Graphene as an Enabling Strategy for Dental Implant and Tissue Regeneration

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Abstract Graphene-based approaches have been influential in the design and manipulation of dental implants and tissue regeneration to overcome the problems associated with traditional titanium-based dental implants, such as their low biological affinity. Here, we describe the current progress of graphene-based platforms, which have contributed to major advances for improving cellular functions in *in vitro* and *in vivo* applications of dental implants. We also present opinions on the principal challenges and future prospects for new graphene-based platforms for the development of advanced graphene dental implants and tissue regeneration.

Keywords Graphene · Dental implant · Titanium · Dental tissue regeneration

1 Introduction

The goal of dentistry is to maintain or regeneration a patient's oral health using a realizable plan. Various hard and soft tissue regeneration methods are used to help edentulous patients recover function, esthetics, comfort, or pronunciation. Among these, dental implants are the most powerful, reliable, and effective method compared to traditional methods in dentistry. A dental implant is defined as

an artificial tooth root and is used to support a restoration that is similar to a natural tooth [1]. It is usually a metal foreign reconstructing appliance that is placed in oral tissue such as mucosa, periosteum, and bone. Then, the restoration or upper structure is placed on top of the implant and serves as the crown of the artificial tooth as a fixed or removable prosthesis [2]. Although a variety of metals and other materials are used to manipulate implants, titanium (Ti) is widely used as an implant material because of its biocompatibility and great osseointegration [3]. After modern dental implantology was developed in the 1950s and 1960s by the orthopedic surgeon Brånemark [4], many studies showed a five-year success rate of 90–95% for upper and lower jaw implants [5]. Although they have shown a very high success rate, sometimes these treatments fail for various reasons, often related to an incomplete osseointegration process [6]. In other words, there are many limitations for current implant materials and surgical techniques [7]. Moreover, even with a completely successful implant treatment, patients experience masticatory difficulty during the 3–6 months osseointegration period [8].

The use of implants with different diameters and length, changes to the implant design, and implant surface treatment have been proposed to overcome these limitations.

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Out of these various methods, progress has been made in implant surface treatment to enhance the performances of titanium implants [9]. The methods used to increase the quantity and quality of the osseointegration between the titanium implant and bone tissues can be divided into physical and chemical methods [10]. First, a physical method increases the contact area of the titanium implant by increasing the roughness [11]. Second, a chemical method involves the addition of other materials to increase the reactivity of the titanium implant. As an example of the current chemical methods, calcium (Ca) [12], hydroxyapatite (HA) [13], or another mineral [14] is commonly used to increase the reactivity of the titanium implant by raising the surface roughness to the nanometer level, which offers further potential for bone regeneration, antibacterial effects, and good tissue engineering using new materials.

Graphene, which is a single layer of sp² hybridized carbon atoms in a honeycomb lattice, is a basic component of both fullerenes and carbon nanotubes, and has drawn much attention in the physical, chemical, and biomedical fields because of its various significant characteristics, including remarkable mechanical properties, outstanding electrical conductivity, and very high surface area [15–17]. In the early research on graphene, it gained much attention because of its potential application to electronic and energy storage [15]. More recently, it has also been used in biomedical fields such as for tissue engineering [18], implants [19], antibacterial materials [20], and drug delivery devices [21]. In particular, graphene has shown a high biocompatibility and good differentiation of stem cells such as osteogenesis [22], neurogenesis [23], and myogenesis research [24]. These properties of graphene could provide insight into the design and manipulation of graphene-based dental implants to overcome the problems associated with traditional titanium-based dental implants.

In this work, we review the current progress of graphene-based approaches, which have been influential in the design and manipulation of dental implants for regeneration of tooth, hard tissue and soft tissue. To this end, we first provide basic information about titanium dental implants and their limitations. We then introduce the most recent progress for using a graphene-based platform as a dental implant. Finally, we present opinions on the principal challenges and future prospects for the use of new graphene-based platforms for the development of advanced graphene dental implants.

