Graphene Applications in Dentistry

Di Carlo Stefano[1], Brauner Edoardo[2], Di Carlo Fabio[3], Visca Annalisa[4], Piccoli Luca[2], De Angelis Francesca[2]

1. Associate Professor, Department of oral and maxillofacial sciences, Sapienza University of Rome, 6 – 00161, Rome.
2. Researcher, Department of oral and maxillofacial sciences, Sapienza University of Rome, 6 – 00161, Rome.
3. Lecturer at the master's degree in Aesthetic and Wellness Medicine, University of Chieti, Via dei Vestini,31, Chieti, Italy.
4. Department of oral and maxillofacial sciences, Sapienza University of Rome, 6 – 00161, Rome.

Abstract

The purpose of this study was to evaluate the scientific literature related to the use of graphene and its derivatives in dentistry. Electronic research was carried out on PubMed, Scopus and Web of Science. The studies found included the following topics: the use of graphene in tissue engineering, implantology, as an antibacterial material and in the manufacture of dental materials. The studies analyzed have provided promising results for a wide range of applications in dentistry and for this reason the use of graphene in dentistry deserves further investigation to define the benefits of these materials and their use in dental treatments.

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Introduction

Graphene is a crystalline form of carbon isolated for the first time in 2004 by Novoselov and Geim. It consists of a single layer of sp2 hybridized carbon atoms arranged in a two-dimensional (2D) honeycomb lattice; this fundamental unit repeated itself to form a planar crystalline structure called "sheet". In this sheet, each atom of carbon binds to other three that are placed at 120 ° from each other, with 1.42 Å interatomic distance and 2.46 Å center-center distance. Each carbon atom forms three bonds σ and a π bond placed outside the plane that can bind with neighboring atoms. These double conjugate bonds, placed above and below the molecule plane, are able to give a very high electrical conductivity and a particular stability.[1-3] Graphene is a very resistant material, in fact, the Young's modulus of single- and bilayer graphene are 2.4 ± 0.4 and 2.0 ± 0.5 TPa, respectively and it has a 130 GPa fracture strength. Graphene is also an extremely light material, with a density of only 0,77 mg/m^2 and it has a good electrical and thermal conductivity (about 5000 $Wm^{-1}K^{-1}$), high intrinsic mobility (200 000 $cm^2 v^{-1}s^{-1}$) and a good optical transparency with a transmittance about 97.7%.[4-8]

***Corresponding author:**
De Angelis Francesca, PhD,
Department of oral and maxillofacial sciences
Sapienza University of Rome
Via Caserta, 6 – 00161 Rome
E-mail: francesca.deangelis@uniroma1.it

Graphene can be synthesized by two approaches: top-down and bottom-up.[9]

The top down approach consists in the production of graphene by separation, peeling, cleavage or mechanical, electrochemical, solvent-based exfoliation of graphite or its derivatives.[10]

The bottom-up approach, instead, includes standard techniques such as chemical vapor deposition (CVD), pyrolysis, chemical synthesis, arc discharge, decompression of CNT, solvothermal, epitaxial growth and electrically assisted synthesis.[11]

Various shapes, sizes and compositions of graphene and also different possibilities for its subsequent functionalization are obtained from these different techniques.[12]

Graphene can be transformed in zero-dimensional (0D) nanomaterials (such as fullerenes), rolled into one-dimensional (1D) nanotube or manipulated in 3D graphite. Graphene's sheets exist in bi-layers and multi-layers. As the number of layers increases, the properties of the material get modified. When the number of layers is greater than 10, the material shows graphite's similar characteristics.[3]

By chemical and physical modifications, graphene's sheets can be transformed into its derivatives, such as: pristine graphene (pG), graphene oxide (GO), and reduced graphene oxide (rGO).[1,13]

The first one is a pure carbon thin layer. The second one is a graphene's hydrophilic oxidized form. Its structure is a single atomic layer with functional groups such as carboxylic acid, epoxide and hydroxyl and it contains carbon atoms with sp3 hybridization located slightly above or below the graphene plane. Because of these differences in hybridization, orientation and defect patterns, the surfaces of GO sheets appear rougher. The highest surface of the GO and its unique amphiphilic surface properties allows the adsorption of proteins, dye molecules and water-insoluble drugs. The rGO, instead, is produced by reducing the GO through thermal, chemical or UV exposure processes.[12,14-17]

