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Research Article

Composite Scaffolds Based on Silver Nanoparticles for Biomedical Applications

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This paper presents the synthesis, characterisation, and *in vitro* testing of homogenous and heterogeneous materials containing silver nanoparticles (nanoAg). Three types of antiseptic materials based on collagen (COLL), hydroxyapatite (HA), and collagen/hydroxyapatite (COLL/HA) composite materials were obtained. The synthesis of silver nanoparticles was realized by chemical reaction as well as plasma sputtering deposition. The use of chemical reduction allows the synthesis of homogenous materials while the plasma sputtering deposition can be easily used for the synthesis of homogeneous and heterogeneous support. Based on the *in vitro* assays clear antiseptic activity against *Escherichia coli* was relieved even at low content of nanoAg (10 ppm).

1. Introduction

Silver nanoparticles are of increasing interest for scientists due to their very good biological properties and limited side effects. Used since 1000 BC, silver proved its biocidal activity for a wide number of bacteria and recently it was also known to be active in the treatment of cancer [1]. As a consequence of silver multifunctionality (antiseptic [2], antitumoral [3, 4], and IR-sensitizing agent [5]) the number of published papers dealing with silver nanoparticles increases exponentially yearly, at present over 10 000 papers [6] being indexed on SCOPUS database. The distribution of the published papers, per year, can be visualized in Figure 1.

Silver nanoparticles are widely used for their biological activity as colloidal suspension [7–10] or in association with other materials [11–14]. Silver nanoparticles were associated with different components such as manganite [15], carbon nanotubes [16], hydroxyapatite [17, 18], and chitosan [19]. Mostly, silver nanoparticles play antibacterial [17] and antitumoral [3] role.

Collagen is widely used for many biomedical applications [20–22]. Adding of calcium phosphates to collagen resulted in composite materials which proved to be remarkable bone grafts [23–31]. A common shortcoming of these grafts is related to the high incidence of infection [32]. Most surgical interventions involve antibiotic administration [33] which could be avoided by using silver nanoparticles [10, 34, 35].

In the case of bone cancer, many times surgical resection is necessary. In order to treat bone cancer the multifunctional COLL/HA-Fe_3O_4 composite materials were proposed. The composite support assures a faster healing of the bone defect while magnetite can assure the necessary hyperthermia to

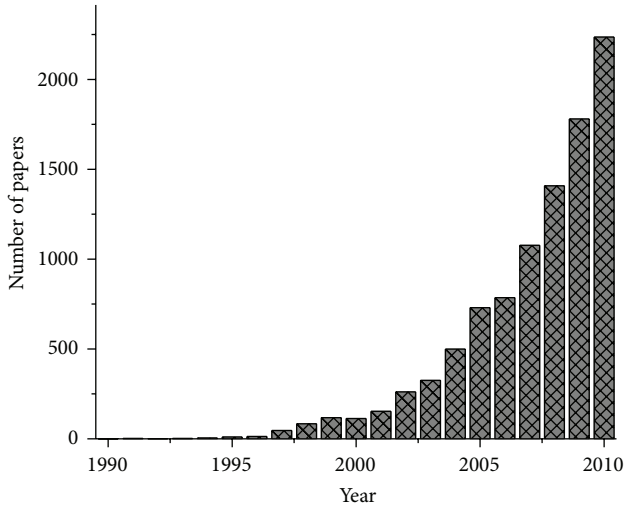

Figure 1: The evolution of the number of papers dealing with "silver nanoparticles."

induce tumoral cells death. It is also important to mention that magnetite can be activated, any time, by applying a proper, external electromagnetic field [36].

The current paper presents the synthesis and characterisation of new antiseptic materials based on silver nanoparticles embedded in collagen, hydroxyapatite, or collagen/hydroxyapatite composite material. Silver nanoparticles were synthesised by two different methods: chemical reduction and plasma sputtering. The obtained materials are intended to be used as bone grafts.

2. Materials and Methods

Type I fibrillar collagen (C) gel having about 300000 Da, concentration of 1.6% (w/w), and pH 7.4 was extracted from calf hide as previously described [20, 25].