2 Dental Implants

Dental implants are classified as subperiosteal, transosteal, and endosseous implants according to their design and placement within the tissue [25]. Among these, the most

common is the endosseous type, which is commonly discussed and developed in a variety of medical fields [26]. As previously mentioned, the structure of an endosseous implant is composed of a fixture that is placed on the bone, along with an abutment screwed to the top of the fixture and the upper prosthesis, as shown in Fig. 1 [2]. Successful implant treatment involves a strong structural and functional connection between the bone and fixture interface (Fig. 1) [6]. Albrektsson et al. [27] suggested six factors for an osseointegration: the implant biocompatibility, implant design, surface condition, location, surgical methods, and post-operative weight control. Under these conditions, many dental clinicians have reported satisfactory esthetic results, with the restoration of function in a short time [28]. However, under uncontrolled conditions such as poor quality for the installation location and bone, the inherent implant material properties played an important role in treatment [29]. Many commercialized implants are well known to have great biocompatibility, providing an appropriate surface for bone repair and recovery, but implants that can compensate for an unstable bone condition have not yet been discovered. Therefore, further studies on the implant surface are needed for successful osseointegration based on bone regeneration ability and biocompatibility by specific materials [30].

The basic purposes of the surface treatment are i) to increase the surface area to improve the mechanical fixation between the implant and bone after placement, ii) provide a surface structure for blood clot maintenance, and iii) provide a surface structure for the acceleration of bone recovery [31]. For these purposes, the current commercial implant surfaces include a machined surface, titanium plasma spraying surface, HA plasma spraying surface, resorbable blasted media surface, sandblast large-grit acid-etched surface, and anodic oxidation surface (Fig. 1; Table 1) [32–35]. However, most surface treatment methods are usually structural approaches designed to increase the surface area [10]. When inserting the implants to the alveolar bone, widening the surface area on dental implant fixtures is known as one of the efficient methods to increase osseointegration. However, this conventional surface treatment method had the disadvantage in the periodontitis site. Since a widened surface area could increase not only osseointegration but also adhesion of contaminants such as periodontal pathogens (*Porphyromonas gingivalis*, *Prevotella intermedia*, *Actinobacillus actinomycetemcomitans*, *Treponema denticola*) [41]. In addition, there is a lack of rapid osseointegration, bone differentiation promotion, and antibacterial effect from implant surface treatment. To add these special functions, various biologic methods have been proposed using fixed proteins, enzymes, and peptides that can induce specific cell and tissue reactions on the implant surface [42, 43]. These methods can be divided

Fig. 1 Graphene as new strategy for designing and manipulating dental implants and tissue regeneration. **A** Endosseous dental implant structure composition: (1) prosthesis part for mastication (restoration), (2) titanium abutment for the connection of fixture and prosthesis, (3) titanium implant fixture with surface treatment. **B** Process osseointegration: *left* after 4 weeks, mineralized new bone be contacted implant fixture surface, *right* after 12 weeks, processing bone remodeling, bone be surrounded by blood, adipose cell, collagen

fiber, monocyte, and so on. So, the titanium implant was more difficult to remove. **C** Various titanium implant surface treatment for osseointegration, biocompatibility, and antibacterial effect: machined surface for only mechanical effects, anodic oxidation surface by nanotube, resorbable blast media(RBM) surface using calcium phosphate, and RBM + anodic oxidation surface. Dental implant using graphene showed that enhanced cell viability and stem cell differentiation

Table 1 Surface treatment of dental implant

Classification	Surface treatment	Summary	Product example	Reference
1st Generation	Machined surface	Cutting or formation of thread, machining lines, fits, groove on implant surface	MK III TM : Noble Biocare	[13]
2nd Generation	Hydroxyapatite (HA) coating	HA particle spray for rapid osseointegration	SteriOss TM : Noble Biocare	[36]
	Titanium plasma spray (TPS) coating	Titanium particles were dissolved and then sprayed onto the surface of the implant	Bonefit TM : Institute Straumann	[37]
3rd Generation	Sand-blasted with large grit and acid etched (SLA) surface	Sandblasting for create roughened surface and chemical acid-etching for remove residual materials	SLA TM : Straumann	[38]
	Resorbable blasted media (RBM) surface	Spreading absorbent material (ex. calcium phosphate)	Tioblast TM : AstraTech	[39]
4th Generation	Anodizing Electrochemical oxidation	An electrochemical oxidation method to increase the oxide layer thickness of the surface	TiUnite TM : Noble Biocare	[40]

