An Alternative Pre-Treatment Sterilization Solution Synthesis Utilizing Boric Acid Doped Graphene Oxide

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ABSTRACT: *In medicine and dentistry, the sterilization process is mandatory for the benefit of both patients and healthcare personnel. Tools used in treatment interventions should lack microorganisms and must be sterilized after every treatment intervention. Before sterilization, chemical cleaning solutions are used in washing machines in order to remove not only biological debris but also composite and adhesive materials remaining on stainless steel hand instruments. Before sterilization by use of an autoclave, additional mechanical cleaning is performed for still remaining debris according to the performance of the chemical procedure. Boron and boron products are found in the composition of many medical products such as lens solutions, creams, mouth rinse, and eye drops due to their antimicrobial properties. Graphene oxide is a water-dispersible graphene counterpart and with this fundamental property, it is often preferred in the formation of water-based hybrid materials. In this study, a boron-doped graphene oxide sterilization pre-treatment solution was produced in order to clean hand instruments used in medicine and dentistry, and performance is compared with market products. Graphene Oxide (GO) was synthesized by the Modified Improved Hummers method without using sodium nitrate. Boron atoms were successfully doped into the GO structure with an atomic percentage of 2 (% w/w) by using boric acid (B) as a precursor. Surface structures and elemental analysis of the synthesized GO and Boron-doped GO (BGO) were made by X-Ray Diffraction (XRD) and Scanning Electron Microscope (SEM). BGO bond was observed by Fourier-Transform InfraRed (FT-IR) analysis. The efficiency of the BGO solution was tested and compared using cleaning process monitoring indicators.*

KEYWORDS: *Boric acid; Graphene oxide; Sterilization pretreatment solution; Hand instrument.*

INTRODUCTION

In order to avoid cross infections, hand instruments and equipment used in the health field should be cleaned and sterilized. Cleaning is the first and most essential step of the sterilization cycle like removing dirt, tissue residues, foreign materials and particles from the surface of instruments. The sterilization efficiency is affected by many factors such as microorganism type, concentration, and structural character (such as biofilm) as well as

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the power of sterilization applied, contact time, and environmental properties (temperature, pH, etc.). Materials used for cleaning and sterilization, besides demonstrating a high efficiency, should be compatible with the instruments, safe for the user, patient, and the environment, easy features such as rinsing, not be adversely affected by environmental conditions, practical and economical [1-3].

Boron is an important element for humans, which is found in the form of compounds in nature and used in many different fields such as defense, agriculture, pharmaceutical industry, and energy [4]. Boron plays an important role in many processes, including bone growth [5,6], the immune system [7] an antioxidant defense system, and wound healing [8]. Boric acid (H_3BO_3) is obtained from boron with a bleaching process and is an effective cleaning agent that is used as a germicidal [9]. In the medical field, it is frequently studied to remove biofilm (plaque) from the inner and outer surfaces of dental equipment [10-13]. H_3BO_3 is also used for eye infections as an optical rinse, mouth rinse, sterilization processes, and antibacterial pomades, as it is known as antiseptic and anti-infective in the medical and cosmetic industry [14-16].

Zan et al. used different concentrations of boric acid to determine their antibacterial effect on *E. Faecalis* biofilms in human root canals. They found that the most effective bactericidal conditions in the disinfection of root canals are 6% boric acid concentration with long irrigation times [17].

Graphene Oxide (GO) is the oxidized derivative of graphene and its obtained by oxidation and exfoliation of graphite. The main reason for GO's excellent solubility in aqueous solutions is that it contains oxygen-rich hydrophilic groups such as hydroxyl, epoxy, and carboxyl. The extraordinary surface activity caused by many functional groups on the surface of GO allows its easy functionalization [18-20].

After the use of carbon-based materials in various fields such as electronics, energy, and materials science, studies have been intensified in the fields of medicine and dentistry to improve the physical, mechanical, and biological properties of biomaterials [21-23].

Palsan et al. used activated carbon, derived from coconut shells to synthesize charcoal-based cosmetics and personal care products. Apart from being a natural exfoliant, they observed that it can act as an effective adsorbent that removes excess oil, dirt, and other contaminants [24].

