a dialysis membrane that helps in the removal of excess ions and at the same time deposit destabilized HAP nanoparticles on graphene flasks. As soon as the HAP nanoparticle is entrapped within the network of the 3D graphene, a homogenous graphene-HAP gel is formed and ready to be used. Similarly, through hydrogen bonding and electrostatic interactions, rGO sheets and HAP microparticles can attach to each other [123, 128]. The calcium moiety present on the surface of the HAP microparticles can be immobilized to the hydroxyl and carboxyl groups on the surface of the reduced graphene oxide sheets. This is possible as a result of the electrostatic interaction between the calcium moiety that is positively charged and the carboxyl and hydroxyl groups that are negatively charged. Although, the bonding of these materials (rGO sheets and HAP microparticles) can also occur as a result of an induced hydrogen-bonding interaction between the hydroxyl group present in the HAP microparticles and groups containing oxygen in reduced graphene oxide sheets. A couple of other studies have shown the enhanced cellular viability of reduced graphene oxide nanocomposites on MC3T3-E1 cells. Result from one of the studies shows that rGO sheets and HAP microparticle nanocomposite had a higher cellular viability when compared to HAP microparticles. Also, spontaneous osteodifferentiation of preosteoblasts (MC3T3-E1) was enhanced in cell groups that were grown on rGO/HAP nanocomposite. Additionally, an invitro evaluation shows a significant increase in calcium deposition, as well a higher expression levels of osteopontin and osteocalcin in rGO/HAP nanocomposite grown cells [123]. At the invivo stage of implanting rGO/HAP nanocomposite in a huge bone defect model, from observations, the inflammatory response was reduced and the formation of a new bone was stimulated [128]. Graphene based materials have also been combined with strontium and calcium silicates in order to investigate their effect(s) on osteogenic differentiation. Strontium particles were embedded in the network matrix of GO and rGO. A continuous release of the strontium ion from the scaffold composite was seen to stimulate cell proliferation and osteogenic differentiation [129]. Likewise, the addition of rGO to $CaSiO_3$ matrix stimulated ALP activities and cell proliferation of human osteoblasts cells more than calcium silicate ceramics when compared [33].

2.3.2 Dental implants

The heterogeneous and dynamic anatomical structure of the teeth makes it quite difficult to treat and manage. This tissue which consists of dentin-pulp complex, cementum, periodontal ligament, enamel and alveolar bone is limited in its ability to undergo self-repair when injured or diseased [130]. Cementum and dentin can regenerate although at a very slow rate for

cementum, dental pulp is able to regenerate partially while enamel tissue cannot regenerate at all [131]. However, in the last decade, a lot of focus has been on overcoming these limitations by several researchers [132-138]. One of the ways they have addressed these challenge is the use of scaffolds made of polymers and nanomaterials amongst other materials [139-143]. Recently, graphene based materials emerged as one of such nanomaterial used in dental applications [33] and a couple of studies have been carried out by researchers to evaluate the effect of graphene based materials on dental cells. In a study on dental pulp stem cells (DPSCs), Rosa and fellow workers compared the effect of graphene oxide scaffold and glass substrate on the proliferation and differentiation of the cells [144]. They reported that the cells (DPSCs) attached to both glass and GO surface without a significant difference in the proliferation rate of the cells. However, a significant higher level of mRNA expression for all the genes (Msh homeobox 1 (MSX-1), Paired box 9 (PAX-9), RUNX2, COLI, Dentin Matrix acidic Phosphoprotein 1 (DMP-1) and Dentin Sialophosphoprotein (DSPP)) was observed in cells treated with GO compared to the glass substrate. This result suggests that GO substrate has the potential to enhance the expression of odontogenic genes opening new opportunities to the use of graphene based material. In another similar experiment, the potential of GBM to induce odontoblastic or osteogenic differentiation of DPSCs without using any kind of chemical inducers was evaluated [145]. From their result, it was observed that the gene and protein expressions of RUNX2 and OCN was more increased by the GBM when compared to the glass substrate. Thus, it suggests that GBMs have the ability to induce odontogenic differentiation of DPSCs but not as much as they can induce osteogenic differentiation of DPSCs. Some other studies have investigated the effects of GBMs on another dental cell: Periodontal Ligament Stem Cells (PDLSCs). These are cells responsible for the maintenance of the periodontium (structures which surround and support teeth. In one of the limited studies done on these cells, an evaluation on the effects of GO, Silk Fibroin(SF) and the combination of both (GO+SF) was done [79]. The researchers investigated the cells adhesion, proliferation, viability and expression of MSCs markers. In their experiment, healthy molars were extracted, cultured for 10 days on the different substrates (GO, SF and GO+SF) and a plastic substrate which served as the control. The immunofluorescence staining of the actin cytoskeleton showed that the cells adhered most to the GO substrate while the MTT assay showed highest rate of proliferation when compared to SF and GO+SF substrate. In addition, it was concluded that the incorporation of GO with SF improved the performance of the fibroin films. Hence, GO can serve as a better alternative to coat fibroin. Furthermore, the ability of SF and GO (in combination) to promote the differentiation of PDLSCs was investigated by the same set of