into dipping, covalent bonding of a biomolecule, and coating [44–46] according to the additional materials. Also, the carbon based materials are trying to combine for improving the surface treatment and there are being recognized as emerging functional materials recently (Table 2). In this review, we focus on the application of graphene to dental titanium implants and tissue regeneration. Unlike previous surface treatments, which have multiple effects based on increased surface area, graphene would be considered to be a very suitable material for application to implant surfaces because of its high biocompatibility, antibacterial property, as well as and improved osseointegration regardless of changes in surface area.

3 Graphene Dental Implants

3.1 Graphene-Based Platforms

Titanium is widely used for the fabrication of dental and orthopedic implants because of good biocompatibility, very high corrosion resistance [59], and long-term performance [60]. However, titanium has relatively poor shear strength. Thus, there is a tendency for severe wear and tear when titanium is used in a dental implant [61]. When natural teeth were replaced by titanium implants, which had unsteady osteoinductivity and osteoconductivity, tissue regeneration was discouraged on the inserted implant surface [62]. Accordingly, a titanium dental implant needs an

Table 2 Carbon materials on titanium alloy

Form of carbon materials	Titanium alloy	Method	Supplement	Cells	Application	Reference
Carbon nanotube (CNTs)	Titanium	Dip-coating	Collagen	MC3T3-E1 Cells	Good biocompatibility	[47]
					Cell proliferation, viability, and growth	[48]
		Coating			Increased osteoblast proliferation, differentiation, and matrix mineralization	[49]
					Good cell proliferation and adhesion	[50]
	Ti-6Al-4 V	Electro deposition	Hydroxyapatite	G292 Cells	Cellular proliferative and cell adhesion	[51]
					Good biocompatibility	[52]
		Sol-gel method		hBMSCs	Excellent corrosion resistance	[53]
					Cell adhesion and growth	[54]
Detonation nanodiamonds	Ti ₆ Al ₇ Nb	Electrophoretic deposition	Hydroxyapatite and chitosan	MC3T3-E1cells	Increased surface roughness, HA formation, and cell proliferation.	[55]
				MG63 cells	Improved adhesion, cell viability, and good biocompatibility	[56]
Carbon fiber	Titanium	Electrochemical immobilization	Phosphate groups		Increased adhesion to bone	[57]
			Poly (methyl methacrylate)		Higher bond strength	[58]

Fig. 2 Four main surface treatment methods. **A** Layer-by-layer assembly, with a multilayer consisting of two different chemical groups (GO-NH₃³⁺/GO-COO⁻) coated on a titanium substrate. **B** PMMA-mediated method, with the adhesion between the graphene and Ti substrate increased by transferring and annealing.

C Electrophoretic deposition, with positive charged particles, including graphene, deposited on the negative charged Ti surfaces. **D** Spin coating, with the negative charged GO solution spin coated on the positive charged APTES-titanium surface at 100–2000 rpm. A platform with a thin and uniform thickness was fabricated

effective surface treatment for tissue regeneration [63], and various treatment methods have been used to enhance the osteoblast responses. Recently, the surface treatment of dental implants using carbon nanomaterials, including carbon nanotubes, graphene, and fullerenes, has been studied [64]. Research is being introduced on the application of graphene to titanium, which is a main ingredient in dental implants.

In 2007, Rojas and Leiva proposed a modified graphene sheet decorated with titanium [65], which has also been increasingly used for implants in orthopedics. La et al. [66] reported the use of layer-by-layer assembly (LbL assembly) to fabricate titanium substrates coated with graphene oxide (GO) and loaded with bone morphogenetic protein-2 (BMP-2) (Fig. 2A). GO-NH₃⁺ (positively charged GO) and GO-COO⁻ (negatively charged GO) were piled on top