In this study, H_3BO_3 doped GO (BGO) sterilization pre-treatment solution was synthesized to be used in the cleaning step of the dental hand instrument before sterilization. GO was synthesized by the Modified Improved Hummers method without using toxic sodium nitrate. The hand instrument cleaned with the BGO solution was compared with the counterpart solution used in dentistry. The effectiveness of the synthesized sterilization pre-treatment solution was carried out using washer-disinfector indicator and cleaning monitor test sheets indicators in accordance with the TS EN ISO 15883-1 standard [25]. To the best of our knowledge, there is no obvious report regarding the fabrication of BGO sterilization pretreatment solution for hand instruments.

EXPERIMENTAL SECTION

Graphite flakes (99% carbon basis, 325 mesh), potassium permanganate ($KMnO_4$, 99%), sulfuric acid (H_2SO_4, 98%), hydrochloric acid (HCI, 37%), hydrogen peroxide (H_2O_2, 30%), boric acid (H_3BO_3, ≥99,5%) were all bought from Sigma Aldrich.

Preparation of GO

Graphene oxide was synthesized using a new method called modified improved Hummers which we mentioned in our previous study [26]. Briefly, graphite and H_2SO_4 were mixed at temperatures below 5 °C. $KMnO_4$ was gradually added with stirring. Deionized (DI) water was added and mixing was continued. Then, H_2O_2 and HCl were added to the mixture respectively and centrifuged at 7000 rpm for 40 minutes and at 9000 rpm for 10 minutes.

Preparation of H_3BO_3 Doped GO (BGO) Pre-Sterilization Solution

The experimental part consists of four stages: optimization of H_3BO_3 concentration, measurement of the washing efficiency of the optimized BGO solution with test indicators, and pre-sterilization studies of contaminated hand instruments in BGO solutions under the same conditions. In order to optimize the amount of H_3BO_3 in BGO pre-sterilization solution different concentrations (2% and 6% w/w) of H_3BO_3 solutions were prepared and cleaning efficiency was measured by using the washer

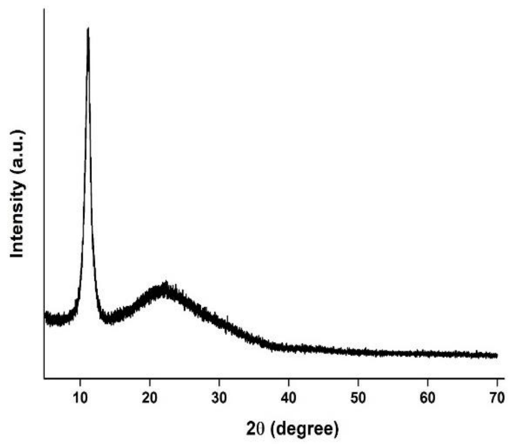

Fig. 1: XRD patterns of GO.

disinfector cleaning efficiency. Three different groups were formed;

Control group: 0.5% (w/w) concentration of cleaning solution used in Dentistry available in the market.

Group 1: 1 g of GO was added to 100 mL of distilled water and dispersed for 2 hours. Then, 2 g of boric acid was added and sonicated in an ultrasonic bath for 1 hour.

Group 2: 1 g of GO was added to 100 mL of distilled water and dispersed for 2 hours. Then, 6 g of boric acid was added and sonicated in an ultrasonic bath for 1 hour.

The solutions were taken to the magnetic stirrer and gradually increased to 90 ˚C. Washer disinfector cleaning efficiency indicators made of stainless steel material coated with agar were dipped into the prepared solutions and their efficiency at different temperatures was tested.

After optimizing H_3BO_3 concentration in BGO the pre-sterilization solution, cleaning monitör test sheets indicators were dipped into the prepared solutions and their efficiency at different temperatures was tested.

RESULTS AND DISCUSSION

Characterization of GO

XRD spectrum for GO (Fig. 1) indicates graphite is oxidized. Because of the intercalation of functional groups between graphite layers interlayer space increased to 0.81 nm which belongs to GO located at about 2ϴ = 10.5 nm [27].