Graphene has several fields of application including engineering, electronics, energy and biomedical area.[18] In the last area, graphene is used for drug and gene delivery, cancer therapy, biosensing, bioimaging and creation of antibacterial material.[9,19] The use of graphene for the regeneration of cardiac, nervous, skin / adipose, cartilage and bone tissues has also been studied.[17]

In this review the existing literature on the use of graphene in dentistry was analyzed. An electronic search until December 2018 was performed in three Internet databases: PubMed, Scopus and Web of Science. The search terms used were: "graphene AND dentistry OR dental materials". They were used together with their all known synonyms. The studies found covered the following topics: the use of Graphene in tissue engineering, implantology, the use of graphene as an antibacterial material and the use of graphene for the creation of dental materials.

Tissue Engineering

In dentistry, fields such as tissue engineering and regenerative medicine are spreading widely. Graphene-based nanomaterials, especially GO, are used as scaffolds in tissue engineering to support cellular attachment, proliferation and differentiation and numerous stem cell studies have been carried out to explore these properties.[20-22]

Xie et al.[23], for example, evaluated the use of graphene to stimulate odontogenic and osteogenic differentiation in dental pulp stem cells (DPSC). DPSCs were seeded on graphene or glass (Gl) and was observed that graphene caused higher levels of mineralization compared to Gl. The odontoblastic genes (MSX-1, PAX and DMP) were down-regulated and genes and osteogenic proteins (RUNX2, COL and OCN) were significantly upregulated on graphene compared to Gl.

Radunovic et al.[24], instead, have investigated the effect of GO-coated collagen membranes on DPSCs. The DPSCs were cultured both on uncoated membranes and on GO-coated membranes. The authors observed that GO's coated membranes promote a greater proliferation of DPSCs differentiation into odontoblasts/osteoblasts and at the same time they can control the onset of inflammatory events.

In the same year, Park et al.[25], evaluated the use of GO in the GBR procedure. The authors carried out a study to determine whether GO titanium membranes (GO-Ti) in different concentrations (10, 100, 1000 mg/ml) were able to improve the osteogenesis of MC3T3-E1 pre-osteoblasts and promote new bone formation. The authors noted that the GO-Ti membranes significantly stimulated the activity of alkaline phosphatase (ALP). In this study the GO-Ti membranes were also used in GBR procedure for the treatment of calvary defects of rats and it was observed that GO-Ti membranes have led to better bone regeneration compared to control group (titanium membranes).

In another research, Kim et al.[26] evaluated the effect of biphasic calcium phosphate (BCP) coated with rGO as bone grafting materials in bone regeneration. The study was carried out on 48 calvary bone defects of rats. The animals were divided into four groups depending on bone graft material used (only BCP, rGO: 28 μg/mL and BCP, rGO: 56 μg/mL and BCP, rGO: 140 μg/mL and BCP) and the results were obtained by micro-CT and histological analysis. The results showed that the bone volume formed was significantly higher in the groups with biphasic calcium phosphate (BCP) coated with reduced rGO compared to the control group.

Graphene and its derivatives have also been used to prevent bone resorption and remodeling occurring after tooth extraction. this procedure is indicated to counteract bone loss and optimize the subsequent positioning of the implant and the prosthetic rehabilitations.[27-28]

Nishida et al.[29] have used collagen sponge coated with 1 μL / mL of GO for the treatment of post-extraction sites of the dogs. The bone formation was evaluated histologically and by means of radiographic images carried out immediately after the intervention and 2 weeks after scaffold implantation. The sockets treated with GO scaffold showed an increase in radiopacity and the histological sample have revealed that GO scaffold promoted new bone formation.

The same scaffold was used by Kawamoto et al.[30] for the treatment of II grade's furcation defects in dogs. Eighteen class II furcation defects were randomly divided in two groups: test group and control group. In the first one the scaffold was used with GO while in the second one only collagen scaffold was used. Periodontal healing was histologically assessed 4 weeks postoperatively. The authors observed that the GO scaffold resulted in better bone and periodontal regeneration compared to the control group.