of each other using the LbL assembly, with the substrate composed of 15 bilayers. The BMP-2 could then be loaded on the outermost coating layer of GO-COO⁻, and sustainably released. Therefore, this dental implant platform provides enhanced osteointegration through effective drug delivery. Furthermore, the previously fabricated platform added the delivery of substance P (SP) [67], which is a greatly obtained 11 amino acid neuropeptide mediating pain perception [68]. This work showed that the SP stimulated cell migration and dual delivery system for BMP-2 and SP enhanced the bone regeneration, improving the osteoinduction and stem cell recruitment agent. To overcome the weakness of Ti, Kalisz's group used the poly(methylmethacrylate) (PMMA)-mediated method [69] when graphene was transferred to the substrate (Fig. 2B) [70]. After PMMA was spin coated on the graphene and copper substrate, it was transferred to a titanium alloy substrate and the PMMA was removed. The fabricated specimens had the same mechanical properties (0.02 $\mu\text{A}/\text{cm}^2$) as the titanium alloy and improved corrosion properties (5.63 GPa), which allowed them to perform better as dental implants. Jung et al. prepared two types of samples, a disc sample and dental implant made of Ti13Nb13Zr (MPCR-TNZ), which had high mechanical strength and solid fatigue characteristics [71, 72]. The oxidized titanium was immersed in 2% (3-aminopropyl) triethoxysilane (APTES) ethanol solution to create the surface with positive charged amine group. Afterward, GO solution was spin coated on the APTES functionalized titanium (MPCR-TNZ), negatively charged GO can electrostatically react to APTES-Ti. The surface treated titanium could carry osteogenic dexamethasone (DEX) for efficient osseointegration, which can be through π - π stacking because DEX has π electrons on the structure (Fig. 2D). The dental implant shape (MPCR-TNZ) combined with graphene through immersion in a graphene oxide solution. The result using RGO-MPCR-TNZ-Dex showed significantly increased growth, differentiation, and calcium deposition. If additional research is carried out for this dental implant (RGO-MPCR-TNZ-Dex), it is expected to produce fine results for dentistry.

Recently, research has been done on GO, chitosan, and an HA composite coated with Ti through electrophoretic deposition, which is a colloidal coating technique using a stainless steel electrode (anode) and Ti substrate (cathode) under continuous stirring (Fig. 2C) [73]. Titanium with a coating showed a greater hydrophilic surface and offered low corrosion. It also exhibited excellent biocompatibility when cells were cultured on the substrate. In contrast, the attachment of bacteria on the fabricated composite was often reduced. Thus, its potential as a dental implant was confirmed [74].

Because various bacteria are present around the teeth, great care is needed when an implant is placed in the mouth. A recent report showed that GO has an effective antibacterial property for typical dental pathogens such as *S. mutans*, *P. gingivalis*, and *F. nucleatum*, which lost their integrity, with their intracellular contents leaking out onto the graphene sheet [75]. The adhesion of the gram-positive bacterium *Staphylococcus aureus* (*S. aureus*) was reduced on titanium with a graphene oxide/chitosan/hydroxyapatite nanocomposite (Fig. 3E) [74]. Graphene has a bactericidal property that disrupts and damages cell membranes through membrane and oxidative stress [76, 77]. Recently, antibacterial materials have drawn attention in the field of dental implants, and graphene with its antibacterial property will be available in dentistry (Table 3).

3.2 Graphene-Based Implant: *In Vitro* Study

Graphene has been used a powerful tool in various applications. However, its use in dentistry is just beginning [64]. Primary cell lines that included MG63 cells and MC3T3-E1 cells were studied. MG63 cells provided good biocompatibility, along with enhanced cell adhesion, differentiation, and proliferation compared with pure titanium [74, 80]. The proliferation and differentiation of pre-osteoblast cells (MC3T3-E1 cells) were also studied, which were confirmed by alizarin red staining, an alkaline phosphatase activity test, immunocytochemistry, and real-time PCR (Fig. 3A, B) [24, 64]. Bit et al. [79] showed that the viability of adherent MG63 cells could be assessed by fluorescence and enhanced calcium deposits of MSCs using alizarin red S staining on the GO with titanium through electrophoretic deposition. Bone formation is a crucial prerequisite for a dental implant, and much research has reported osteoblast proliferation on graphene with titanium. Zhou's group reported on periodontal ligament stem cells (PDLSCs) with GO coated on titanate, which showed the enhanced osteogenic differentiation of PDLSCs on GO-Ti [81]. Rosa et al. demonstrated that a GO substrate allowed the attachment and proliferation of dental pulp stem cells (DPSC), and enhanced the expression of various genes to induce mineralization [82]. The osteogenic differentiation of hBMMSCs was increased on a GO-coated Ti substrate as a carrier for the delivery of BMP-2 or BMP-2 with SP, which was confirmed by measuring the alkaline phosphatase (ALP) activity and immunocytochemical staining (Fig. 3C, D) [66, 67]. The effect of graphene has been proven through various tests, and there are greater effects when graphene is combined with another supplement (Table 4).

