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Multi-walled carbon nanotubes/graphene oxide hybrid and nanohydroxyapatite composite: A novel coating to prevent dentin erosion

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ABSTRACT

To date is emergent the development of novel coatings to protect erosion, especially to preventive dentistry and restorative dentistry. Here, for the first time we report the effectiveness of multi-walled carbon nanotube/graphene oxide hybrid carbon-base material (MWCNTO-GO) combined with nanohydroxyapatite (nHAp) as a protective coating for dentin erosion. Fourier transform Raman spectroscopy (FT-Raman), scanning electron (SEM), and transmission electron (TEM) microscopy were used to investigated the coatings and the effect of acidulated phosphate fluoride gel (APF) treatment on bovine teeth root dentin before and after erosion. The electrochemical corrosion performance of the coating was evaluated. Raman spectra identified that: (i) the phosphate ($\nu_1 PO_4^{3-}$) content of dentin was not significantly affected by the treatments and (ii) the carbonate ($\nu_1 CO_3^{2-}$) content in dentin increased when nHAp was used. However, the nHAp/MWCNTO-GO composite exposited lower levels of organic matrix (C—H bonds) after erosion compared to other treatments. Interesting, SEM micrographs identified that the nHAp/MWCNTO-GO formed layers after erosive cycling when associate with APF treatment, indicating a possible chemical bond among them. Treatments of root dentin with nHAp, MWCNTO-GO, APF_MWCNTO-GO, and APF_nHAp/MWCNTO-GO increased the carbonate content, carbonate/phosphate ratio, and organic matrix band area after erosion. The potentiodynamic polarization curves and Nyquist plot showed that nHAp, MWCNT-GO and nHAp/MWCNT-GO composites acted as protective agents against corrosion process. Clearly, the nHAp/MWCNTO-GO composite was stable after erosive cycling and a thin and acid-resistant film was formed when associated to APF treatment.

1. Introduction

Dentin is a complex structure of hydrated biological material that has a distinct microstructure characterized by tubules [1]. It contains approximately 50 vol% mineral in the form of a carbonated apatite, 30 vol% organic material (the majority of the organic material is Type I collagen), and 20 vol% fluid [1].

The main constituent of dentin is the basic mineral hydroxyapatite (HAp) with a chemical composition of $Ca_{10}(PO_4)_6(OH)_2$. HAp is inherently susceptible to the etching and dissolving action of acids, which result in tooth decay including dental caries and dental erosion [2,3]. Erosion may occur when the teeth are exposed to low-pH solutions ($pH < 4$) from non-bacterial sources (acidic foods and beverages, or gastric fluid) [2,3].

Dentin demineralization is a gradual and irreversible loss of tooth structure [4]. Initially the mineral loss in dentin occurs at the border between the peri- and intertubular dentin. Subsequently, there is loss of peritubular dentin, enlargement of the tubules, and finally demineralization of the intertubular dentin with exposure of the organic matrix

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[5]. The tooth wear process by friction and acid erosion can result in the loss of enamel and dentin exposure. If the erosion process continues without any treatment, dentin sensitivity may appear, causing pain [6]. At high-level of erosion, oral rehabilitation with prosthetic materials is necessary.

Fluoride-based products have been employed to protect and remineralize the dentin. Topical fluoride applications may arrest or reharden the dental caries lesions in dentin [7]. In the case of dental erosion, no subsurface lesion is available for remineralisation, and therefore the precipitation of CaF_2-like layers had been assumed as an only mode of action [8]. All these effects can be attributed to the formation of fluorides precipitates that were able to hamper the demineralization due to formation of a protective barrier on the teeth surface. Contradictory, researchers attributed the fluorides protective action to slight reduce its solubility due to adsorption onto the crystalline surface altering the biological dynamic equilibrium [9]. Synergistically, all these mechanisms are further discussed and need to be more explained. Therefore, the fluoride's alone is not able to drive the remineralization process due to the lack of calcium and phosphate ions [10]. For this, are very interesting to develop protective thin films that may be capable to accelerate the natural fluoride precipitation and to available calcium and phosphate ions in biological medial especially around dental hard tissue.

To date, nanomaterials have been applied to combat early caries lesions and to protect erosion, especially to preventive dentistry and restoration dentistry. Nanohydroxyapatite (nHAp) surges as an excellent candidate to prevent erosion due to their chemical and biological similarity to the mineral part of teeth and bone [11]. Recently, several authors showed that nHAp has some potential to repair dental enamel [12–16], but the published results were contradictory, opening perspectives to perform further studies about that. Briefly, authors showed that nHAp paste decreased enamel demineralization [17,18]. On the other hand, other studies showed that nHAp solutions [19,20] and nHAp toothpaste [21] would be efficient to enhance the remineralization process. It is know that nHAp may be effective to enhance biomineralization process different from larger HAp particles [22]. Nonetheless, a recently published paper suggested that an association between nHAp and soft drink decreased the erosive potential due to calcium and phosphate supersaturation [23]. In summary, it is not clear the effect of nHAp by itself as a protective erosion agent, highlighted the developing of nanocomposites that are effective to erosion protection.