Fig. 2 shows the morphological changes of graphite during the oxidation to GO. As can be seen in EDS analyses, functional groups are added between layers as a result of the oxidation of graphite from the layered GO morphology (Fig. 2 a-b) [28].

Thermogravimetric analysis (TGA) of GO (Fig. 3) showed the minimal weight loss (~5%) before 100 °C corresponds to the loss of water molecules. The major weight loss (~30%) in the range of 150–280 °C and weaker mass loss (~10%) in the range of 280–600 °C are attributed to the oxygenated functional groups [29].

Optimization results of BGO pre-sterilization solution

Fig. 4 shows the agar-coated washer-disinfector indicator results showing the cleaning efficiency of the BGO solution containing 2% and 6% H_3BO_3 at different temperatures.

It was observed that the agar coating was removed from the indicator in certain areas in 2% BGO, while the agar remained still on stainless steel material with 6% BGO. Also, pH value of the solution was measured 4.59 and 3.68 respectively. Acidic solutions may adversely affect the surface of hand instruments.

As a result of the pH value of the 6% boric acid solution being more acidic than the 2% boric acid solution and the cleaning efficiency of 2% BGO being more effective than 6% BGO, it was decided to optimize the boric acid amount to 2 g.

Characterization results of 2% BGO pre-sterilization solution

Fig. 5 shows the XRD spectrum of the H_3BO_3 and 2% H_3BO_3 doped GO (BGO) solution. The severe diffraction peak seen at 27.15° in the spectrum and low-intensity peaks at about 14° and 57° belong to the H_3BO_3 [30]. It has been seen that the diffraction peak at 2θ = 10.91º observed in the spectrum attributed to GO that intensity has decreased in boric acid additive (2%) (Fig. 5b).

Fig. 6 shows FT-IR spectrum of H_3BO_3 and BGO solution. The presence of a peak at 3219 cm^{-1} shows the stretching vibrations of the O-H bond. Strong intermolecular hydrogen bonds have been formed between the hydroxyl groups of H_3BO_3 and GO structure. The peaks at 581 and 671 cm^{-1} belong to O-B-O ring stress. The peak at 1162 cm^{-1} is attributed due to the B-C stretching vibrations, it confirms the boron doping in the carbon network. The band seen in the spectrum at 1637 cm^{-1} shows the –C = C– bond of GO These bands observed in the FT-IR spectrum of BGO confirm the incorporation of H_3BO_3 in the GO layers [31] (Fig. 6b).