Implantology. Tooth loss is a public health problem worldwide. Loss of teeth and alveolar or basal bone can lead to significant problems during mastication, affecting the nutritional status. To date, there are several therapeutic options for the rehabilitation of edentulous patients and fixed implant-supported restoration has established itself as a popular and effective procedure for replacing missing teeth.[31-38]

The fixed prosthesis guarantees stability, reduces inflammation of the mucous membranes and correctly restores their function and aesthetics. In addition, this procedure also has a positive social and psychological impact on the patient and improves his quality of life.[39-42]

Today, titanium is the material used for dental implants due to its excellent biocompatibility (due to the formation of a stable oxide layer on its surface), corrosion resistance, specific resistance, toughness, resilience and low rigidity.[43] Over the years, different alloys, surfaces and coatings have been used to try to maximize bone at implant contact values (BIC).[40,44] Recently, graphene has also been used as implant's coating. The transfer of graphene to the titanium surface can be carried out by two methods: wet and dry. Wet transfer is the most commonly used method but often causes water trapping between the target substrate and the graphene film which, after evaporation, creates large folds and cracked areas. To overcome these problems, dry transfer techniques have been developed, such as roll-to-roll, thermal release tape and direct dry transfer techniques.[45-47]

Jiajun et al.[48] studied the properties and the osteogenic activity of three-dimensional modified titanium in porous nanosheets (rGO @ Ti). Mesenchymal stem cells of rat bone (rBMSCs) were seeded on various samples and incubated for 1, 4, and 24 h. The authors observed that rGO @ Ti showed a high hydrophilicity and biocompatibility. Furthermore, it could improve the activity of ALP, extracellular matrix mineralization (ECM), collagen secretion of rBMSCs and the expression of genes related to osteogenesis containing ALP, morphogenic protein of bone 2 (BMP-2), osteocalcin (OCN) and osteopontin (OPN).

Gu et al.[49] evaluated, in vitro and in vivo, the effects of graphene coating on titanium implant on adhesion, proliferation and osteogenic differentiation. Human gingival fibroblast (hGF), stem cells derived from human adipose tissue (hASC) and human bone marrow mesenchymal stem cells (hBMMSCs) cultures on graphene samples were made. Graphene caused an increase in the adhesion of hASC and hBMMSCs to the substrate, so the graphene's coating on Ti substrates could improve the interaction between the material and the surrounding soft tissue. Graphene-coated titanium can also promote the osteogenic differentiation of hASCs and hBMMSCs but no significant differences in cell proliferation and growth were observed on the surfaces of the studied samples.

Dubey et al.[50], also, investigated the osteogenic and antibacterial potential of graphene coating on commercially pure titanium (CpTi). The coating was made both with the dry technique (DGp) and the wet one (WGp). The authors observed that titanium coated with graphene was cytocompatible and didn't induce cell membrane damage. Moreover, both WGp and DGp increased the expression of all osteogenic-related genes, osteocalcin gene and protein expression.

Suo el al.[51], instead, have made a composite coating of GO / chitosan / hydroxyapatite (GO / CS / HA) on titanium. The authors evaluated the adhesion, vitality, differentiation and cellular mineralization in vitro while the osteogenic properties in vivo by conducting a study on animals comparing the GO / CS / HA-Ti with HA-Ti, CS / HA-Ti and GO / HA-Ti.

The GO / CS / HA-Ti samples showed better adhesion, proliferation and differentiation of BMSC and osseointegration compared to other samples too.

Antimicrobical Effects

Carbon-based nanomaterials have been reported to have antimicrobial effects.[52] This effect is based on three different mechanisms. Firstly, 2D nanostructures are able to wrap cells by inducing mechanical stress and limiting nutrient absorption. The second interaction mechanism is due to the edges of the nanostructure acting as nano-knives, penetrating and disrupting the cell membrane. Finally, the third mechanism is based on the production of oxidative stress.[53,54]

Rago et al.[55] analyzed the antimicrobial properties of graphene nanoplatelets (GNP) against S. Mutans. The strain used in this study had been isolated from plaque and saliva samples taken from pediatric patients. Graphene nanoplatelets have led to a reduction in the number of S. Mutans. From the SEM analysis it was found that graphene determines mechanical damage to the bacterial surface. Bergnocchi et al.[56], instead, studied the use of GNP as a filler of dental adhesives in order to obtain an antibacterial and anti-biofilm activity. It has been observed that the nanomaterial determined a reduction in the adhesion and growth of S. Mutans in vitro without altering adhesive's properties of the material itself.