Fig. 3 Graphene-based cell culture and tissue regeneration. Various cells and bacteria were incubated on graphene with titanium substrate. **A** The alizarin red staining, immunofluorescence related to osteocalcin of MC3T3-E1 cells on MPCR-TNZ, RGO-MPCR-TNZ, and Dex/RGOMPCR-TNZ in osteogenic media (scale bar black 500 μ m, white 25 μ m). The results showed that the proliferation and differentiation were increased on the graphene-based platform with a supplement. **B** Relative quantification of alizarin red staining and ALP activities of MC3T3-E1 cells. The Dex/RGO-MPCR-TNZ group has the most calcium nodule deposition and enhanced ALP activity compared to cells on nonosteogenic media. **A, B** adapted with permission from Jung et al. ACS Appl Mater Interfaces. 2015;7:9598–607, copyright 2015 American Chemical Society [72]. **C** Immunocytochemical staining for osteocalcin, which is an osteogenic (OC) differentiation marker (green). The cell nuclei were counterstained with DAPI (blue) (scale bar 100

μ m). **D** Real-time PCR analysis to detect the mRNA expression levels of alkaline phosphatase (ALP) and OC in hBMMSCs at 2 and 3 weeks. The increased osteogenic differentiation of hBMMSCs cultured on Ti/GO/BMP-2 compared with bare Ti ($n = 5$). $*p < 0.05$ versus with Ti/BMP-2 group, $\$p < 0.05$ versus Ti/GO-. **C, D** adapted from La et al. Small. 2013;9:4051–60, with permission from John Wiley and Sons [66]. **E** SEM images of the *S. aureus* after incubation for 12 h, showing that the bacteria are more sensitive to graphene. **E** adapted from Shi et al. J Mater Sci-Mater Med. 2016;27:1–13, with permission from Springer [74]. **F** The bone regeneration of a titanium ring implant with graphene was investigated *in vivo* with Goldner's trichrome staining (scale bar 1 mm). **G** The histological analysis revealed BMP-2/SP and the carrierdependent formation of new bone ($n = 5$). $*p < 0.5$ between designated two groups. **F, G** adapted from La et al. Int J Nanomed. 2014;9:107–16, with permission from DOVE Medical Press [67]

3.3 Graphene-Based Implant: *In Vivo* Study

A graphene with titanium implant was researched *in vivo* by evaluating bone formation in a mouse with calvarial defects. A study using a graphene/titanium/BMP-2 implant showed enhanced mature bone formation with lamellar structures and osteocytes after 8 weeks, which was confirmed by micro-computed tomography and a histological

analysis using Goldner's trichrome staining [66]. They demonstrated that BMP-2, which was loaded on the graphene surface, stimulated osteointegration. In a second study, they added a substance (P) related to wound healing [83] and angiogenesis [84]. For 8 weeks after implantation, the new bone area increased dramatically in the mouse calvarial defect model. The potential for dental or orthopedic implants using dual drug delivery was confirmed

