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RESEARCH PAPER

A Novel Indirect Functionalized of Graphene Nanosheets (Graphene/ SiO_2ATES) for Flexural Strength of Bone Cement

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ABSTRACT

The low resistance to fracture has limited the use of indirect composite resins for bone tissue repairs, especially in areas subjected to strong occlusal forces. To address this challenge, various reinforcement methods for composites have been introduced, with graphene and functionalized graphene being among the most promising options. The primary objective of this study was to assess the flexural strength and resistance of a commercially available indirect composite resin, specifically Sinfony by 3M/ESPE, following the integration of graphene into its structure. The study also aimed to determine the effects of different treatment approaches, including the presence or absence of silanization, on the overall mechanical properties of the composite, to better understand how these modifications could enhance its performance and durability in dental applications. Composite resin samples were created using a Teflon mold. The resin was prepared following the manufacturer's guidelines (with 10 specimens per group), incorporating graphene at three different concentrations. Transmission electron microscopy (TEM) was used to examine the graphene and graphene/SiO_2-ATES samples. Additionally, a flexural test was performed in accordance with ISO 4049/2009 standards. The resulting flexural strength values, measured in MPa, were analyzed using one-way ANOVA followed by Tukey's post hoc test, with a significance level set at $p<0.05$. In comparison to the control, the addition of graphene did not significantly enhance the flexural strength of the indirect composite resin, regardless of the concentration used ($p>0.05$). However, when evaluating the two types of additives, graphene/SiO_2-ATES demonstrated significantly greater flexural strength than all three concentrations of pure graphene ($p<0.05$). While silanization enhances the mechanical properties of graphene, altering the chemical bonding between graphene and graphene/SiO_2-ATES in varying concentrations did not lead to an improvement in the flexural strength of the composite resin.

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INTRODUCTION

Bone tissue engineering and regenerative medicine have increasingly focused on developing advanced materials with enhanced mechanical and biological properties [1-3]. Among these, nanomaterials, particularly graphene nanosheets, have garnered significant attention due to their exceptional strength, flexibility, and biocompatibility [4-6]. Functionalizing graphene nanosheets can further improve their integration with biological tissues and tailor their properties for specific biomedical applications [7-9]. Despite the promising potential of graphene in enhancing the mechanical properties of biomaterials, there remains a need for innovative approaches to optimize its performance for bone-related applications. Indirect functionalization techniques offer a pathway to modify graphene surfaces without compromising their intrinsic properties, potentially leading to improved interactions with the bone matrix and increased mechanical stability [10, 11].

One of the most widely employed strategies to enhance the mechanical performance of bone-inspired composites involves the reinforcement with advanced nanomaterials, such as graphene and its derivatives [12-14]. Incorporating these nanomaterials into polymeric or ceramic matrices can significantly improve their strength, toughness, and overall durability, making them more suitable for load-bearing applications. The exceptional mechanical properties of graphene, including its high tensile strength and elastic modulus, contribute to the reinforcement effect, fostering better load distribution and resisting fracture under stress [15-17]. Additionally, surface functionalization of graphene nanosheets can further optimize interfacial bonding with the matrix, facilitating effective stress transfer and reducing the likelihood of failure [18-20]. This approach not only enhances the biomechanical integrity of composite materials but also aligns with the ongoing trend of developing biomimetic solutions that closely replicate the hierarchical structure and function of native bone tissue. Consequently, nanomaterial reinforcement remains a pivotal technique in the pursuit of designing next-generation biomaterials for orthopedic and reconstructive applications.

In the field of enhancing the mechanical properties of bio-composites, various nanocomposites incorporating different nanomaterials have been extensively studied [21-23]. For instance, nanographene and its derivatives, nanocellulose, bioactive nanoparticles, and ceramic nanoparticles such as aluminum oxide and titanium dioxide have all been explored. The addition of nanographene to polymeric resins or phosphate-based materials has resulted in significant improvements in flexural strength and tensile durability, thanks to graphene's layered structure and high surface area, which facilitate better load distribution [24, 25]. Similarly, nanocellulose, given its structural similarity to natural bone and favorable mechanical properties, has been integrated into composites to enhance physical and biological performance [26-28]. Moreover, ceramic nanoparticles like aluminum oxide and titanium dioxide have been used to develop composites with high fracture and wear resistance [29, 30]. Focusing on these nanocomposites and optimizing their fabrication and surface modification methods are crucial for advancing biomaterials with superior mechanical properties, paving the way for improved implant and regenerative applications [31].