researcher [146]. The results of their study showed that as the cells treated with low amounts of GO and high amount of SF had a consistent improvement in the rate of proliferation and differentiation. They also stated that the proliferation rate of the cell is most enhanced when the cells are treated with only GO and a 1:3 ratios of rGO: rSF. In addition, the gene expression of the cells was further analyzed in order to evaluate the effects of these scaffolds on PDLSCs differentiation into osteo/cementoblast-like cells [147]. The experiment was done without the use of a chemical inducer in the medium. It was observed that the over expression of early osteoblast/cementoblast markers such as BMP2, RUNX2, ALP and COLI was induced by GO-SF composites especially in their reduced states (rGO, rSF and rGO-rSF). On the other hand, a down regulation of osteoblast markers Osterix (OSX) and Osteocalcin (OCN) was observed in all substrates. Implants osseointegration has also be seen to improve as a result of the use of graphene based materials. Titanium (Ti) is a material that has history in dental implant application for teeth replacement due to its reliability, mechanical strength, biocompatibility and predictability [148, 149]. However, as a result of its inertness which may cause the development of fibrous tissue and subsequently implant failure, studies on how to modify its surface has been embarked upon. Some studies have revealed the benefits of associating graphene based material with implants for dental application. Graphene based materials have been found to be excellent implant-coating for dental application. A research done by Zhou and colleagues is one of the studies have revealed the benefits of associating graphene based material with implants for dental application. They investigated and compared the morphology, proliferation and osteogenic differentiation potential of PDLSCs seeded on GO-Ti scaffolds with sodium titanate (Na-Ti) substrates [150]. Observations made from the study are: higher proliferation rate of cells seeded onto GO-coated Ti-scaffolds and higher ALP activity was exhibited when compared to cells seeded with Na-Ti substrate. In addition, the gene expression levels of osteogenesis-related markers (COLI, ALP, Sialoprotein (BSP), RUNX2 and OCN) was up regulated in cells seeded with GO-coated Ti-scaffolds. Also, at the protein level, an enhancement in the expression of RUNX2, BSP and OCN was associated to the presence of GO. It was concluded that GO is a promising material in dentistry especially in Ti dental implants. Another set of researchers also functionalized GO-Ti implants via different methods with a synthetic glucocorticoid in order to improve stem cells osteogenic differentiation [148, 151]. GO-coating of Ti implants improved biocompatibility, cell proliferation cell osteogenic differentiation as observed in both methods that were used in the functionalization and coating of the Ti implant. Furthermore, it has been discovered that bioactive proteins such as BMP can enhance osseointegration when they are incorporated in implants [152, 153]. This therefore

