A BIOACTIVE HYBRID NANOSTRUCTURE FOR ORTHODONTIC APPLICATIONS

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ABSTRACT

The bioactive hydroxyapatite (HAP) ceramic/polymer composites have received an increased interest for endodontic/orthopaedic applications in recent years. In this work, an antimicrobial DGEBA thermosetting epoxy composite was prepared from diglycidyl ether of bisphenol-A (DGEBA), nanostructured HAP and eugenol for use in endodontic or orthopedic areas. The purpose of this work was to study in vitro the biological properties of DGEBA/HAP composites. HAP was synthesized by using $Ca(OH)_2$ and H_3PO_4 as precursors in a classical wet chemical method. The synthesized HAP powder and DGEBA/HAP composite were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM) techniques, respectively. The DGEBA/HAP exhibit no significantly citotoxic response. The antimicrobial activities of the DGEBA/HAP were assessed against Staphylococcus aureus and Escherichia coli. The composite DGEBA/HAP showed better antimicrobial activity against E. Coli than against S. aureus.

Keywords: DGEBA, Cytotoxicity; 1-(2-(aminoethyl)piperazine), Hydroxyapatite.

INTRODUCTION

Hydroxyapatite (HAP) is a calcium phosphate ceramics has been widely used in medicine for the replacement of the bone tissue [1]. The HAP has similar composition with natural bone and biocompatibility properties that propitiates a easily bonding to bone [2].

HAP ceramics may be combined with polymers that are generally bioinert to provide the composite bioactivity. The composite implants thus are able to form chemical bond with the host tissue, and the fixation of implants is accelerated. Moreover, the elastic modulus of the composite can be adjusted to approach that of the human bone by altering the content of ceramic. It is known that the match of elastic modulus between implants and the human bone favors the avoidance of ‘‘stress shielding’’ and the sequent bone absorption, which is often caused by implants with high elastic modulus [3].

In orthopaedics applications, composite materials have to possess good biocompatible harmony with human tissue and excellent mechanical property under body fluid. At this sense, HAP is commonly combined with bioinert polymers to provide the composite bioactivity. The composite implants thus are able to form chemical bond with the host tissue, and the fixation of implants is accelerated. Moreover, the elastic modulus of the composite can be adjusted to approach that of the human bone by altering the content of ceramic.

The main objective of this work was to develop a biologically active hybrid epoxy network based on diglycidyl ether of bisphenol-A (DGEBA) and nanostructured HAP containing eugenol for use in endodontic or orthopedic areas. Eugenol (4-allyl-2-methoxy-phenol) is an essential oil of cloves with anesthetic and antimicrobial properties. The experiments are focused on the DGEBA/HAP composite preparation and biological response of mammalian and microbial cells to the composite DGEBA/HAP.

EXPERIMENTAL PROCEDURE

2.1. Nanopowder HAP synthesis

HAP was synthesized by the classical wet chemical method using $Ca(OH)_2$ and H_3PO_4 as precursors. The resulting HAP was centrifuged, washed thoroughly with double distilled water and dried in air oven at 90°C. The synthesized HAP powder was then sintered at 900 ^oC for 8 hours and characterized by X-ray powder diffraction technique (Rigaku D/MAX 2100-PC, CuK_α:1.5405 A, 40 kV, 20 mA).

2.2. Bioactive Composite preparation

The polymer material used in this work was epoxy resin diglycidylether of bisphenol A (DGEBA, Down) and 1-(2-aminoethyl)piperazine (AEP, Sigma-Aldrich) was used as a hardener. The ratio of epoxy to hardener was kept constant at 100/20 according to the manufacturer's data sheets. HAP powder, with silane treatment and eugenol at 2% (m/m) was added into the stirred epoxy at the HAP/epoxy weight ratio of 40% (m/m). The mixtures were then heated at 130 ^oC for 1 h and then cast into the Teflon® molds.

2.3. Cytotoxicity properties

The composite DGEBA/HAP was tested for cytotoxicity by using a ROS 17/2.8 rat osteoblast-like cell line [4]. ROS 17/2.8 osteoblast-like cells originate from rat bone sarcoma and have been reported to contain a large proportion of proliferative, phenotypically immature cells that closely resemble progenitor osteoblast cells [5]. Cytotoxicity of the composite was assessed after the initial 72-hour setting period (ie, week 0) and for five succeeding weeks, in according to the method reported by Azar et al. [6]. The surface area-to-volume ratio of the specimen to medium was approximately 150 mm^2/mL (within International Organization for Standardization (ISO) 10993 specifications) [7]. The cellular mitochondrial activity was determined by estimating their succinate dehydrogenase (SDH) activity using the 3-(4,5-dimethylthiazole- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [8].

