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Synthesis, morpho-structural properties and antibacterial effect of silicate-based composites containing graphene oxide/hydroxyapatite

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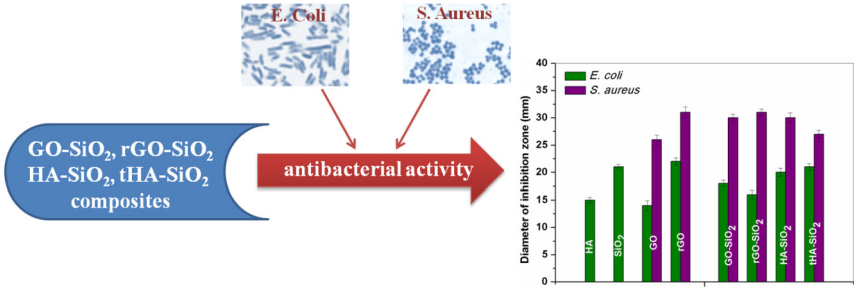
HIGHLIGHTS

- GO-SiO_2 rGO-SiO_2 composites were prepared via chemical-thermal method.
- HA-SiO_2 and tHA-SiO_2 composites were obtained by wet chemical techniques.
- The morpho-structural characteristics of composites were evaluated and compared.
- The rGO-SiO_2 and tHA-SiO_2 showed the highest antibacterial effect on *E. Coli* and *S. Aureus*.

GRAPHICAL ABSTRACT



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ABSTRACT

Silicate-based biomaterials play an important role in formulations applied in medicine. Graphene oxide-SiO_2 (GO-SiO_2) and reduced graphene oxide-SiO_2 (rGO-SiO_2) composites were prepared by a two-step (chemical and thermal) method. Hydroxyapatite-SiO_2 (HA-SiO_2) and thermally treated (900 °C) hydroxyapatite-SiO_2 (tHA-SiO_2) composites were obtained by wet chemical techniques. The obtained materials were characterized by Transmission Electron Microscopy (TEM), X-ray powder Diffraction (XRD), Fourier-Transform Infrared Spectroscopy (FTIR) and Raman spectroscopy. The results indicated the presence of amorphous silica phase, graphene oxide and nanocrystallites of hydroxyapatite in the prepared composite powders. *In vitro* antibacterial activity was tested by agar well diffusion method. The morpho-structural properties and antibacterial effect on *Escherichia coli* and *Staphylococcus aureus* bacteria of GO-SiO_2 and rGO-SiO_2 composites were compared with those of HA-SiO_2 and tHA-SiO_2 composites. The rGO-SiO_2 and tHA-SiO_2 showed higher antibacterial effect than GO-SiO_2 and tHA-SiO_2 composites, on both bacterial strains.

1. Introduction

Biomaterial science and engineering has become an area of active research worldwide, due to its direct relationship with human health. Besides the appropriate mechanical properties, the antibacterial effect and biocompatibility are important features of biomaterials [1,2].

Recently, the excellent optical, thermal, mechanical and biomedical properties of graphene and its derivatives (such as graphene oxide and reduced graphene oxide) have provided a promising potential for various biomedical applications (bio-sensing and imaging, drug/gene delivery, photothermal therapy and tissue engineering) [3,4]. Moreover, graphene oxide (GO) can be chemically modified due to the abundant

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oxygen-containing groups (epoxy, hydroxyl, and carboxyl) attached to its honeycomb structure composed of sp^2-bonded carbon atoms. Reduced graphene oxide (rGO) is a graphene derivate obtained by restoring the graphene network through thermal, chemical or photochemical treatment [5]. Thus, the easy functionalization of GO and rGO facilitate targeted imaging and drug delivery [3]. Besides these attractive physico-chemical properties, the cytotoxicity, biocompatibility and antimicrobial activity of graphene and its derivatives should be considered, despite contradictory or inconclusive results obtained in different studies. Various intrinsic parameters (such as size, shape, or surface chemistry) could influence the biological response of the graphene-based materials and define their cytotoxic effects, biocompatibility and antimicrobial properties [6–10].

Hydroxyapatite (HA), composed of calcium and phosphate, is a bioactive ceramic similar in crystallography (hexagonal structure in teeth and bones; mono-clinical structure in dental enamel) and chemical composition (stoichiometry Ca/P ratio ranging from 1.55 to 2.2) to bone apatite [11–13]. Hydroxyapatite was proposed for a wide range of orthopedic (from thin coatings on metallic implants to synthetic bone grafts) and dental (repair of enamel, implant coating, cosmetic dentistry) applications due to its excellent affinity to biomolecules, favorable bioactive and osteoconductive properties, and also antimicrobial activity [12,14–17]. However, the medical applications of synthetic HA are restricted due to its poor mechanical strength and fracture toughness [13,14]. Various methodologies were tested in order to improve the physico-chemical properties of HA, such as different preparation methods (sol-gel, co-precipitation, hydrothermal/solvothermal synthesis, microwave and ultrasound-assisted approaches), sintering techniques or adding reinforcing materials (*i.e.*, alumina, silica, zirconia, titania, carbonaceous materials, bioglasses/glass ceramics, synthetic polymers or metallic agents) [14,18]. Even if silica (SiO_2) behaves as a bio-inert material, it is one of the most widely used filler in dental restoration due to its promising properties such as low refractive index and wear resistance [19]. Previous studies already suggested that HA-based materials containing SiO_2, with high bioactivity, osteoconductivity and suitable mechanical properties could be promising composites to different biomedical applications [20–30].