prompted La and team to evaluate the efficiency of GO coated on Ti substrate for the delivery of BMP-2 (one of the most potent osteoinductive proteins) and a stem cell recruiter protein (Substance P). Results of the invitro evaluation showed that the difference between the release of SP from Ti and Ti-GO was not significant. However, BMP-2 release from Ti substrate occurred within 24 hours while its release from Ti-GO substrate was maintained for two weeks. La and team went further to do an *invivo* study on the bioactivity of the proteins when loaded on implants. They implanted Ti-BMP-2, Ti-SP-BMP-2 and Ti-GO-SP-BMP-2 on the calvaria of mice. Ti-GO-SP-BMP-2 showed the greatest extensive formation of bones compared to other groups. Thus, it suggests that the presence of GO has the ability to preserve the bioactivity of recruiter and osteoinductive proteins [154, 155]. The antibacterial properties if graphene based materials have also been discovered to be advantageous in dental implant applications. This was demonstrated by functionalizing Ti coating with GO and antibacterial substances. In one of such experiment, minocycline hydrochloride was included in a GO-coating in order to enhance the anti-bacterial activity. He effectiveness of this designed implant was tested against aerobic or facultative anaerobic bacteria (*Staphylococcus aureus*), facultative anaerobic bacteria (*E. coli*) and anaerobic bacteria (*S. mutans*). A synergic effect was observed between GO and minocycline hydrochloride as seen in the death of the bacteria [156]. Similarly, the anti-microbial activity of GO-silver coating on Ti against *S. mutans* and *P. gingivalis* was investigated by Jianfeng and coworkers. They reported the significant efficacy of the GO-silver-Ti implant and suggested that the nanocomposite may help in averting infections that are associated with implants [157].

2.3.3 Drug delivery implant

Research on drug delivery implant is growing day by day due to the need for a safe and better method of delivery pharmaceutics to targeted sites in the body. These studies include both *invivo* and *invitro* evaluation of different graphene based materials for drug delivery implants. Therapeutic agents such as doxorubicin and curcumin have been loaded unto graphene based materials because of some intrinsic properties they possess. The major properties harness in drug delivery application include high surface area and sp2 hybridization [158] as these allows for loading of a larger amount of drugs. One of the graphene based material that has received significant attention in drug delivery implant application is graphene oxide. Nanocarrier (GO) synthesized by vigorous oxidation of graphite using Hummers technique is known to be ideal for drug and gene delivery. Usually, the GO nanocarrier suitable for this application has a thickness of 1-2 and consists of between 1 to 3 layers with size of about few nanometers to

several hundred nanometers [35, 60, 159, 160]. The ability of the reactive COOH and OH groups present on the surface of GO permits its conjugation with polymers [161], biomolecules (biotargeting ligand [60], DNA [162], protein [163-165], quantum dots [166], Fe_3O_4 nanoparticles [167], and others [168]. Hence, the application of GO can be seen in various biomedical fields.

Liu and co-workers have carefully reviewed the advantages of a large surface area and the presence of functional groups such as hydroxyl, carboxyl, epoxy on graphene based materials [81]. It was reported that these factors permit the immobilization of drug molecules in targeted drug delivery. This suggests that graphene based materials such as GO a potential candidate for successful drug delivery. Other studies have also demonstrated the ability of GO and its derivatives to serve as a drug delivery implant as well as a photothermal therapeutic agent capable of enhancing cytotoxicity [169]. This was seen in an experiment where anticancer drugs; SN38 and doxorubicin were loaded on the nano-GO [170, 171]. In their experiment, a six armed polyethylene glycol molecule terminated by an amine group was linked with nanoscale graphene oxide (NGO). After which, a simple non covalent adsorption method was used to load the anticancer drug on the NGO-PEG composite through π-π stacking. The NGO-PEG nanocarrier was used to deliver drugs to HCT-116 and CPT-11 cells respectively. They reported that NGO-PEG loaded with SN38 was highly cytotoxic for HCT-116 cells but far more potent than CPT-11 cells. Furthermore, decitabine (drug) was loaded by Lu et al on a hybrid drug delivery vehicle consisting of GO and aptamer [172]. Cancer cells were selectively targeted by synthesizing nano-GO. The adjustment of the concentration and pH during the process of synthesis, led to an alteration in the drug loading ability of the GO [173]. Although, it was discovered that a double load of drug on nano-GO are more cytotoxic when compared to nano-GO with a single drug load [174]. In another evaluation done by the same researcher, facile amidation technique was used to attach polyethyleneimine to GO through covalent bonding [174]. Drugs in combination was loaded and delivered to targeted cells. Results reveal the enhanced anticancer performance of the drugs as a result of the synergistic effect exhibited. Bcl2-siRNA and DNA synthesis were both inhibited [175]. Weaver and his group have also shown that controlled drug delivery on GO electrically is possible [176]. In this research, the drug dexamethasone was loaded on a GBM-polymer scaffold and by adjusting the voltage stimulation, the drug was released in a controlled linear fashion. In another work, Rituxan (CD20+ antibody) was conjugated with NGO-PEG for targeted drug delivery [60]. The drug release was seen to be pH dependent, thus suggesting a pH-controlled drug release. Other

