ORIGINAL RESEARCH PAPER

Dental Science

GRAPHENE NANOMATERIALS – A BOOM IN PERIODONTAL REGENERATION

KEY WORDS: Graphene, Graphene oxide, tissue Engineering, scaffolds, periodontitis

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ABSTRACT

Graphene is a two-dimensional single layer with hexagonal configuration. Graphene was building block of graphitic carbon molecule include graphite, diamond, nanoribbons and fullerenes. Its potential applications including various biomedical applications such as drug delivery carriers, imaging agents, biosensors, bimolecular analysis, and tissue engineering scaffolds. Graphene oxide facilitates connection with various materials including polymers, biomolecules, DNA, and proteins. Graphene, when combined with other compounds, showed improved synergistic antimicrobial, antibiofilm, and antiadhesive properties. Graphene is the most biocompatible scaffolds for Mesenchymal stem cells adhesion, proliferation, and differentiation. Application of graphene inhibits periodontal pathogens responsible for periodontitis. Other application includes tissue engineering, coated dental implants, barrier membrane, irrigant in endodontics, adhesive in restorative dentistry. This article aimed to focus on importance of graphene, antibacterial effects and tissue engineering scaffolds.

INTRODUCTION

Nanotechnology is defined as the study and manipulation of matter at sizes ranging from 1 to 100 nm. Nanomaterials come in a variety of forms, including silver, gold, diamonds, zinc, zeolite, and others.

Graphene is a well-known nanomaterial. Graphite, the most stable form of carbon is utilized as a lubricant in industry and as the 'lead' in pencils.[1] Graphene is a 2D single layer of sp2 hybridized carbon atoms with a hexagonal packed configuration.

Graphene family nanomaterials (GFNs) include ultrathin graphite, few-layer graphene (FLG), graphene oxide (GO; from monolayer to few layers), reduced graphene oxide (rGO), and graphene nanosheets. Graphene oxide (GO) is one of the most important chemical graphene derivatives which could be produced through energetic oxidation of graphite using oxidative agents.[2]

First graphene was extracted from graphite using a technique called micromechanical cleavage and the Nobel Prize in 2010 was awarded jointly to Konstatntin Novoselov and Andre Geim.[3] Graphene and its derivatives have been studied and used in tissue engineering, biosensors, drug delivery.[4]

These materials were also broadly studied in regenerative dental research, such as dental hard and soft tissue regeneration, as well as periodontal tissue and bone regeneration.

Graphene oxide-based materials, such as graphene oxide-fibroin, were reported as promising in tissue engineering for their biocompatibility, bioactivity, and ability to enhance cell proliferation properties in periodontal ligament stem cells.[5] This review attempt to understand the importance of graphene in periodontal regeneration and dental implants.

Historical Background

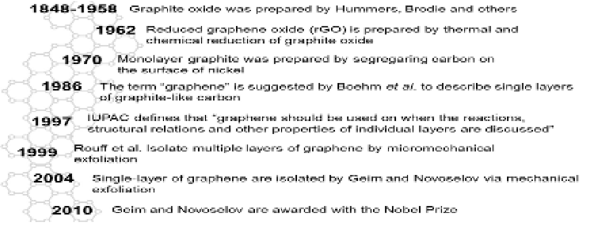

Figure 1 – Key milestones on graphene development.[6]

Antibacterial Effect

Porphyromonas gingivalis is regarded as a keystone periodontal pathogen. GO nanosheets could effectively suppress the viability of Porphyromonas gingivalis stopping growth at a concentration of 40 µg/Ml. The mechanisms are involved in destroying the cell wall and membrane, thereby resulting in plasma leakage. The antibacterial mechanism of graphene involves both physical and chemical modes of action. Physical damages are the most common and are induced by direct contact of the sharp edge of graphene with bacterial membranes. The photo-thermal ablation and wrapping are also involved in the mechanism associated with physical damage. The chemical modes of action are associated with oxidative stress generated by charge transfer and a reactive oxygen species (ROS). Graphene on a Ti surface could destroy the intact structures of polymicrobial biofilms, including P. gingivalis, F. nucleatum, and S. mutans. The antibacterial activity of two graphene-based materials: graphene oxide (GO) and reduced graphene oxide (rGO) nanosheets, which significantly inhibited the E. coli bacterial growth.[7]

Bactericidal activity of GMs: Membrane stress, oxidative stress, and wrapping isolation. For membrane stress, GMs destroy bacteria by piercing and extracting phospholipids from the membrane. Oxidative stress involves reactive oxygen species (ROS) such as superoxide radical ($O_2^{\cdot-}$), hydroxyl radical (OH–), and hydrogen peroxide (H_2O_2) that can be generated by GMs in the presence of bacteria.