Graphene possesses outstanding mechanical strength, electrical conductivity, rheological characteristics, thermal conductivity, stability, and flame resistance. As a result, integrating graphene into polymer composites can markedly improve these properties [32-35].

Numerous studies have demonstrated the various applications of graphene, including the fabrication of reinforced fibers and nanocomposites [36, 37]. The properties of graphene and its functionalized forms can vary considerably depending on factors such as the synthesis method, purification process, suspension techniques, filling agents, and functionalization procedures. Additionally, strong interfacial adhesion between the polymer matrix and graphene, along with its uniform distribution throughout the composite, are essential for optimizing the mechanical performance of polymer-graphene materials [38, 39]. Based on these considerations, graphene presents a promising option for incorporation into laboratory-manufactured resin composites to enhance their flexural strength, although this area remains underexplored. The aim of this research was to assess the flexural resistance of a commercial composite resin after the addition of graphene. Furthermore, the impact of silanization of graphene on its flexural strength was examined.

It is anticipated that achieving proper dispersion and bonding of graphene within the dental resin could lead to substantial improvements in its mechanical properties.

MATERIALS AND METHODS

Preparation of graphene and silanization of graphene

Graphene oxide (GO) was synthesized using the modified Hummer's method. Initially, 1.0 g of natural graphite powder (99.99% purity, Sigma-Aldrich) was added to 23 mL of concentrated sulfuric acid (H_2SO_4, 98%, Merck) under continuous stirring in an ice bath to prevent temperature escalation. To this mixture, 0.5 g of sodium nitrate ($NaNO_3$, Merck) was gradually introduced while maintaining vigorous stirring. Subsequently, 3.0 g of potassium permanganate ($KMnO_4$, Merck) was slowly added to the suspension over 30 minutes, ensuring the temperature remained below 20°C. The reaction mixture was then stirred at room temperature for 2 hours to facilitate oxidation. Next, 46 mL of deionized water was carefully added to the mixture while stirring; this caused an exothermic reaction, and the temperature was maintained below 20 °C by placing the vessel in an ice bath. After complete addition, the mixture was stirred for an additional 2 hours at room temperature. To terminate the reaction and reduce residual permanganate, 140 mL of deionized water was added gradually, followed by 10 mL of hydrogen peroxide (H_2O_2, 30%, Merck). The mixture's color changed to bright yellow, indicating oxidation completion. The resultant GO suspension was washed by repeated centrifugation and filtration until the pH of the supernatant reached neutral (around pH 7). The purified GO was then dried under vacuum at 40°C for 24 hours and stored in a desiccator for subsequent use. This process yielded graphene oxide with a high degree of oxidation suitable for further reduction or functionalization steps [40].

The silanization procedure was conducted following the method described by Zhang et al [41]. First, graphene was coated with nano-silica (SiO_2). The process began by sonication of graphene powder in ethanol for 30 minutes to facilitate dispersion. Subsequently, the graphene suspension was incubated with 5 mmol/L of 3-aminopropyltriethoxysilane (APTES) for 30 minutes to facilitate surface functionalization. After incubation, the graphene-APTES was thoroughly washed with ethanol and then ultrasonically dispersed in 20 mL of ethanol for an additional 30 minutes. Next, 200 µL of concentrated ammonia solution ($NH_3 \cdot H_2O$) and 100 µL of tetraethyl orthosilicate (TEOS) were added to the dispersion. After 30 minutes, an additional 100 µL of TEOS was introduced. The hydrolysis and condensation reactions were carried out under ultrasonic conditions for 90 minutes to form a silica coating on the graphene surface. The resulting SiO_2-coated graphene was then separated and washed multiple times with ethanol. To further modify the surface, the graphene-SiO_2 was treated with 5 mmol/L of allyltriethoxysilane (ATES) in ethanol, producing graphene-SiO_2-ATES. After washing with ethanol, the samples were dried at 120 °C and stored in a desiccator for preservation.