Table 3 Graphene on titanium alloy

Coating method	Titanium alloy	Graphene type	Supplement	Fabrication process	Surface features	Reference
Spin coating	Titanium	Reduced graphene oxide (RGO)	Osteogenic dexamethasone (Dex)	Piranha solution → (3-aminopropyl) triethoxysilane (APTES) ethanol solution → RGO coating	Hydrophobic surface	[71]
		Hummer's method graphene oxide (GO)	Sodium (Na)	Three step spin coating (200 rpm–300 s → 500 rpm–20 s → 2000 rpm–160 s)	Increasing of surface roughness, hydrophilicity and corrosion resistance	[78]
Electrophoretic deposition	Ti6Al4 V	Hummer's method GO		GO sheets → HNO ₃ and NH ₄ OH → etching and oxidizing (HNO ₃ : HF: H ₂ O = 28.8:2.4:70)	Homogeneous dispersion of GO	[79]
	Titanium	GO	Chitosan (CS) and hydroxyapatite (HA)	Stainless steel electrode (anode) and a Ti substrate (cathode) → ethanol/HA/CS/GO	Hydrophilicity and corrosion preventing	[24]
				Ti or anodized Ti (anode) and platinum (cathode) → Isopropyl alcohol/GO	Anodized Ti has smooth surface than Ti	[64]
			HA	Ti (cathode), graphite rod (counter-electrode) and a saturated calomel electrode (electrode) → Ca(NO ₃) ₂ , NH ₄ H ₂ PO ₄ , NaNO ₃ and H ₂ O ₂ /HA/GO	Dense and uniform surface	[80]
PMMA mediated method	Ti–Al–V	CVD graphene		PMMA spin coated on graphene with copper (Cu) → Cu etching → ion particle removing	Same surface hardness with Ti and uniform coating	[70]
Layer by Layer assembly	Titanium	Hummer's method GO	Bone morphogenetic protein-2 (BMP-2)	Ti substrate → dipped in GO-NH ³⁺ → GO-COO ⁻ (15 bilayer)	Smooth, dense, and uniform surface coating of graphene	[66]
			BMP-2 and substance P (SP)		Hydrophobic and color changed to black	[67]

(Fig. 3F, G) [67]. Jung et al. [71] showed regenerated bones on Dex/RGO/Ti using synchrotron X-ray images, a histological analysis after hematoxylin and eosin (H&E), and collagen staining in rats. They confirmed that a modified titanium implant with drug-loaded graphene could be used as an implant with bone tissue engineering (Table 3). Further studies are needed to learn how the bone dramatically regenerated in the graphene with titanium and to develop a variety of *in vivo* approaches.

3.4 Mechanism in Graphene-Induced Cell Behaviors and Tissue Regeneration

The mechanisms underlying the effects of graphene on cellular behaviors and tissue regeneration remain unknown well. Although detailed basic researches should be performed including specific signaling pathway, recent studies have suggested some possible reasons how graphene-based platforms enhance the cellular behaviors and tissue

regeneration [22, 86, 87]. It has been reported that the unique properties of graphene including nanoscale structure, strong stiffness, roughness, functional groups, absorption of biomolecules might affect the cellular behaviors and tissue regeneration [86].

It has been generally accepted that the cellular behaviors and tissue regeneration are regulated by nanotopographical cues [22]. The graphene-induced nanoscale topographical cues might stimulate the differentiation of cells (e.g., osteogenesis of stem cells), which might enhance the bone tissue regeneration [22]. That is, the unique characteristics of the nanoscale topographical cues of graphene and its secondary effects such as stiffness and roughness might play a crucial role in the enhancement of cellular behaviors and tissue regeneration. It is also reported that the surface oxygen content of graphene-based platforms might affect the behaviors of osteoblasts [22, 86, 87]. For example, the functional groups of graphene with negatively charged surface may lead to various interactions such as