studies that suggest the possibility of drugs loaded onto a graphene based material is released in a pH-controlled manner includes works done by Shen, Depan-Misra and their colleagues [65, 177, 178]. A thermoresponsive drug delivery implant which consists of poly(N-isopropylacrylamide) and graphene sheets have also been designed by Pan and coworkers [179]. Few researchers have employed the use of graphene based materials with multiple drugs since the discovery of the use of multiple drug to combat drug resistance associated with several disease condition such as cancer [180, 181]. One of such study on graphene based material is that of the use of GO for the targeted delivery of two chemical drugs [178]. In the study, GO containing folic acid and SO_3H groups was loaded with doxorubicin (DOX) and camptothecin (CPT). This was achieved in a controlled manner through π-π stacking. Upon a successful loading of these drugs, it was tested against MCF-7 human breast cancer cells. Result reveals there was more specific targeting of the cells in the group that was treated with GO-folic acid-DOX-CPT when compared to the group treated with a single drug. Also, a much higher toxicity to the cells was observed in the group treated with both drugs. In another recent study, the use of chitosan-grafted GO for delivery of Ibuprofen, an anti-inflammatory drug was evaluated by Rana and team [182]. From their reports, by adjusting the pH value, a controlled release of the drug is achievable. In addition to all these different studies, Yang and co-workers did a study on a graphene based material for an enhanced anticancer effect on SK3 human breast cancer cells [183]. A magnetic and bio-dual targeting drug delivery vehicle made of GO-Fe3O4 nanoparticle hybrid was designed by this set of researchers. Results from their invitro evaluation suggests that the drug delivery cargo is capable of specific cell targeting. While, the invivo study was aimed to show the magnetic field-guided and bio-targeted ability of the GO-Fe3O4 nanoparticle hybrid.

2.3.4 Biosensor implants

In simple terms, biosensors are devices or systems that can be used for analytical purposes. The first biosensor devices used to monitor chemical components in the blood and quantitative recording of the biomolecules in the blood was introduced by Clark and Lyons [184]. From that time on, the use of biosensor in healthcare and biomedicine has become essential. Biosensors have been found relevant for analysis [185-187], diagnosis of diseases [188-192] and in food safety [193]. It consists of both biological and electrical component [38, 194, 195]. The biological components interact in such a way that the analyte is recognized, then a signal is further generated with the aid of the electrical component. The biological components include tissues, enzyme, nucleic acid, antibodies and microorganisms. The main function of a

biosensor is to target a particular biomolecule in a given sample. In the design of a biosensor, it is important to incorporate receptors that are very selective and specific to biomolecules. In addition, the transducer should be ultrasensitive and should be reproducible for reliable real time measurement [71]. In order to obtain a strong and precise signal, it is advised that the labelling technique be employed in the presence of a chemical binding or biological molecule specific to an analyte. Although, this process involves the use of fluorescent dyes, chemiluminescent molecules, photoluminescent nanoparticles and quantum dots [196-204]. On the other hand, the label free technique prevents interferences from the labelling process and provides direct information about the targeted molecule. Most cancer diagnostics and drug development application uses the label free method due to the need for a highly sensitive biosensor [191, 205-207].

Graphene based materials are being employed as biosensors majorly because of their great electronic, electric and florescence properties [57, 208]. These properties enable the design of tools and devices that can be used for monitoring and diagnosing acute and chronic disease conditions [70]. However, certain factors are often considered before graphene based materials such as graphene oxide (GO), reduced graphene oxide (RGO) and graphene-based quantum dots (GQDs) are used as biosensor in biomedical field. These include the electrostatic forces, charge-biomolecule interactions at π-π domains and charge exchange. In addition, the effect of defects, disorder and the chemical functionalization for immobilizing the molecular receptors onto the surface of the graphene based material is put into consideration [71]. It has been established that the presence of functional moieties on graphene based materials makes them very reliable to capture molecules as well as analyze their interaction with the specific biomolecule of target. Graphene at the oxidized stage (GO) generates groups such as hydroxyl, carbonyl, carboxyl and epoxide which are rich in oxygen. Thus, they possess surface charges that enables specific interactions easier [209-212]. The most commonly used functional groups used for analysis in bio-sensing are carboxyl and epoxide moieties. This as a result of the very important central role they play in immobilizing biomolecules [213-215]. Carbonyl groups are believed to have the ability to adjust the defect in the carbon-carbon bond in the base of graphene [210]. Due to the different properties of graphene based materials, various biosensors have been developed.