Lee et al.[57] examined the addition of graphene oxide nanosheet (nGO) in quantities (0.25, 0.5, 1.0, or 2.0% by weight) to polymethyl methacrylate (PMMA) powder. The authors found that nGO caused a bacterial adhesion's reduction on the material. Furthermore, the addition of nGO to PMMA has led to an increase in the surface roughness and hydrophilicity of the material without compromising its properties.

The antimicrobial activity of graphene can also be exploited in implantology.

The implant surfaces can be colonized by bacteria that represent a potential source of tissue inflammation.[58,59] The inflammatory process of peri-implant tissues may result in biological failure and progressive loss of osseointegration.[60] As has recently been reported[49,50], graphene coating reduces the number of viable bacteria and the formation of biofilm on implant surface.

Dental Materials

Graphene can be mixed with other materials in order to improve its properties.[11]

Lee et al.[57] in their work observed that the resistance to 3-point flexion and Vickers microhardness were increased thanks to the addition of 0.5% of nGO to PMMA compared to control samples.

Alamgir et al.[61], instead, studied the addition of TiO2 and GO to PMMA as reinforcement. Two different nanocomposites (PMMA / GO and PMMA / GO + TiO2) were prepared. The structural, thermal, and mechanical characterizations were performed. Nanocomposites exhibited different thermal and microstructural characteristics and a greater resistance to localized deformation when they were compared to pure PMMA. The PMMA / GO + TiO2 nanocomposite had higher Young module and scratching force compared to PMMA/GO and PMMA samples.

Sun et al.[62] analyzed the mechanical properties, wear resistance and antibacterial properties of glass ionomer cements with the addition of fluorinated graphene (FG) in different percentages (0.5 wt%, 1 wt%, 2 wt% and 4 wt%). The addition of FG caused an increase in Vickers microhardness, compressive strength and a reduction of the friction coefficient compared to non-reinforced materials without changing the properties of the glass ionomer cements. A decrease in the number of S. aureus and S. Mutans colonies was also observed.

Dubey et al.[63], instead, evaluated the effects of graphene nanosheets (GNS) on bioactivity and on the physical, mechanical and chemical properties of two cements: Biodentine (BIO) and Endocem Zr (ECZ). GNS was combined with the two cements in different concentrations (1, 3, 5 and 7 wt %). The addition of the GNS did not interfere in the composition of the cements; it reduced the setting time, increased the hardness and didn't alter the pH of both materials. On the other hand, the addition of GNS to ECZ in all the percentages used, caused a significant reduction in the adhesion force.

Lee et al.[64], instead, studied the

mechanical and biological properties of orthodontic bonding adhesive (Transbond XT (LV)) enriched in GO and bioactive glass mixture (BAG) (BAG @ GO) in a ratio of 1, 3 and 5 %. The adhesive with 3%wt and 5%wt of BAG@GO showed a better microhardness compared to LV, while there wasn't statistically significant difference in the shear bond strength test between the LV group and the BAG @ GO group. BAG @ GO group also had high antibacterial and anti-demineralization effect.

Sava et al.[65] investigated the properties of materials composed of a monomeric mixture of Bis-GMA and TEGDMA as a matrix and hydroxyapatite with graphene, bioglass, colloidal silica as a reinforcing filler. The authors observed that in materials with the addition of 5-10% by weight of hydroxyapatite with graphene nanoparticles the Young's modulus, the surface hardness of the material and the flexural strength were increased.

Conclusions

In recent years, graphene and its derivatives have aroused considerable interest in the biomedical field due to their structures and properties. They are very versatile materials with unique characteristics such as large surface area, high mechanical strength, electrical conductivity and thermal stability. They can be transferred to different substrates to give existing materials greater bioactivity and optimized physical, chemical and mechanical properties. In this review, we discussed recent studies on the use of graphene in tissue engineering and implantology to assess its potential to promote cell differentiation, proliferation and adhesion. The antibacterial activity and mechanical properties of graphene materials were also evaluated. The studies analyzed have given promising results for a wide range of applications in dentistry and for this reason the use of graphene in dentistry deserves further investigation to establish the benefits of the material and its use in dental treatments.

Declaration of Interest

No conflict interest.

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