Sample Calculation and qualification

For this research, the number of samples was

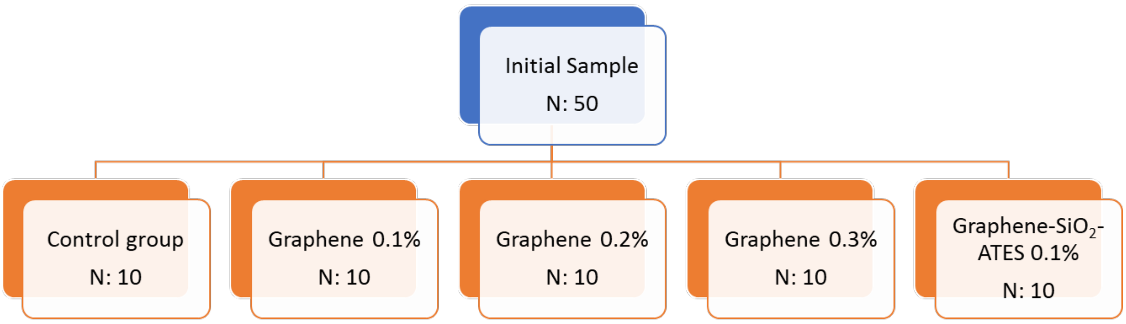

Fig. 1. Flow chart of allocation process.

determined using data from prior studies, with a statistical power of 0.80, resulting in a requirement of 10 specimens for each group. The samples were divided into five different groups for testing (see Fig. 1): a control group without graphene, groups with 0.1%, 0.2%, and 0.3% graphene, and a group with 0.1% graphene modified with SiO_2-ATES.

Incorporation of graphene in the composite resin

The composite resin was formulated in accordance with the manufacturer's guidelines, without any alterations for the control group (lacking graphene). Graphene was incorporated at concentrations of 0.1%, 0.2%, and 0.3% (w/w), while graphene-SiO_2-ATES was added at 0.1% (w/w). The resins were mixed thoroughly using spatulation to simulate the typical clinical preparation process. Following the addition of graphene and graphene-SiO_2-ATES at various concentrations, specimens were shaped using a Teflon mold measuring 25 × 2 × 2 mm. The resin surfaces were pre-cured with a visible-light curing unit (Visio Alfa) for 5 seconds, then subjected to final polymerization in the Visio Beta device for 16 minutes under vacuum conditions. All curing procedures were performed in accordance with the manufacturer's guidelines (3M/ESPE). To achieve a uniform thickness of 2 mm, the specimens were polished using sequentially finer silicon carbide papers (600, 800, 1,000, and 1,500 grit), with thickness verified by a digital micrometer (Mitutoyo Corp., Tokyo, Japan). The finished samples were then immersed in distilled water maintained at 37°C for 24 hours prior to testing.

Data Collection and Data Analysis

Transmission electron microscopy (TEM) was utilized to examine the morphology of the graphene and graphene/SiO_2-ATES specimens. The flexural test was performed in accordance with ISO 4049/2009 standards using an EMIC DL 2000 testing machine, equipped with a 1,000 kgf load cell, at a crosshead speed of 0.5 mm/min. The flexural strength (F) was determined using the following equation: where F represents the flexural strength, Pf is the peak load in newtons, L is the span length between supports in millimeters, W is the specimen width in millimeters, and H is the height of the specimen in millimeters. The resulting flexural strength values, expressed in MPa, were analyzed statistically with one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

RESULTS AND DISCUSSION

This research assessed the flexural strength to examine how effectively graphene with two different chemical surface modifications can reinforce Sinfony via indirect composite techniques. To enhance the material characteristics of composites through the incorporation of graphene as reinforcement, two primary challenges must be addressed. The main challenges involve the interfacial adhesion and uniform dispersion of graphene within the polymer matrix. Graphene is naturally chemically inert and prone to forming aggregates. Additionally, slipping within the bundles and inadequate bonding at the interface lead to poor load transfer, which ultimately lowers the mechanical performance of graphene-enhanced composites. Therefore, surface modification of the particles is necessary to reduce clustering and promote better distribution throughout the matrix. This is typically achieved by attaching functional groups to the nanotube surfaces or through covalent linking.