Table 4 Graphene-based implant: *in vitro* and *in vivo* studies

<i>In vitro/In vivo</i>	Experiment	Effect	Type of graphene	Method	Form of titanium	Reference
<i>In vitro</i>						
HDFn cells	Cell viability assay, immunofluorescence	Cellular viability, proliferation, biocompatibility	Na-loaded on GO	Spin coating	Titanium	[78]
MC3T3-E1 cells	Alizarin red staining, ALP activity, real-time PCR, immunocytochemistry	Enhanced growth and differentiation into osteoblast	Dex-loaded on RGO		Ti13Nb13Zr (MPCR-TNZ)	[72]
	MTT assay, F-actin fluorescence	Higher cell viability, adhesion, proliferation, and differentiation into osteoblast				[71]
hBMMSCs	ALP activity, immunocytochemical staining, qRT-PCR	Osteogenic differentiation	BMP-2-loaded on GO	Layer-by-Layer assembly	Titanium	[66]
	ALP activity, qRT-PCR analysis, fluorescence images	Osteogenic differentiation	BMP-2- and SP-loaded on GO			[67]
MG63 cells/ <i>staphylococcus aureus</i>	ALP activity, cell viability assay, antibacterial adhesion assay	Biocompatibility, antibacterial effect	CS/HA/GO	Electrophoretic deposition		[74]
MG63 cells	CCK-8 assay, ALP activity	Cell adhesion, proliferation, and differentiation	HA/GO			[80]
MG63 cells/ <i>E. coli</i> and <i>S. epidermidis</i>	Alizarin red S staining, fluorescence microscopy, and adhesion assay	Cell proliferation, osteogenic differentiation, antibacterial property, decreasing of bio-film formation	GO		Ti6Al4 V	[79]
MC3T3-E1 cells	MTT assay, cell viability, EDX, XRD	Osteoblast proliferation, biocompatibility			Ti/anodized Ti	[64]
<i>In vivo</i>						
Rat	X-ray imaging, H&E and collagen staining	Bone formation	Dex-loaded RGO	Spin coating	Titanium	[71]
Mouse	Micro-CT and Goldner's trichrome staining	Bone formation and improved osteointegration	BMP-2-loaded on GO	Layer-by-Layer assembly	Titanium	[66]
	Goldner's trichrome staining, histomorphometric analysis	Bone formation	BMP-2- and SP-loaded on GO			[67]

electrostatic forces, hydrogen bonding, and hydrophobic interactions between the graphene-based platforms and cells.

It is also accepted that the cell spreading and interaction between cell–substrate or cell–cell are important factors to be considered for regulating cellular behaviors and tissue regeneration [87]. Kim et al. reported that the graphene-based substrata could influence cell morphology and interaction between cell–cell or cell–substrate, which might be another possible explanation to graphene-enhanced cellular behaviors and tissue regeneration [22].

4 Future Prospects

4.1 Considerations for Design of Dental Implants Using Graphene

Dental implants require the consideration of many factors such as biocompatibility and other factors for adaptation to a special environment such as the oral cavity. First, even a healthy individual has a variety of microorganisms in his mouth [85]. Therefore, their resistance against these oral microorganisms has to be considered first. In addition, the

Fig. 4 Advanced graphene dental implants. The surface treatment can be combined with various technologies. **A** Graphene-blended nanocomposite loaded on the titanium. **B** Drug delivery, including antibacterial, drug, and growth factors on the graphene surface.

C Nanofiber with graphene coated on the titanium surface. **D** Graphene-based DNA sensing platforms, which allows diagnosis and treatment of diseases at the same time

changes upon salivation and sustained food consumption, and the adaptation of the bite force upon mastication, have to be exceptional [88]. Although dental implants are usually placed within bone, it is possible that implants may also contact soft tissue. Thus, it is very important to check the biocompatibility in soft tissue [89].

Losing the appliance coating is recognized as a limitation of all coating techniques applied to the body. The techniques used for dental implants and orthopedic implants are different. Dental implant installation technique has the goal of obtaining the greatest initial bone fixation; it uses drills with a smaller diameter than those used for orthopedic implants to drill into the bone and place the implant fixture. This creates friction between the implant and bone. Therefore, it is critical for the adherence between the titanium surface and coating material to surpass the friction in a clinical appliance [24]. Furthermore, the coating is not simply applied to a smooth surface *in vitro*, but is applied to the rough surfaces of implants with screw lines. Thus, the goal is to reconsider the methodical perspectives of coating methods to obtain a uniform coating surface.