To the best of our knowledge, there are few studies regarding the antibacterial activity of graphene-SiO_2 materials. For example, Jinhua Li et al. [31] observed that the surface of graphene@SiO_2 cannot significantly damage the cell membranes and destroy neither *E. coli* nor *S. aureus* bacteria strains.

The purpose of the present study was to compare the morpho-structural properties and antibacterial activity of graphene-SiO_2 (GO- and rGO-SiO_2) and hydroxyapatite-SiO_2 (HA- and tHA-SiO_2) composites, on *Escherichia coli* and *Staphylococcus aureus*. The selected pathogenic bacteria are commonly associated with hospital-acquired (nosocomial) infections as a result of their multidrug resistance (MDR). In particular, oral colonization by these organisms could influence the equilibrium state of oral cavity and can cause infections and diseases to oral cavity and respiratory tract.

The synthesis of composite material based on hydroxyapatite and SiO_2 nanoparticles may have various advantages, since the silica particles fill the voids between the hexagonal HA and subsequently enhance the packing density and the hardness of the dental materials. The graphene and its derivatives could be competitive candidates for various biomedical applications due to their unique and superior properties that continue to be recognized or discovered. The investigations on the potential of graphene-based materials bring a strong contribution for new trends and future developments in the nanomedicine area.

2. Experimental

2.1. Synthesis of GO-SiO_2 and rGO-SiO_2 composites

Graphene oxide (GO) was prepared from natural graphite, using a modified Hummers' method [32]. Graphene oxide (75 mg) was added into 10 mL double-distilled water and dispersed by ultrasounds for 15 min. Separately, 1.5 g SiO_2 powder (Aerosil 200; Degussa, Germany) was ultrasonically dispersed in 15 ml NaOH solution (pH 9.5) for 15 min. Then, the two suspensions were mixed and the sonication time was extended to 45 min. After that, the colloidal suspension was dried at 50 °C. About half of the resulted GO-SiO_2 composite was annealed for 15 min at 300 °C, under argon atmosphere. Finally, two graphene-SiO_2 composites were obtained: a raw composite material (GO-SiO_2) and a thermally-treated one (rGO-SiO_2).

2.2. Synthesis of HA-SiO_2 and tHA-SiO_2 composites

Hydroxyapatite was synthesized by precipitation method associated with the maturation process, as was reported in our previous study [33]. SiO_2 powder was dispersed in aqueous solution containing 0.60 wt% HNO_3 and mixed on a magnetic stirrer at room temperature, for 2 h. In the next step, hydroxyapatite (in its second maturation stage) was added and stirred for further 3 h. The resulting HA/SiO_2 sample with weight ratio of 40/60 was kept at room temperature for 96 h.

The material was vacuum filtered, washed with distilled water and dried in the oven at 80° C, for 5 h (η = 95%). The obtained powder was denoted HA-SiO_2. An appropriate amount of HA-SiO_2 was subjected to a thermal treatment at 900 °C, for 3 h. The resulted composite was named tHA-SiO_2.

2.3. Characterization methods for composites

Transmission Electron Microscopy (TEM) images were obtained with an H-7650 120 kV Automatic Microscope (Hitachi, Japan). The samples were dispersed in ethanol using an ultrasonic bath, and a drop of each powder suspension was deposited onto carbon-coated grids. X-ray powder diffraction (XRD) patterns were recorded with a BRUKER D8 Advance X-ray powder diffractometer, using CuKα radiation (λ = 0.154 nm). Fourier Transform Infrared Spectroscopy (FTIR) analysis of samples was carried out with a JASCO FTIR – 610 Instrument in the 4000-400 cm^{-1} range, using the KBr pellet. The Raman spectra were recorded at room temperature with a JASCO NRS 3300 spectrometer with an incident laser beam wavelength of 514 nm.

2.4. Antibacterial activity

The *in vitro* antibacterial activity of prepared composites was investigated on *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 as Gram-negative and Gram-positive bacteria by agar well diffusion method. The bacteria strains were provided by the Microbiology laboratory of Bio-Labs (Germany). According to the standard agar well diffusion method, the bacteria were cultured on Mueller-Hinton agar (containing the nutrient medium HiMedia) into Petri plates.

The well–variant of the diffusion assay involved cutting the wells (5 mm in diameter) in bacterial inoculated Mueller-Hinton-agar and placing 20 µl of the composite dispersions (GO-SiO_2, rGO-SiO_2, HA-SiO_2, tHA-SiO_2 and HA) into each well. The plates were incubated at 37 °C for 48 h. The diameter of the clear zone (mm) that indicates the bacterial growth inhibition was measured and the antibacterial activity was also evaluated. All the experiments were carried out in triplicate.

2.5. Statistical analysis

Data were statistically evaluated with repeated measurements analysis of variance (ANOVA). Statistical significance was defined as $P < 0.05$.