Bioactive Materials 3 (2018) 1–18

Contents lists available at ScienceDirect

Bioactive Materials

journal homepage: http://www.keaipublishing.com/en/journals/bioactive-materials/

# An overview of graphene-based hydroxyapatite composites for orthopedic applications

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ARTICLE INFO

*Article history:*
Received 30 August 2017
Received in revised form
21 December 2017
Accepted 2 January 2018

*Keywords:*
Graphene
Hydroxyapatite
Composites
Bone tissue
Biomedical devices

ABSTRACT

Hydroxyapatite (HA) is an attractive bioceramic for hard tissue repair and regeneration due to its physicochemical similarities to natural apatite. However, its low fracture toughness, poor tensile strength and weak wear resistance become major obstacles for potential clinical applications. One promising method to tackle with these problems is exploiting graphene and its derivatives (graphene oxide and reduced graphene oxide) as nanoscale reinforcement fillers to fabricate graphene-based hydroxyapatite composites in the form of powders, coatings and scaffolds. The last few years witnessed increasing numbers of studies on the preparation, mechanical and biological evaluations of these novel materials. Herein, various preparation techniques, mechanical behaviors and toughen mechanism, the *in vitro/* in vivo biocompatible analysis, antibacterial properties of the graphene-based HA composites are presented in this review.



## 1. Introduction

Bone tissues consist of organic and inorganic components, with self-healing ability and great capability to withstand mechanical loading. Fixation of bone fractures and non-unions, correction of spinal deformities and replacement of arthritic joints [1] are major unmet clinical needs. Traditionally, biological approaches for bone repair involve using autografts and allografts of cancellous bone [2]. Nowadays, calcium phosphate ceramics and bioactive glasses are introduced as promising osteoinductive and osteoconductive substitutes for large orthopedic defect remolding or regeneration [3]. In addition, these bioceramics are also utilized as coating on metallic implants to provide long-term performance of the devices and to minimize micromotion between bones and implants during physiologic loading [1,4].

Hydroxyapatite (HA, $Ca_{10}(OH)_2(PO_4)_6$) possesses chemical and crystallographic similarities to inorganic components of the bone matrix and the teeth [5] with excellent osteoconductivity and osteoinductivity. It has been clinically used as bioactive coatings on dental and orthopedic implants, enabling the adhesion and proliferation of osteoblast cells on the prosthetic surface, and resulting in biological fixation between bone tissues and the implant [6]. However, one primary limitation, when used under major load bearing, is its poor mechanical properties, such as low fracture toughness and tensile strength [5]. To address this problem, specific reinforcing materials, such as carbon nanotubes [7], polyethylene [8], $Al_2O_3$ and $TiO_2$ [9], are typically used to prepare HA composites with increased mechanical properties, but these materials result in significantly less bioactivity than that of pure HA.

Graphene is rapidly rising as a promising material for biomedical applications [10,11], featuring two-dimensional nanosheet of hexagonally bonded carbon atoms, with large surface area, high conductivity, strong mechanical properties and good

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Peer review under responsibility of KeAi Communications Co., Ltd.

https://doi.org/10.1016/j.bioactmat.2018.01.001

biocompatibility. The graphene-based composites have great advantages when used in bone repair or regeneration, as it can induce osteogenic [12–14] and chondrogenic [15] differentiation of stem cells. Compared with other reinforcement fillers, graphene can greatly increase the mechanical properties of the composite at low content, and its high elasticity and flexibility (adaptability to flat or irregular surfaces) also renders graphene and its derivatives (graphene oxide (GO) and reduced graphene oxide (rGO)) as promising mechanical fillers for biomaterials.

Recently, biomaterial scientists have explored the possibilities of preparing graphene-based HA composite for orthopedic applications with increased bioactivities and mechanical properties. Graphene-based HA composites can be prepared in the form of powders, bulks, coatings and scaffolds. The powders or bulk composites can be used to repair the bone defects or small non-unions. This novel material can also be coated onto orthopedics metallic implant to increase its bone-binding abilities. As for the large defects or bone loss, three dimensional porous graphene-based HA composites can be incorporated into the damaged hard tissues to accelerate their regeneration.

The related research has begun very recently in 2009 [16]. The chronological tendency of the research papers on graphene/HA system is shown in Fig. 1, showing an increasing interest in this area. A significant growth in the number of publications from 2013 to 2016 highlights the novelty and importance of this topic in the up-to-date scientific community. Therefore, in the foreseeable future, more and more related works will be undertaken, and it is the right time to present a comprehensive review of current achievements and findings in this field, which may provide guidance and future directions for further study.

Herein, we present a comprehensive review on almost all of the available investigations on graphene/HA system. Articles were identified via Web of Science and Google Scholar by searching "graphene" and "hydroxyapatite" which were published up to June 2017. This review paper includes the issues regarding the preparation methods, mechanical properties, i*n vitro* and in vivo biocompatibility of graphene/HA composites, as well as the underlying challenges required to be coped with.