Between tests, the specimens were aseptically removed and rinsed twice with sterile SBF (136.8 mmol/L NaCl, 3.0 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 1.5 mmol/L $MgCl_2$, 0.5 mmol/L Na_2SO_4, 4.2 mmol/L $NaHCO_3$ and 1.0 mmol/L K_2HPO_4 in deionized water, buffered to pH 7.4 with 0.1 mol/L Tris and 0.1 mol/L HCl). Each specimen was then immersed for 4 days in 10 mL SBF before securing in a new cell-plated well and incubated for another 3 days at 37°C and 5% CO_2 atmosphere before the next assay cycle. Cytotoxicity responses were qualitatively rated as severe (<30%), moderate (30%-60%), slight (60%–90%), or noncytotoxic (>90%) relative to the SDH activity of the Teflon controls as well as analyzed quantitatively.

2.4. In vitro antimicrobial properties

The *in vitro* antimicrobial activities against Escherichia coli and Staphylococcus aureus were determined on powdered samples of DGEBA/HAP composite by the cut plug method on agar [9].

RESULTS AND DISCUSSION

Figure 1-A shows the X-ray pattern of the spray dryer prepared HAP. The XRD pattern shows sharp lines indicating the crystalline nature of the sintered samples. The strongest peaks in the XRD pattern were characteristic of pure HAP and closely matched with the JCPDS file No. 09-432 of calcium hydroxyapatite. The size of the crystallites responsible for the Bragg reflection of the (002) and (003) planes determined using Sherrer relationship was found to be approximately 635 nm and 687 nm, respectively. In (Figure 1-B), the intensity of the particle is plotted as a function of the diameter of the particle size, from which the mean particle size of spray dryer prepared HAP is approximately 600 nm with narrow agglomerates distribution.

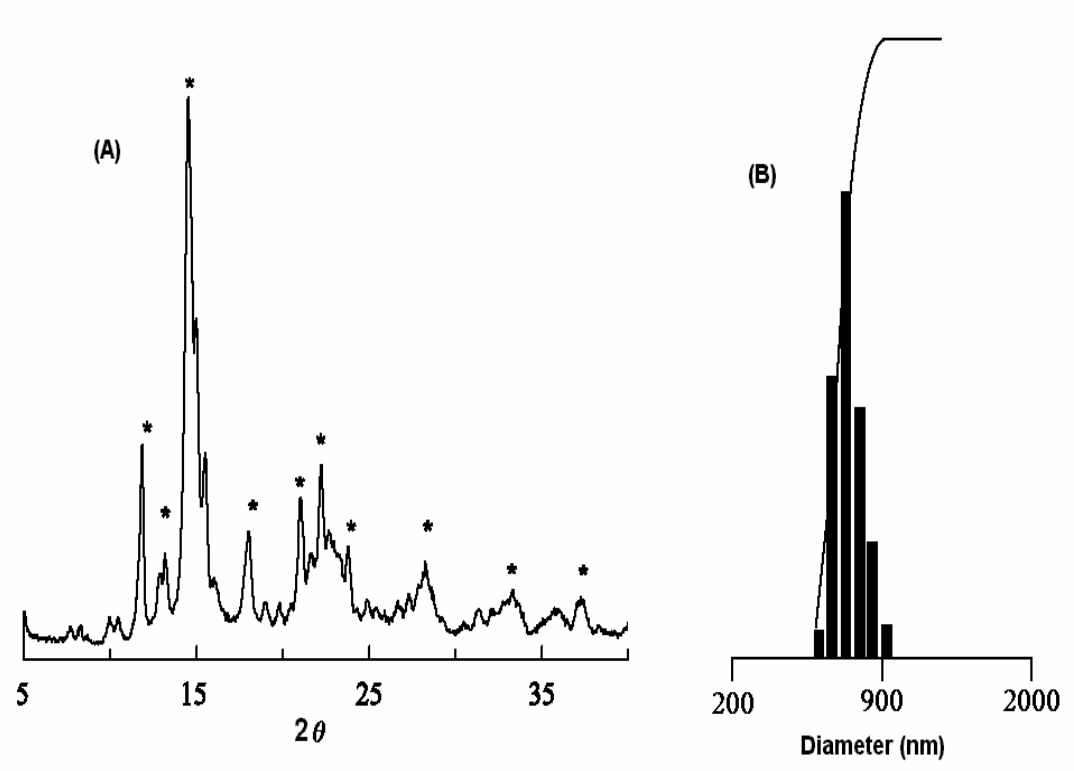

Figure 1. X-ray pattern of spray dryer prepared hydroxyapatite (A) and particle size distribution of HAP (B).