1. Fluorescence resonance energy transfer (FRET) based biosensors have been designed based on the efficient fluorescence quenching ability [216, 217].

2. Highly ultrasensitive biosensors for detecting DNA and other molecules have been built as a result of the controllable self-assembling ability of graphene biomolecules [218-221].
3. FET biosensors have been designed based on the unique electronic properties [222].
4. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry has also been built based on the ability of the matrix of GBM to detect molecules [223, 224].

Furthermore, the huge surface area, excellent electrical conductivity, and good ability of loading a broad range of biomolecules via chemical or physical interactions has led to the development of novel biosensors via electrochemical principle [225, 226]. The study of graphene derivatives for their potential application in bio-sensing and detection of thrombin, ATP, oligonucleotide, amino acid and dopamine have been done by [216, 217, 223, 225]. The application of graphene based materials and DNA hybrid in biosensor is increasing becoming attractive especially in optical systems. This is because graphene base materials are not only effective as fluorescent compounds quenchers, they also have different affinities for free and bound functional DNAs [227]. The use of reduced graphene oxide based biosensor have been demonstrated by Fathalipour and colleagues [228]. The nanocomposite was designed to possess excellent electrocatalytic activity in addition to bacterial inhibitory effects. Results also showed that the functionalized end of the nucleic acid was effectively immobilized on the graphene based material. In the past years, bacteria [229], fungus [230], toxin [231] and protein [232] have been targets to be detected on graphene oxide. However, in addition to these, it has been discovered that biomolecules and small molecules can also be detected easily on the large surface of graphene and sp2 bonded carbon atom. Graphene based biosensor have also been found relevant for the cellular probing, monitoring and detection. This is seen in the probing of Adenine triphosphate molecule in JB6 C1 41-5a mouse epithelial cells by using aptamer-FAM graphene oxide (GO) nanosheet [217]. This was done by connecting the aptamer-FAM/GO nanosheet to the fluorescence microscope and the incubation of JB6 cells was observed. Furthermore, it was seen that hormonal catecholamine molecules in neuroendocrine PC12 rat adrenal medulla cells were detected by graphene-based Field-effect Transistor in another experiment [222]. In addition, it was revealed in a study that apart from living cells, graphene-based biosensor can also detect the circulating tumor cells in prostate cancer [38]. Gu and coworkers have also reported that graphene oxide modified light-addressable potentiometric sensor can serve as a device for molecular analysis [233]. In addition to all these experiments, a couple of reviews exists on the interactions of graphene, GO and RGO-based

biosensors with the molecules of target [57, 207, 234, 235]. Some researchers have carefully reviewed all of the different types of GBM biosensors [18, 35, 236-239] earlier stated and the limitations associated with their use. Challenges faced by GO-based bio-sensing based on FRET principle include inability to tune the electrical properties of GO, irreproducibility, unreliability, high cost, low sensitivity and selectivity. While, GQDs are limited by weak fluorescence intensity (with quantum yield about 10%) and broad emission band (with bandwidth beyond 100 nm). Therefore, in order to enhance the fluorescence quantum yield and other vital properties of GQDs, more attention and efforts to should be put into the design of GQDs with a good control of size and size distribution. Their surface defects and functionalization should also be dealt with in order to develop better biosensors for biomedical applications [177].

2.4 Biodegradation and elimination

Generally, biodegradation is defined as the disintegration of materials via biological means. In theory, graphene based materials are more likely to degrade due to the thin nature of the graphene sheet they contain. However, factor such as colloidal stability which still remains a challenge associated with GBMs will determine their degradability. Graphene based materials have been reported by few researchers to have the ability to biodegrade in the body system. That is, they are able to undergo metabolism or transformation *in vivo* after administration into the body. In recent studies, the biodegradation of graphene based materials with structural changes that are time dependent have been reported in the tissues of mice such as lung, liver, spleen and kidney [70]. In a report, the authors stated that a maximum degradation of GO was observed in the spleen of a mice after three months and this was attributed to the macrophage engulfment [240]. Similarly, in another study, authors reported that there GO and GO-PEG were present in the liver, spleen and lung of mice after three months but at a low retention. Thus, there will be subsequent clearance of the graphene based material from the organs over time [241]. At the moment, very few studies and reports have been made on the metabolism/degradation of GBM *invivo*. Likewise, the understanding of the products formed and their safety in the body is still not well researched and understood [242]. Thus, a need for more studies on the subject of the metabolism of graphene based materials.