Regarding the flexural strength data presented in Table 1, there were no notable differences observed between the graphene/0.1%, graphene/0.2%, and graphene/0.3% groups when compared to the control group. The findings also indicated that the graphene/SiO_2-ATES samples demonstrated greater flexural strength than the other experimental groups, except for the unmodified control resin, which displayed the highest values. As indicated in Table 1, the composite incorporating graphene/SiO_2-ATES demonstrated greater flexural strength compared to the samples with original graphene contents of 0.1, 0.2, and 0.3 wt%. The comparatively lower flexural strength observed in these cases may be attributed to graphene aggregation, which reduces the material's ability to absorb energy during viscoelastic deformation. Another possible reason could be the opacity caused by graphene agglomerates, which might have hindered the monomer's degree of conversion and thus influenced the composite's overall properties. It is more crucial to achieve proper dispersion and effective load transfer between the nano-reinforcement and the matrix than simply increasing the nanotube content. Compared to the composite lacking graphene, the flexural strength of the graphene/SiO_2-ATES material was consistently lower. In this research, graphene was selectively purified to contain carboxylic functional groups, which allow for enhanced chemical modification, as these groups can participate in multiple reactions. The organosilane agent ATES used in the process promotes bonding between the surface hydroxyl groups (Si-OH and OH) of graphene and the organic component, which can subsequently interact with the resin matrix via its C=C double bond.

The TEM image of the pristine graphene (Fig. 2a) revealed that it consisted of few layers, primarily stacked and overlapping each other. The TEM image of graphene following silanization (Fig. 2b) indicated that most impurities had been effectively eliminated, and the dispersion had been enhanced. Additionally, the TEM image of the graphene/SiO_2-ATES composite shows numerous spherical nanoparticles that create a thin coating on the exterior surface of the graphene.

The SEM image of the pristine graphene (Fig. 3a) showed a surface with few layers, mainly appearing as stacked and overlapping sheets. The SEM image of the silanized graphene (Fig. 3b) demonstrated a cleaner surface with significantly fewer impurities and a more uniform, well-dispersed structure. Furthermore, the SEM image of the graphene/SiO_2-ATES composite revealed numerous spherical particles forming a thin coating on the external surface of the graphene sheets. The SEM images provide valuable insights into the surface morphology and dispersion quality of the graphene before and after surface treatment. The pristine graphene's surface appears relatively smooth but with evident layering and aggregation, which could hinder uniform distribution in composites. Post-silanization, the smoother and cleaner surface indicates successful removal of impurities and better individualization of the graphene sheets, which is crucial for improved interfacial bonding. The presence of spherical nanoparticles on the composite surface suggests successful attachment of SiO_2-ATES, potentially enhancing filler-matrix interactions. These morphological features are essential for predicting

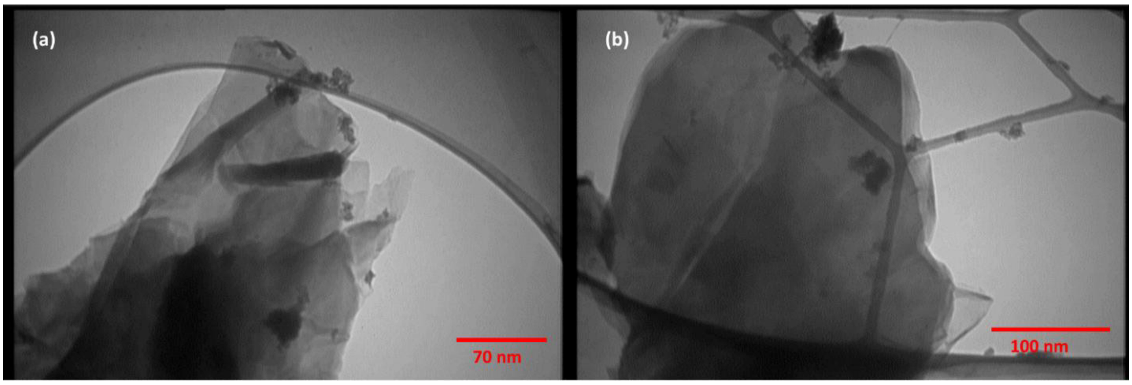

Fig. 2. TEM images of a) pristine graphene, and b) graphene/SiO_2-ATES composite.