4.2 Advanced Graphene Dental Implants

It has been proven that various types of graphene, including GO and RGO, enable greater stability and diffusion when combined with nanocomposites on its surface. Graphene-based nanocomposites such as metals [90], metal oxides [91], polymers [92], quaternary phosphonium salts [93], and ethylenediaminetetraacetic acid (EDTA) [94] have been regarded as promising bio-materials [95]. Although little research has been done for dental implants, this hybrid approach can be further extended using

nanocomposites (Fig. 4A). The potential of graphene was confirmed when it was used in other biomedical applications, including drug delivery [96], tissue engineering [86], and gene transfection [97]. A graphene surface can be combined with numerous drugs [90, 98]. A previous study successfully prepared biocompatible GO-modified materials and observed their antibacterial effect by releasing levofloxacin (Fig. 4B) [99]. In addition, Pandey's group showed the phenomenon of releasing gentamicin sulfate from graphene [100]. An ideal environment could be provided to extract a tooth with periodontitis, or it would be possible to provide an antibacterial agent through effective drug delivery for a successful implant placement. For example, in research on the delivery of drugs such as dexamethasone [24] and BMP-2 [66], an attempt was made to improve the effectiveness of a dental implant with graphene, which has the most potential at present. The soft tissue and bone that support the teeth are often lost during the implant placement. Thus, if we used the tissue regeneration capabilities of a graphene scaffold for a dental implant, an endemic problem could be overcome. A previous study investigated the proliferation of stem cells by a scaffold using GO and chitosan [101], and another study obtained an appropriate scaffold by making GO nanosheets in poly (acrylic acid)/gelatin composite hydrogels [102]. Si et al. [103] reported that GO improved the tensile strength and Young's modulus of a nanocomposite hydrogel, which are essential for a suitable tissue engineering scaffold. Various technologies are being grafted onto dental tissue engineering, which will generate a significant synergy effect. For example, a platform consisting of nanofibers or a nanopattern could offer a micro-environment for cells. In previous research, the adhesion and differentiation of human mesenchymal stem cells (hMSCs) were controlled

using a multiscale patterned patch, and bone regeneration was promoted when the patch was inserted in an animal [104]. NIH 3T3 fibroblasts were cultured on electrospun nanofiber using poly (ϵ -caprolactone) (PCL) and polyethylenimine (PEI), with greater cell adhesion and proliferation provided on the electrospun nanofiber scaffold [105]. Therefore, the micro/nanoengineered pattern or fiber was also better than a flat scaffold because it offered a microenvironment for the regulation of cellular behaviors by mimicking the extracellular matrix (ECM) in a human (Fig. 4C). Although there is no strong interaction with double-stranded DNA (dsDNA), graphene has a strong binding force for single-strand DNA (ssDNA). Thus, various groups have studied graphene-based DNA sensing platforms (Fig. 4D) [106–108]. In addition, graphene has also been used for a gene delivery system. A previous report found that a dsDNA molecule GO-PEI (graphene oxide polyethylenimine) complex showed a high gene transfection efficiency and low cytotoxicity [109]. As the ability of graphene is gradually applied to dental implants, it will create a synergy effect. We expect the success rate for dental implant treatment to be enhanced based on previous graphene effect research.

5 Conclusion

In this work, we discussed the present and potential applications of graphene with titanium for dental implants and tissue regeneration. Because graphene has unique properties such as a very high surface area and biocompatibility, it is being applied in dentistry as a dental material. To overcome the limitations of traditional titanium implants, surface treatment is being introduced for graphene. We discussed the foundation and limitations of dental implants, and the four main surface treatment methods were introduced, which include layer-by-layer assembly using chemical properties, a PMMA-mediated method for increased combination with titanium, the electrophoretic deposition of a charged titanium substrate, and spin coating for an efficient platform. Various cells, from stem cells to primary cell lines, have been incubated on graphene with titanium, and have shown enhanced results in numerous ways such as viability, differentiation, and proliferation, suggesting that graphene-based implants or scaffolds may have great potential as platforms for dental tissue regeneration. Although a graphene-based titanium implant is still required to provide additional evidences such as *in vivo* research, it will be one of the major dental materials for dental implants and tissue regeneration in the future. Furthermore, certain caveats about the use of dental implants were described, and we proposed several options for designing and manipulating

them in the dental field. If further study is implemented, graphene with titanium will take center stage for dental implants and dental tissue engineering applications.

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Compliance with Ethical Standards

Conflicts of Interest The authors have no financial conflicts of interest.

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