Single-walled (SWCNT), multiwalled (MWCNT), oxidized multiwalled carbon nanotubes (MWCNTO) [24] and graphene oxide (GO) [25] have been studied for biomedical applications due to their chemical and physical characteristics, such as chemical stability when functionalized, feasibility to insert functional groups and physical dimensions. Our group exfoliated MWCNT using oxygen plasma etching to promote superhydrophilicity and expose graphene sheets from MWCNT structure. This graphene sheets come from CNT walls and tips and are oxidized by oxygen plasma as we have shown elsewhere [26]. This new nanomaterial named as MWCNTO-GO shows special biological properties when incubated with cells [27]. For this, it is very interesting a combination of MWCNTO, GO and nHAp for promote dentin remineralization and protect the teeth against erosion. In this way, recently our group discussed for the first time that the carboxylic groups attached at the end of MWCNT-GO tips promoted the biomineralization process [26].

To date, few studies have been explored the carbon nanomaterials as agent to protective dental erosion. Zhang et al. [28] evaluated the effects of SWCNT in dental composite resins by flexural strength measurements. These authors improved the flexural strength of the resin by covering it with modified SWCNT. Akasaka et al. [29] showed that MWCNT adhered easily to the dentin and cementum surfaces and did not affect the tensile bond strength of dentin adhesives. Soares et al. [30] evaluated the MWCNTO-GO, nHAp and nHAp/MWCNTO-GO with and without acidulated phosphate fluoride (APF) as protective coating before root dentin erosion. The authors identified that artificial saliva and nHAp/MWCNTO-GO composite promoted a lower dentin demineralization than nanomaterials without APF. When the dentin tubules were exposed, it was observed a better interaction of nanobiomaterials than in smear layer covered dentin. Meanwhile, the association of fluoride to other nanobiomaterials had a positive influence on acid etched dentin. The APF_nHAp/MWCNTO-GO composite promoted a level of demineralization similar to the control group. Clearly, the MWCNTO-GO was able to obtain a more stable surface, protecting the demineralization of dentine, due to their feasibility to recognize the carbonate and phosphate group [26]. It is know that the biomineralization is a natural process for formation of teeth and whether controlled may enhance the remineralization process. We already showed that MWCNTO-GO nanoparticles were efficient to improve the in vitro biomineralization process [27]. For this, our produced nanobiomaterials may be a candidate to protect erosion of teeth.

Herein a simple method was developed to cover and protect the dentin from de- and remineralization cycles using nHAp, MWCNTO-GO and nHAp/MWCNTO-GO composite. FT-Raman spectroscopy, scanning and transmission electron microscopy (SEM/TEM) were used to analyse the phosphate and carbonate contents before and after different treatments. Clearly, dentin coated with nHAp, MWCNTO-GO and nHAp/MWCNTO-GO composite increased the carbonate/phosphate ratio. We identified that nHAp/MWCNTO-GO composite protected the dentin from erosion due to the formation of an acid-resistant surface film. It was evidenced by the enhancement of the carbonate/phosphate ratio bands and electrochemical impedance spectroscopy measures (EIS). As null hypothesis, we assumed that had no significant differences at the inorganic and organic components. In addition, we considered that did not occur any change of morphology of the dentine surfaces coated with the different materials tested.

2. Material and methods

2.1. Synthesis, purification and treatment of multi-walled carbon nanotube

The multi-walled carbon nanotubes (MWCNT) were prepared using chemical vapour deposition (CVD) as previously described [31]. Briefly, a mixture of camphor (85 wt%) and ferrocene was vaporized in a chamber (at 850 °C and atmospheric pressure). The MWCNT production was performed in few minutes. The raw-MWCNT were subject to conventional sonication for 5 h in 10 M of HCl, and then they were washed many times in DI water and dried to remove Fe nanoparticles. The MWCNT functionalization through incorporation of oxygen-containing groups was carried out in a pulsed-direct current plasma reactor. Briefly, the process was carried out using an oxygen flow rate of 1 sccm, at a pressure of 300 mTorr, −700 V, with pulse frequency of 20 kHz for 10 min. The oxygen-functionalized samples were named as MWCNTO-GO.

2.2. Synthesis of nHAp/MWCNTO-GO

Briefly, MWCNTO-GO powder (0.004 g) was diluted in an aqueous solution of $Ca(NO_3)_2 \cdot 4H_20$ (0.167 M) and $(NH_4)H_2PO_4 \cdot NH_4OH$ (0.1 M) ($Ca/P = 1.67$). Thereafter, the precipitations were subjected to ultrasound for 30 min (Ultrasonic Processor 500 W; 20 kHz; 13 mm probe; model: VCX-500, SONICS), maintaining the pH above 10 up to 30 min. The resulting suspensions were left to age for 120 h and then filtered, extensively washed and dried at 60 °C for 4 h. More details about this process follow in elsewhere [32].

2.3. Sample preparation and experimental design

The Ethics Committee of the Universidade do Vale do Paraíba approved this study (CEUA protocol n ° 04/2012). Twenty-one non-damaged bovine incisors were removed from bovine jaws. Later, the remaining soft tissue was removed from the tooth surfaces with a