Thus far, the major elimination route of graphene based material is via the renal pathway. Here, small graphene oxide sheets, graphene quantum dots are able to go across the glomerular filtration barrier which is about 40nm [243-247]. However, the elimination was observed to take place within the first 24 hours upon administration. Although, in another recent study,

authors reported that a larger sheet of graphene based materials were able to cross the glomerular filtration barrier [248]. This was made possible by the sliding or folding of the thin and flexible functionalized graphene sheets across the membrane. Furthermore, after administration of dextran-functionalized GO, it was observed that the GMB was eliminated via the faecal pathway [249, 250]. Also, a complete faecal excretion was noticed when different functionalized GO derivatives were orally administered and no absorption in the alimentary tract was observed as well [251]. Additionally, there have been reports on the clearance of GBM from the cranial mediastinal lymph nodes [42] and hepato-biliary [252, 253] after administration. These reports suggest that GBM can be eliminated from the body hence can be considered safe.

2.5 Toxicity

It has been established that the shape, size, functional group density and ability to transfer charges are key to influencing the interaction of GBM with proteins, cells and other biomolecules. Therefore, the toxicity and mechanisms of toxicity of graphene based materials is an expect that should be carefully considered, researched and understood before they are applied in various biomedical fields [254]. Generally, the generation of intracellular oxygen species that are reactive have been linked to the mechanism of toxicity of graphene and graphene based materials. This in turn leads to protein or/and DNA damage, causing cell death through apoptotic or necrotic pathways [255-257]. Two major mechanism of graphene mediated reactive oxygen species (ROS) have been reported by scientists. The first is the interference of GO with the electron transport system that causes H_2O_2 and hydroxyl radicals to be produced excessively and the other is the activation of MAPK (JNK, ERK, p38) and TGF- β signaling pathways. This in turn leads to the activation of Bcl-2 proteins which results in activated mitochondria-induced apoptosis [255]. In the first mechanism of graphene based material toxicity, cardiolipin is oxidized by H_2O_2 and hydroxyl radicals, hemoprotein is then released and translocated from mitochondrial inner membrane to the cytoplasm. Cell death therefore occurs when caspase 3 and 7 is activated by caspase 9 and calcium (released from the endoplasmic reticulum) which was induced by the release of cytochrome c complex (cyt c) [256]. Apart from GO causing ROS induced cell death, the activation of toll-like receptors and induced autophagy via inflammatory pathways can be caused by GO [258]. Several researchers have reported on the toxicity of graphene based materials. Various *invitro* studies have been done by [115, 258-273] while *invivo* studies were carried out by other researchers such as [42, 246, 247, 251, 274-282]. In addition to these studies, antimicrobial and environmental toxicity

study of GBM have been evaluated by [283-285] and [267, 286, 287] respectively. The figure below shows various ways in which the toxicity and health effects of graphene based materials have been investigated and studied. Although, more studies are still on going in these areas in order to ensure a clarity on the health impact of graphene based materials.