Bacterial nucleic acid, membrane lipids, and proteins exposed to oxidative stress will be oxidized and degraded leading to cell destruction. Lipid peroxidation is a critical phenomenon responsible for the disorganization of cell membrane upon exposure to ROS-generating GMs. ROS react with membrane lipids leading to the formation of lipid peroxides, which in turn oxidize and degrade other membrane components. Wrapping isolation occurs when bacterial cells are encased in GM sheets and thus separated from their growth. This mechanism has been observed with both Gr and GO nanosheets preventing nutrients to pass through the cell membrane and leading to growth inhibition.[8]

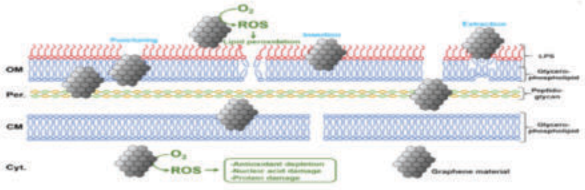

Figure 2 – Membrane stress and oxidative stress

Structure Of Graphene

Graphene is a carbon nanomaterial made of two-dimensional layers of a single atom thick planar sheet of sp^2-bonded carbon atoms packed tightly in a honeycomb lattice crystal. It is a carbon allotrope in the structure of a plane of sp^2 bonded atoms with a molecule bond length of 0.142 nm.

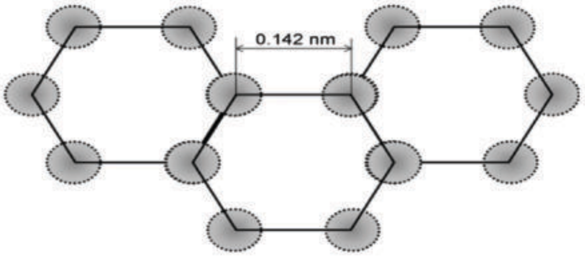

Figure 3- Structure of graphene.

Layers of graphene are piled together to form graphite, with an inter-planar spacing of 0.335 nm. The thickness of graphene is only 0.35 nm which is 1/200,000th the diameter of a human hair. The connection between carbon atoms is tough enough to endure external force by a twisting lattice plane to avoid the reconfiguration of atoms. Graphene, with a limited structure, can exist as a nanoribbon such that an energy barrier occurs near the central point due to a lateral charge movement. Such an energy barrier is increased with a decrease in width of the nanoribbon. Graphene can be classified into zigzag and armchair according to different carbon chains. A graphene nanoribbon with a zigzag edge usually behaves like a metal while a nanoribbon with an armchair edge could conduct electricity like either metal or a semiconductor.[9]

Properties Of Graphene

Electronic Properties

The remarkable electronic and optical properties observed for graphene crystallites are the primary reasons for the exclusive focus of experimental and theoretical efforts on graphene while ignoring the existence of other 2D materials. The electrons in graphene have long mean free paths without disrupting the electron-electron interactions and disorder. Therefore, the properties of graphene differ from those of other common metals and semiconductors associated with the physical structures and electronic properties.

Quantum Hall Effect (QHE)

The QHE is another factor indicating the system's outstanding electronic quality. Because the temperature range of the QHE for graphene is 10 times broader than that of other 2D materials, the QHE in graphene can be seen at room temperature.[9]

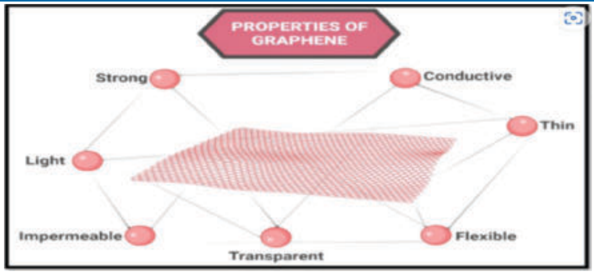

Figure 4- Properties of graphene.

Graphene In Tissue Engineering

Tissue engineering is an interdisciplinary science which aims at developing biological substitutes to restore, maintain, or improve tissue function by using a combination of cells, scaffolds and suitable biochemical factors. Scaffolds represent the key element in tissue engineering. Three-dimensional (3D) scaffolds have been successfully developed and employed for various tissues, such as skin, cartilage, muscle, vasculature and bone. All the periodontium is constantly maintained by Periodontal Ligament Stem Cells (PDLSCs), which have the ability to differentiate into cementoblasts, odontoblasts and fibroblasts. GO may upregulate β-catenin protein expression and activate catenin/Wnt signalling pathway, increasing the degree of proliferation and differentiation of cultured cells, and led to acceleration of bone formation. Another stem cell from dental tissue that can be influenced by graphene scaffold is periodontal ligament stem cells. It is capable of differentiating into both cementoblasts and collagen forming cell. GO in cell proliferation and mesenchymal phenotype expression of PDLSCs. Collagen is the main components in soft and hard tissues and is widely distributed throughout bones, tendons, skin, and teeth. It is commonly used in bone tissue engineering due to its excellent biocompatibility, adhesion, osteogenic induction properties, and degradability. Collagen-based scaffolds have been modified with GO and rGO to increase mechanical stiffness and bioactivity to improve hard tissue regeneration. GO coated membranes increased DPSC differentiation into odontoblasts and osteoblasts by stimulating the production of BMP2 genes and RUNX2 transcriptional modulators that induced osteoblastic differentiation.[10]