## 2. Preparation of composites

Various preparation techniques could be exploited to make this

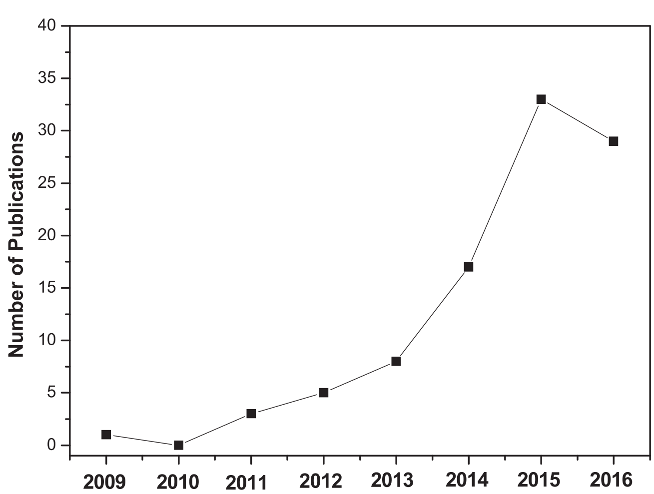


**Fig. 1.** The number of publications on Graphene-based HA composites from year 2009–2016 (2009 [16], 2011 [17–19], 2012 [20–24], 2013 [25–32], 2014 [33–49], 2015 [50–82], 2016 [83–112]). This figure does not include the papers published in 2017 [113–130].

interesting composites, which is summarized in Fig. 2. In most cases, the composite prepared under high temperature or high pressures have high crystallinity and mechanical properties, such as hydrothermal synthesis, spark plasma sintering and hot isostatic sintering. However, thermal spraying techniques usually lower the crystallinity of the HA coating. HA could be synthesized onto graphene and its derivatives and be directly mixed with these nanofillers by ultrasonic dispersion and ball milling.

### *2.1. Graphene/HA composite powder*

#### *2.1.1. In situ synthesis*

Nano HA particles are successfully fabricated on GO [30], chitosan functionalized GO [30] and rGO [29] surfaces using *in situ* synthesis methods. Usually, as shown in Fig. 3a, graphene-based powders are first dissolved and exfoliated in DI water by ultrasonic dispersion to obtain a uniform solution; then $Ca(NO_3)_2$ is added into the graphene-based solutions by stirring for a desired time; afterwards, the pH of the suspension is adjusted to 9–10 using ammonia water, and $(NH_4)\ _2HPO_4$ was added dropwise into the mixture [30]. The resulting composite solutions are recommended to be aged for days to ensure the fully transformation of apatite into hydroxyapatite with good phase purity and well crystallinity. During the synthesis, the oxygen-containing functional groups on GO surfaces behave as receptor sites for $Ca^{2+}$ through electrostatic interactions; these anchored cations can *in situ* react with the phosphate ions to obtain apatite nanoparticles. The underlying reaction mechanism has been proposed and discussed by Li et al. [30]; the distribution and the microstructures of HA on graphene are mainly influenced by (1) the amounts and types of the oxygenous groups on the graphene-based templates and (2) the concentration of the reagents ($Ca^{2+}$ and $HPO_4^{2-}$), solution pH values and so on. Besides, $Ca(OH)_2$ and $H_3PO_4$ are also utilized by Gururaj et al. to *in situ* deposit HA on rGO nanosheets [29].

Composite, prepared in this method, is expected to increase the interfacial bonding strength between graphene and HA, facilitating the stress transfer from the matrix to the graphene-based nanofillers. This facile approach is economical and can be industrially mass-produced.

#### *2.1.2. Biomimetic mineralization*

Biomimetic mineralization is a facile and environmental friendly method to synthesis bone-like apatite under ambient conditions in aqueous environments. Usually graphene and its derivatives are immersed in a supersaturated or unstable solution with calcium ions and phosphate ions concentrations similar to simulated physiological condition, and apatite will nucleate and precipitate on the surface of those graphene-based materials. During the mineralization process, GO greatly enhance the nucleation and crystallization of HA, resulting in a hybrid homogeneous GO/HA coatings with dense and fine flake-like HA nanocrystalline [54]. Usually, graphene and its derivatives are surface-functionalized by bioactive materials to endow the composite with novel properties and facilitate the biomimetic deposition of HA. The GO can be modified by gelatin to mimic the charged proteins in extracellular matrix for regulating bone formation, and the presence of gelatin improves the attraction of calcium ions and promotes the nucleation of HA [37]. Besides GO can be also biofunctionalized by polydopamine [24], casein phosphopeptide [26], carrageenan [35], chitosan [104,131], fibrinogen [33] or peptide [78] to improve the mineralization process.

#### *2.1.3. Hydrothermal synthesis*

Hydrothermal synthesis of graphene/HA composite involves of dispersing graphene or GO into aqueous solutions containing