Table 1. Screening of flexural strength (MPa) as bone cement.

Group	CG	graphene/SiO_2-ATES	Graphene, 0.1%	Graphene, 0.2%	Graphene, 0.3%
Mean value	120.42	109.90	102.29	100.89	100.03
SD value	1.5	1.62	1.26	2.45	3.11
	A	B	C	C	C

Abbreviation: CG: control group; SD: standard deviation; Note: Along the horizontal axis, mean values marked with the same capital letters were not significantly different ($p > 0.05$).

the reinforcing effectiveness of graphene-based fillers in composite materials, where uniform dispersion and strong interfacial bonding are key factors for optimal mechanical properties.

When comparing our findings to those of Zhang et al., some discrepancies become apparent. Zhang and colleagues (41) added silanized graphene at a weight ratio of 0.1 (graphene to paste) into a composite material and observed significant enhancements in flexural strength in specimens containing the modified graphene, relative to Durafill without graphene. These differing results may stem from variations in experimental methods and the types of composites used. Specifically, their study employed a blend of urethane dimethacrylate as the monomer with the silanized graphene, and the low viscosity of their resin likely facilitated better particle dispersion and organization within the matrix.

The Sinfony system is a microhybrid indirect composite designed for layering techniques due to its flowable consistency. In this study, graphene was directly mixed into the resin paste through spatulation. This method may have influenced the resin's properties, as a substantial amount of filler was added to a limited organic matrix, potentially leading to a reduction in the flexural strength of the graphene-enhanced Sinfony resin. To achieve optimal performance, nanosheets should be evenly distributed as separate, individual layers, each coated with polymer for better integration.

In a separate study (42), researchers observed that composites containing silanated nanofillers, compared to those with untreated nanofillers, not only exhibited enhanced mechanical properties but also demonstrated greater resistance to water absorption and overall wear. These findings are consistent with our results, where the graphene/SiO_2-ATES group showed higher flexural strength than the graphene/0.1%, graphene/0.2%, and graphene/0.3% groups that were not silanized. Additionally, TEM images of silanized graphene revealed silicon oxide nanoparticles growing on the surface, indicating successful surface modification. However, despite these confirmations of silanization, the process may not have been entirely effective, since the control group (CG) displayed higher flexural strength than the graphene/SiO_2-ATES group. This suggests that graphene may serve as a site of weakness, possibly due to inadequate chemical bonding between the nanofiller and the organic phase of Sinfony. A similar argument could be made when comparing graphene/SiO_2-ATES to the non-silanized groups. Within the limits of this study, the results imply that incorporating graphene may