GO has also been combined with natural biopolymers other than collagen. Inclusion of GO at 0.25% (w/v) in chitosan/gelatin scaffolds prepared through freeze-drying promoted osteogenic differentiation of C3H10T1/2 mouse MSCs through upregulating RUNX2, the master transcription factor of osteogenesis, and osteoblast marker genes such as ALP, COL1, and OCN. Graphene and its derivatives have further been employed to enhance the performance of nanofibrous scaffolds. The osteogenic effects of graphene and carbon nanotubes were compared by incorporating them into poly-L-lactide acid (PLLA) nanofibrous scaffolds fabricated through thermal-induced phase separation. Hydrogels also display improvements in their physicochemical and biological properties upon GO incorporation. The inclusion of GO into chitosan/glycerophosphate thermosensitive hydrogels improved their protein adsorption, swelling behavior, and biomineralization capacity, as well as their ability to promote osteogenic differentiation in MSCs.[11]

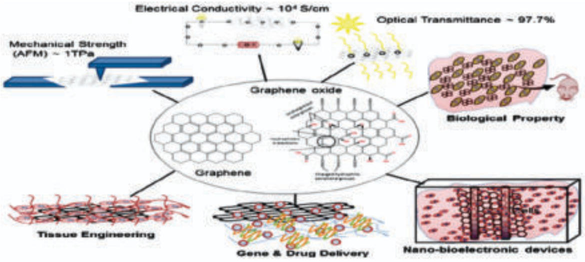

Figure 5- Graphene in tissue engineering.

Graphene And Dental Implants

Osteogenic properties of implant material are essential for osseointegration while at the soft tissue interface, to ensure a tight epithelial seal preventing bacterial invasion. Leak of seal at either interface may result in bacterial contamination and colonization, which may eventually impair osteogenesis and induce bone loss. Modifications on titanium implant features such as surface composition, hydrophilicity, surface roughness topography, and geometry can affect the rate and quality of osseointegration. Due to graphene's potential osteogenic and antibacterial ability, it appeared to be an excellent implant coating material to favor better osseointegration. When graphene is coated on titanium substrate, the hydrophobic character of graphene film exerted self-cleaning effect on its surfaces decreasing the adhesion of microorganism including S. sanguinis and S. mutans. Graphene possesses osteogenic property enhancing the expression of osteogenic related genes RUNX2, COL-I, and ALP, boosting osteocalcin gene and protein expression. GO-Ti substrate provided a suitable environment for the attachment, proliferation, and differentiation of PDLSCs. Coating of titanium with graphene could be a promising strategy to improve osseointegration and prevent biofilm formation on implants and devices. Hydroxyapatite (HA) coating is widely applied as an osteoinductive modification of titanium implant. Graphene oxide, chitosan and hydroxyapatite-titanium (GO/CS/HA-Ti) is produced by incorporating GO and chitosan (CS) into hydroxyapatite-titanium substrate through electrophoretic deposition method. Direct fabrication of graphene on NiTi based dental implant using chemical vapor deposition technique upregulated the expression level of osteogenic related genes (OCN, OPN, BMP-2, and RUNX2) and promoted expression of integrin $\beta1$. Graphene and its derivatives when coated on titanium implant have remarkable abilities to improve properties of titanium, enabling binding of biomolecules, and induce osseointegration.[2]

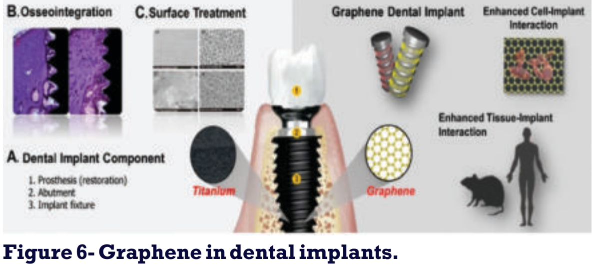

Figure 6- Graphene in dental implants.

CONCLUSION

Graphene-based materials have been shown to have promising results as antibacterial agents. Antibacterial and antibiofilm properties for dental implants are crucial since bacterial infections are often the causes for implant failure and integration with the bone. Graphene nanoparticles have been found to have adequate initial biocompatibility in the mouth when it comes to bone and tissue engineering. They have been shown to possess good osteoblastic differentiation tendencies to increase osseointegration and improve bone tissue development.[10]

Future Perspectives

There is still much investigation to perform in order to assess the potential long-term toxicity of graphene and its composites and their effects on different cells, tissues and organs, including those of oral cavity.

Moreover, in-depth studies are necessary to understand cell-signalling, metabolic pathways and osteogenic effects triggered by graphene-based nanomaterials. Overall, we believe that the use of graphene-based nanomaterials in the periodontal field deserves to be deeply explored as it can lead to even more reliable periodontal treatments in the near future.[10]

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