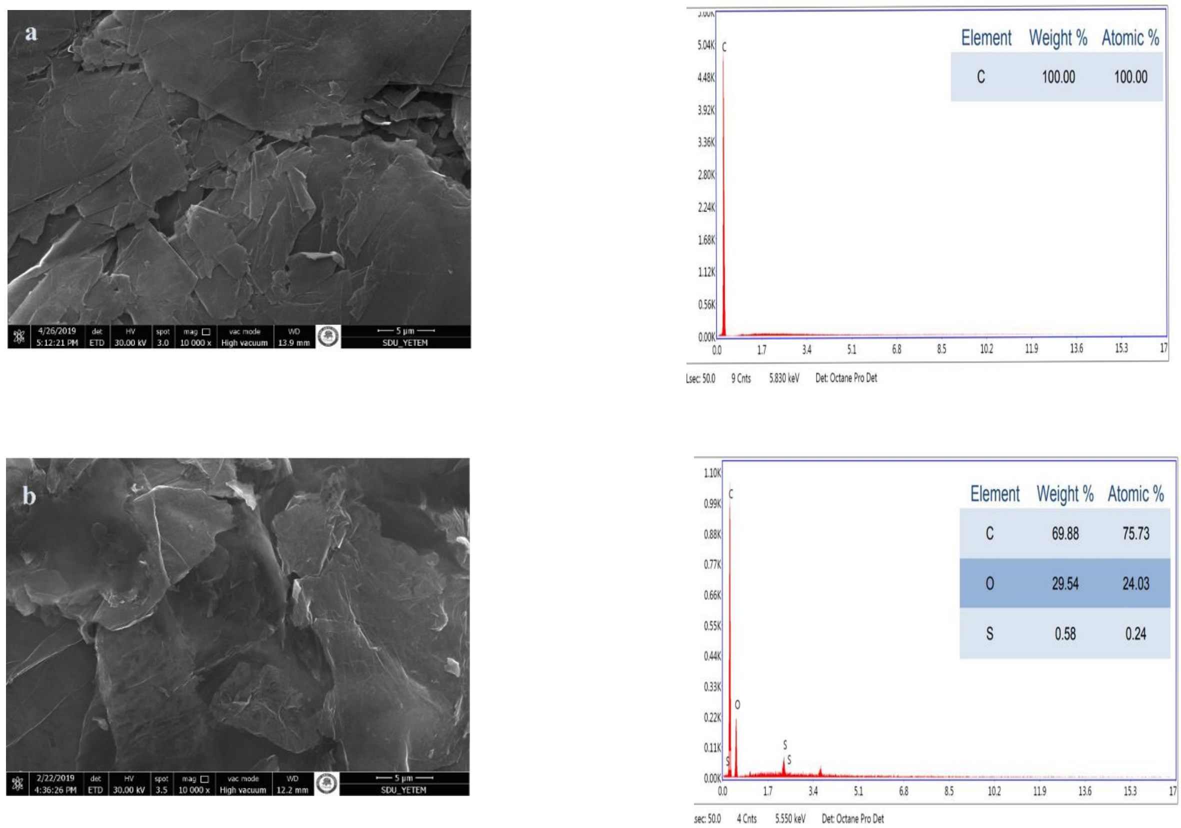

Fig. 2: SEM/EDX image of graphite (a) and GO (b).

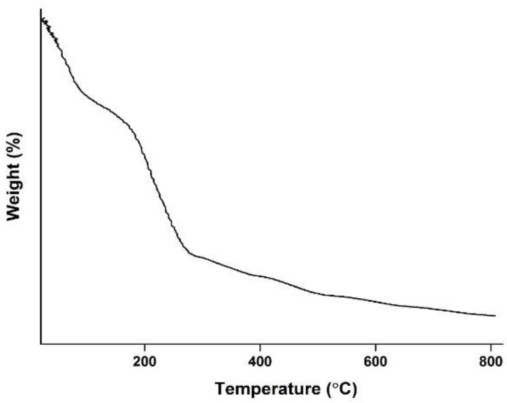

Fig. 3: TGA curves of GO.

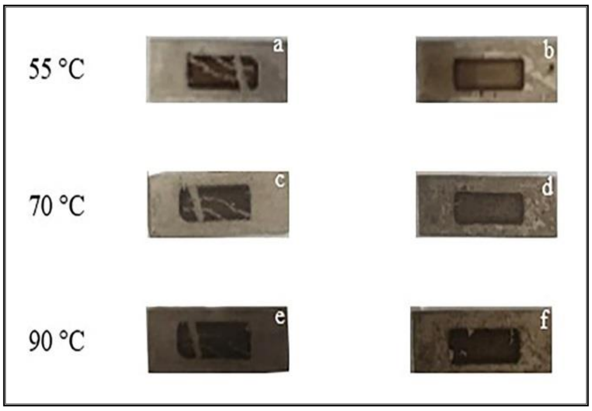

Fig. 4: Images of the washer-disinfector cleaning indicators 2% Boric Acid (a, c, e) and 6% Boric Acid (b, d, f) BGO solution at different temperatures (55 °C, 70 °C, 90 °C).

The SEM image of H_3BO_3 consists of granular structures of different sizes (Fig. 7). In the BGO structure, it is seen that the granular and irregular H_3BO_3 crystals settle between the layers of GO and form a homogeneous structure (Fig. 7b).

Fig. 8 shows the cleaning monitor test sheet indicators results which dipped in 2% BGO, 2% H_3BO_3 solution, and also added in control solution used in dentistry available in the market (0.5%) at different temperatures.