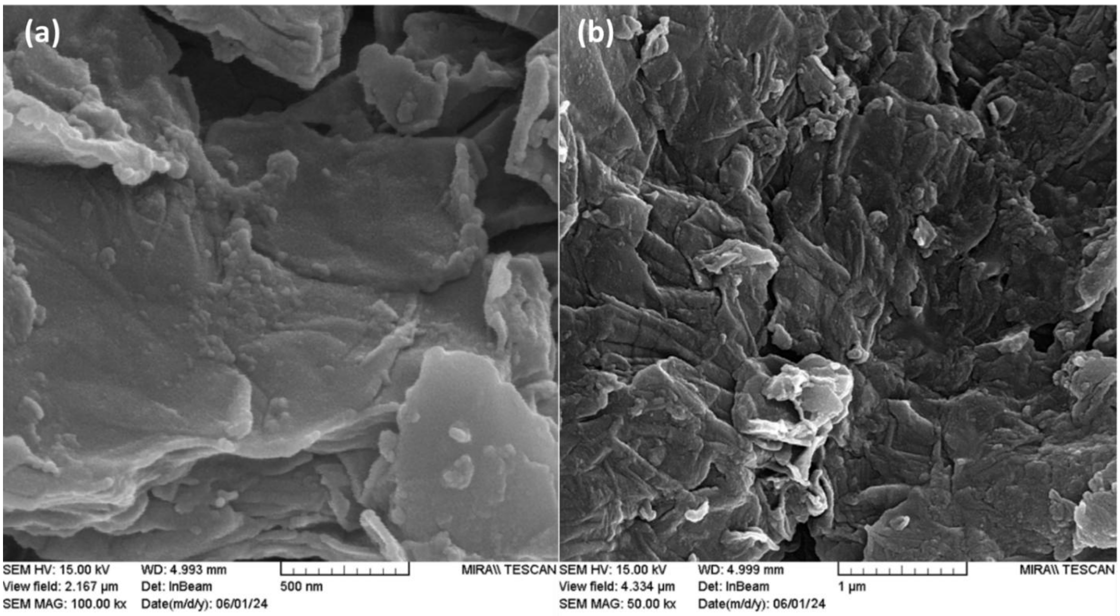

Fig. 3. SEM images of a) pristine graphene, and b) graphene/SiO_2-ATES composite.

be a promising way to enhance composite flexural strength. Nonetheless, such integration should be approached cautiously, as the amount of graphene added can influence the wettability of the resin and its degree of polymerization. Future research should explore alternative surface treatments for graphene and investigate how its inclusion affects dental composite properties.

Future directions and challenges

Future research in the development of functionalized graphene nanosheets, such as graphene/SiO_2-ATES, for enhancing the flexural strength of bone cement should focus on optimizing surface functionalization techniques to improve interfacial bonding and dispersion within the polymer matrix [43, 44]. Advanced surface modification methods need to be explored to ensure uniform nanoparticle distribution and strong chemical integration, thereby minimizing the risk of agglomeration and weak points. Moreover, comprehensive in vivo studies are necessary to evaluate the biological compatibility, long-term stability, and potential cytotoxicity of these nanocomposites to ensure their safe application in clinical settings. The challenges of scalable manufacturing, cost-effectiveness, and reproducibility of functionalized graphene nanosheets also require attention to facilitate commercial translation. Additionally, understanding the long-term mechanical and biological behavior under physiological conditions remains crucial to predict the durability and performance of these nanocomposites in real-world biomedical applications. Overcoming these challenges will be vital in advancing the practical use of graphene-based nanomaterials to enhance the mechanical properties and longevity of bone cements in regenerative medicine and implantology.

CONCLUSION

This study explores the reinforcement potential of graphene nanosheets, particularly focusing on functionalized variants, to improve the mechanical properties of dental composite resins. The primary aim was to assess how the incorporation of graphene—synthesized via modified Hummer's method and surface-functionalized with silane agents—affects the flexural strength of the commercially used Sinfony resin. The experimental setup involved preparing multiple composite groups with varying graphene concentrations (0.1%, 0.2%, 0.3%) and a silanized graphene variant (graphene/SiO_2-ATES), then evaluating their properties through TEM, SEM, and mechanical testing aligned with ISO standards. The findings reveal that adding unmodified graphene at different concentrations did not significantly enhance the flexural strength compared to the control. In contrast, the silanized graphene (graphene/SiO_2-ATES) improved flexural strength over unmodified graphene groups, though still not surpassing the control resin. However, despite the surface modifications, the results suggest that graphene may act as a site of weakness, likely due to insufficient chemical bonding within the resin matrix, as evidenced by higher strength in the control group. The study emphasizes the importance of optimal dispersion and bonding over mere filler content increase. Future research should focus on refining surface treatment techniques, improving dispersion methods, and exploring how different functionalization approaches can enhance the overall biomechanical performance of graphene-reinforced dental composites. Finally, while graphene and its derivatives show promise as reinforcement agents, their effective application requires careful surface treatment, dispersion, and integration strategies to maximize their potential for biomedical use, especially in load-bearing bone and dental restorations.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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