The distribution of the HAP particles in the epoxy matrix can be seen by SEM micrographs in Figure 2. A Typical dispersed particle morphology with the epoxy forming a continuous phase can be observed. These trends indicate that the addition of HAP increases the links between epoxy networks and modified the molecular structure stability within epoxy matrix,

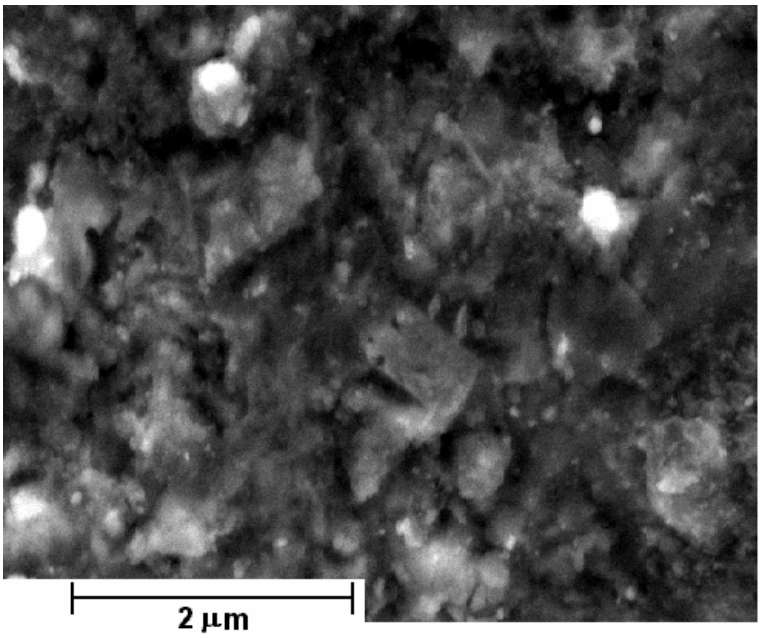

Figure 2. Micrograph of the fractured surface of the composite HAP/Epoxy .

The evaluation of DGEBA/HAP composites after repeated immersion in SBF simulates the differential changes in cytotoxicity levels over time after diffusion of eugenol from the material into the surrounding environment. Due to the presence of eugenol the DGEBA/HAP a cytotoxic response was initially observed. However, this response showed different degrees of toxicity reduction in function of time. The results of the MTT assay over the six time periods are graphically represented in Figure 3. The DGEBA/HAP was moderately cytotoxic than negative control (Teflon) after the first cycle of SBF immersion (week 1), became only mildly cytotoxic at weeks 2 and 3, and was noncytotoxic after week 3.