(a) (b)

Fig. 5: XRD patterns of (a) H_3BO_3 and (b) BGO solution.

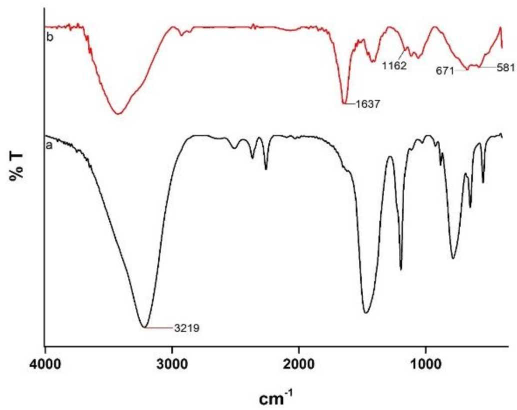

Fig. 6: FTIR spectra of (a) H_3BO_3 and (b) BGO solution.

Efficiency results of 2% H_3BO_3 doped GO Pre-sterilization solution

Fig. 8 demonstrates, at low temperatures, the washing efficiency of the 2% H_3BO_3 doped GO solution is similar to the 2% aqueous H_3BO_3 solution, with increasing temperature the washing efficiency of 2% BGO solution is better than aqueous boric acid.

CONCLUSIONS

Boric acid has been investigated in various studies in the field of medicine. However, the effects of cleaning efficiency on hand instruments have never been investigated by clinicians. Cleaning is the first and most important step before any disinfection or sterilization process is carried out. Our study emphasized that 2% BGO solution may be an alternative to the cleaning solution used for hand instrument cleaning in dentistry.

Current commercial chemical alternatives for cleansing and sterilization contain chlorine and ammonia. More importantly, such substances have been clinically proven to be highly hazardous to live creatures, especially sterilization staff. Therefore, it is extremely important for the preservation of mankind thus alternative natural substances be utilized as soon as possible. The process includes a unique natural sterilization solution including boric acid and graphene oxide combination which is a unique blend. GO is used to be an effective surfactant due to its amphiphilic properties and high specific surface area and also forms a protective layer on steel hand tools with its lubricating feature.

We performed the GO synthesis by using a sufficient amount of $KMnO_4$, without using $NaNO_3$ and extra acid by using the Modified Improved Hummers method. In order to optimize the H_3BO_3 concentration in the pre-sterilization solution agar coating test solution was used. The concentration of H_3BO_3 optimization in BGO pre-sterilization results showed that 2% BGO solutions are more effective than 6% BGO in removing agar from the surface. Due to the pH value of 6% boric acid solution being more acidic than 2% boric acid, considering that it will create a more abrasive effect on the surface in repeated cleaning of hand instruments, the concentration of H_3BO_3 in BGO is set to 2%. The crystalline structure of BGO was confirmed by XRD. FT-IR confirmed the presence of B-C and B-O bonds and SEM/EDS analysis evidenced the H_3BO_3 doping in BGO.

Efficiency results of 2% H_3BO_3 and 2% H_3BO_3 doped GO pre-sterilization solution were measured by using cleaning monitor test sheet indicators. The results showed