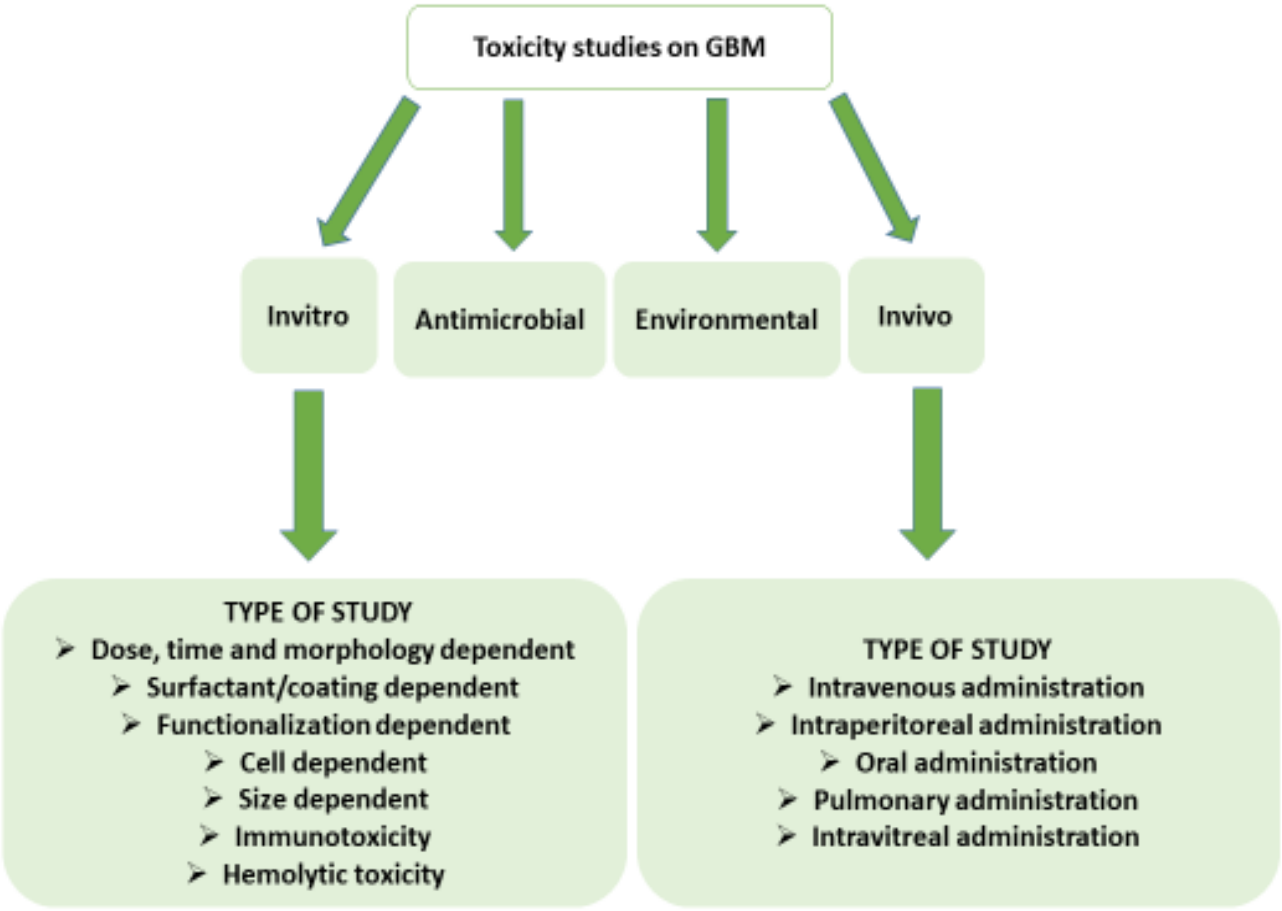

Figure 1: Various toxicological studies done on graphene based materials

Conclusion

Graphene is a carbon allotrope with amazing properties, various methods of its synthesis have resulted to the production of a number of graphene based materials. The discovery of these graphene based materials with outstanding properties has led to their many practical applications in different fields such as biomedicine. There has been a lot of encouragement and excitement as a result of the progressive expansion in their fields of applications. They have become potential materials in solving a wide range of medical conditions affecting millions of people globally. Their application as implants for gene and drug delivery, bio-imaging and biosensor is one of the field in biomedicine that has significantly harness the interesting properties of these graphene based materials. A better performance and effectiveness in by using graphene based materials have been proven by different researcher from various institutions. Graphene based materials have the ability to revolutionize many fields in biomedicine if a comprehensive knowledge and understanding of the functioning principle is well grasped. In terms of sustainability, graphene based materials are known to have low negative impact on the environment and are nontoxic. In several applications of graphene based materials, they are able to conserve resources. Therefore, there is little or no concern about environmental and health issues on unclean earth (pollution). However, there are still few concerns about their toxicity at the *invitro* and *invivo* levels as there are currently debates surrounding their safety. Despite the successes that have been recorded by using these materials in different biomedical applications, more research studies can still be undertaken in order to discover the untapped benefits of these materials or otherwise.

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