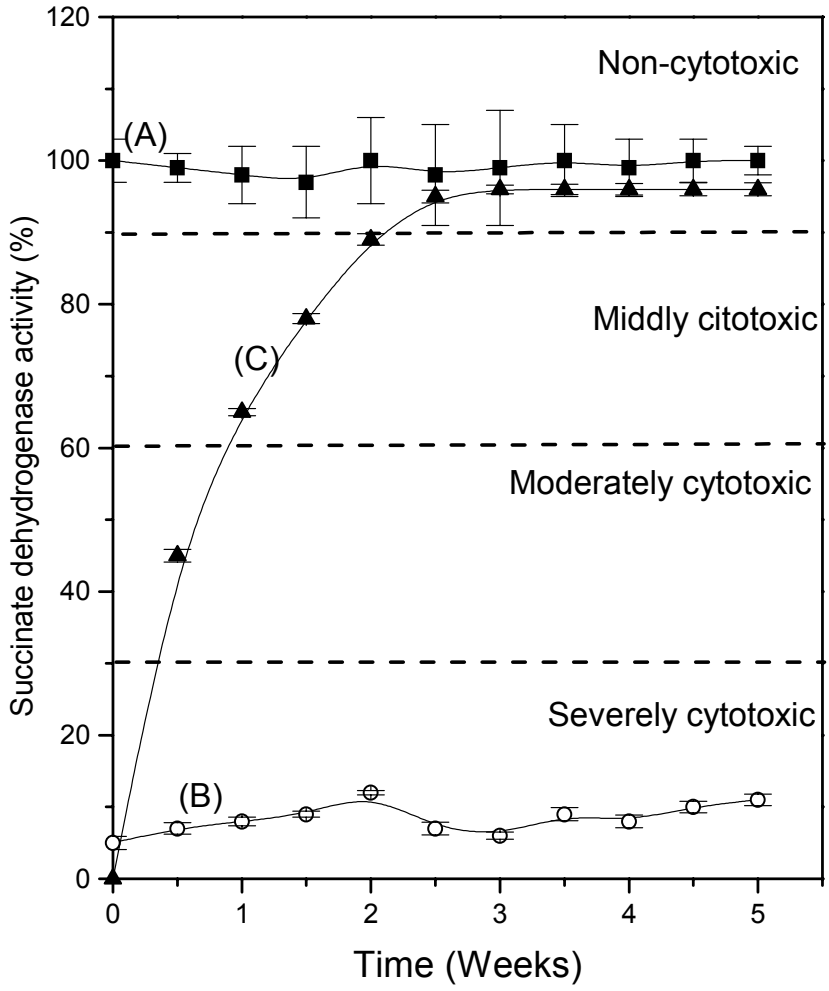

Figure 3. Cytotoxicity expressed in function of succinate dehydrogenase with time after the repeatedly immersion of DGEBA/HAP composite in simulated body fluid (SBF). Values were expressed relatively to the Teflon (A) (negative control, 100%) and are classified as severe (<30%), moderate (30-60%), slight (60-90%) or noncytotoxic (>90%). Poly(methyl methacrylate) (PMMA) (B) was used as positive control. The curve (C) represents the DGEBA/HAP composite.

The antibacterial activities of DGEBA/HAP against *E. coli* and *S. aureus* were explored by the cut plug method. The ability of the prepared composite to inhibit the growth of the tested microorganisms in solid media is shown in Figure 4. The composite DGEBA/HAP inhibited the growth of the tested bacteria with an increasing inhibitory effect in the order *S. aureus* < *E. coli*.

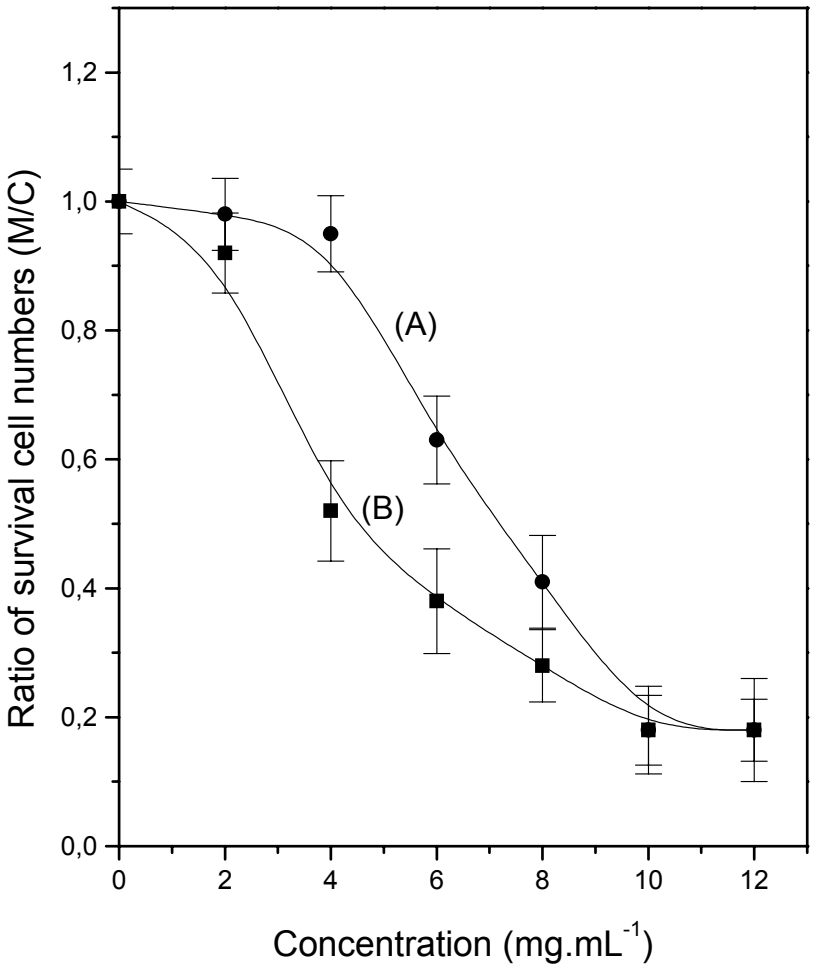

Figure 4. Growth inhibition of different concentration of the composite DGEBA/HAP. Inoculation: $5x10^4$ cells.mL^{-1}, E. coli (A) and S. aureus (B), respectively.

M/C represents the ratio between the surviving cell number in the medium containing the polymer (M) to that without the polymer (C).

CONCLUSIONS

The cytotoxic response and antimicrobial properties demonstrate that the DGEBA/HAP composite should be interesting for endodonty/orthopaedic applications. However, it must also be emphasized that although the results of this study have been considered clinically relevant, the results of this study should also be confirmed by *in vivo* tests. Collectively, *in vitro* and *in vivo* data should provide the best assessment of the overall biocompatibility of this new bioactive DGEBA/HAP composite.

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