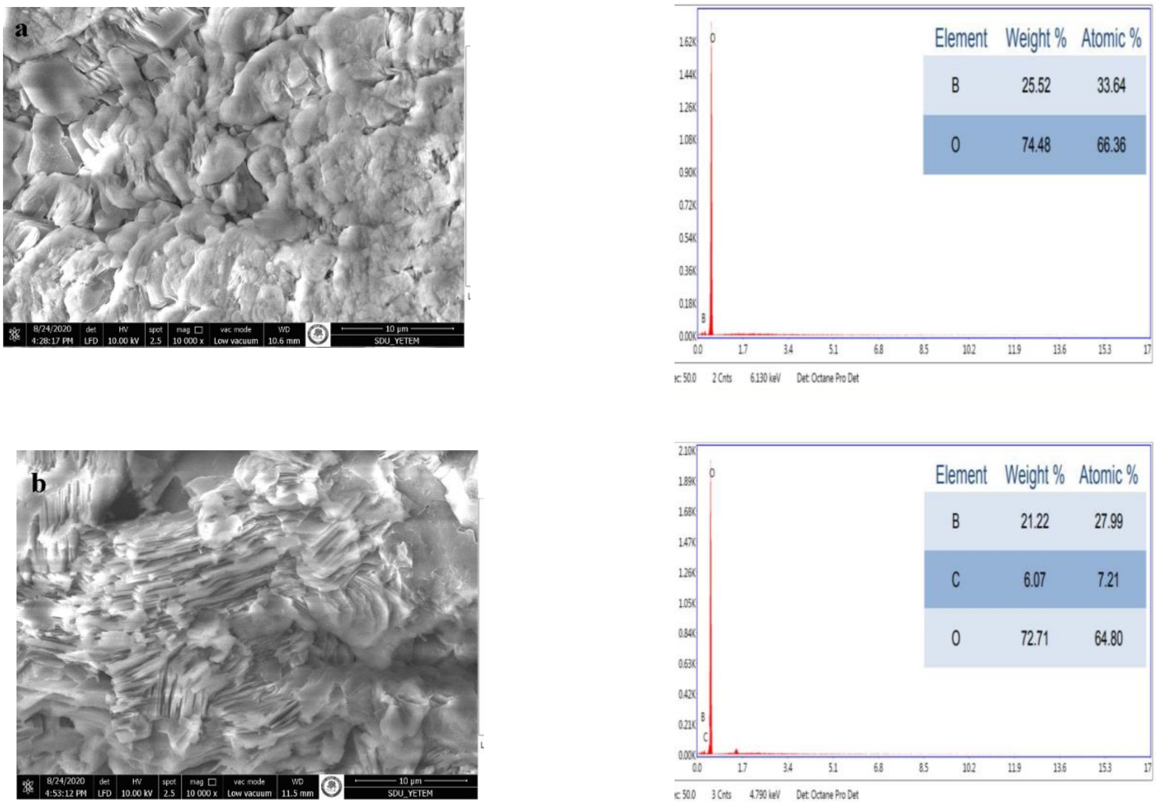

Fig. 7: SEM / EDS results of a) H_3BO_3 and b) BGO solution.

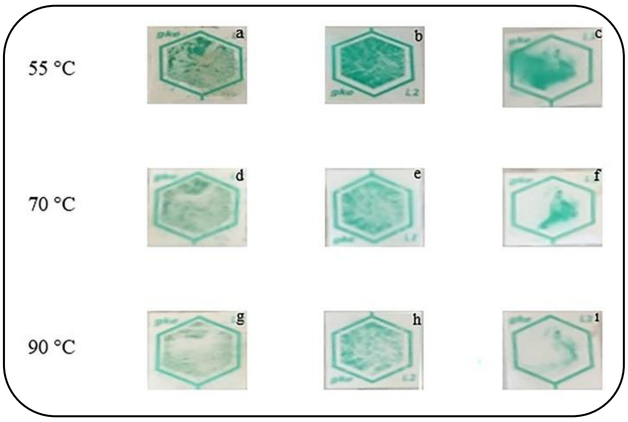

Fig. 8: Images of the cleaning monitor test sheet indicators 2% BGO (a, d, g), 2% H_3BO_3 (b,e,h), and control solution used in dentistry (c, f, ı) at different temperatures (55 °C, 70 °C, 90 °C).

that; at low temperatures, the washing efficiency of the 2% BGO solution is similar to the 2% H_3BO_3 solution, with increasing temperature the washing efficiency of 2% BGO solution is found to be more effective. GO acts as a surfactant, reducing the surface tension of the solution at the interfaces, allowing better cleaning of hand instruments. The pre-sterilization solutions evaporate due to heat media during the cleaning of instruments. Hazardous effects may occur due to inhalation. Natural minerals like H_3BO_3 may diminish the hazardous effects. Further investigation may demonstrate the benefit of these natural minerals in these fields.

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