Antiseptic collagen sponge was obtained by chemical reduction of Ag^+ in the presence of glucose and by plasma sputtering of Ag nanoparticles onto the collagen sponge. In both cases collagen sponge was obtained by cross-linking of the collagen gel with glutaraldehyde. For cross-linking 0.5% glutaraldehyde, reported to dry collagen, was used.

The reduction of Ag^+ occurs in the presence of glucose which undergoes an oxidation process as presented in the following reaction:

$$Ag^+ + C_6H_{12}O_6\ (\text{glucose}) \longrightarrow Ag^0 + C_6H_{12}O_7 \qquad (1)$$

Antiseptic HA powder was also obtained by the same two methods starting from HA powder obtained by coprecipitation from $Ca(OH)_2$ and NaH_2PO_4 [23].

The antiseptic composite materials were obtained by a similar way as antiseptic collagen sponge but starting from mineralized collagen gel and COLL/HA composite sponges, respectively.

The COLL/HA composite material was synthesised as we described in our previously published papers [25, 37]. Briefly, the collagen gel (when plasma sputtering method is used) or silver containing collagen gel (when chemical method is used) was neutralized with $Ca(OH)_2$ 24 h and then the proper amount of NaH_2PO_4 was added and let for other 24 h to interact. During these steps which lead to the HA nucleation on the collagen, the pH was set at 9. The final steps consist in cross-linking followed by freeze-drying.

Plasma sputtering of silver nanoparticles was realised using a BAL-TEC SCD005 Sputter Coater with nitrogen plasma and the deposition current was 59 mA while the deposition time was set at 60 s.

The obtained materials were investigated by X-ray diffraction, IR spectroscopy, scanning electron microscopy, transmission electron microscopy, and antimicrobial activity against *Escherichia coli*.

X-ray diffraction analysis was performed using a Shimadzu XRD 6000 diffractometer at room temperature. In all the cases, Cu K_α radiation from a Cu X-ray tube was used. The samples were scanned in the Bragg angle, 2θ range of 10–70.

For IR spectroscopy (Shimadzu 8400 FTIR Spectrometer) measurements, the spectra were recorded in the wavenumber range of 400–4000 cm^{-1}, with a resolution of 2 cm^{-1}.

SEM analyses were performed on a HITACHI S2600N electron microscope on samples covered with silver layer.

The transmission electron images were obtained on finely powdered samples using a Tecnai G^2 F30 S-TWIN high resolution transmission electron microscope (HRTEM) equipped with STEM-HAADF detector, EDX, and EELS. The microscope was operated in transmission mode at 300 kV while TEM point resolution was 2 Å and line resolution was 1 Å.

The antibacterial activity was evaluated in triplicate against *Escherichia coli*. *Escherichia coli* (K 12-MG1655) were cultured in a tube containing Luria-Bertani (LB) medium [38] at 37°C (LB medium composition: peptone, 10 g/L; yeast extract 5 g/L, NaCl 5 g/L). Sterile samples were incubated for 18 hours in test tubes containing 5 mL culture of *Escherichia coli*. Culture was obtained from a volume of 100 mL sterile culture medium. The sterile medium was inoculated with 1 mL of *Escherichia coli* (1%). Once obtained 5 mL of culture was placed over the samples. Optical density was determined after 18 hours of incubation. Incubation was performed in the incubator Laboshake Gerhardt. The bacterial growth was determined by measuring optical density for the four samples and control (*Escherichia coli* culture without sample) at 600 nm using UV-VIS spectrophotometer (Jenway Spectrophotometer).

The antibacterial activities were determined by calculating the inhibition of growth using [39]

$$I\% = \frac{[(B_{18} - B_0) - (C_{18} - C_0)]}{(B_{18} - B_0)} \cdot 100, \qquad (2)$$

where I is the inhibition of growth, %, B_{18} is the blank-compensated optical density at 600 nm (OD_{600} = 3.36 of the positive control of the organism at 18 h), B_0 is the blank-compensated OD_{600} of the positive control of the organism at 0 h (OD_{600} = 0.049), C_{18} is the negative control-compensated OD_{600} of the organism in the presence of test sample at 18 h, and C_0 is the negative control-compensated OD_{600} of the organism in the presence of test sample at 0 h.

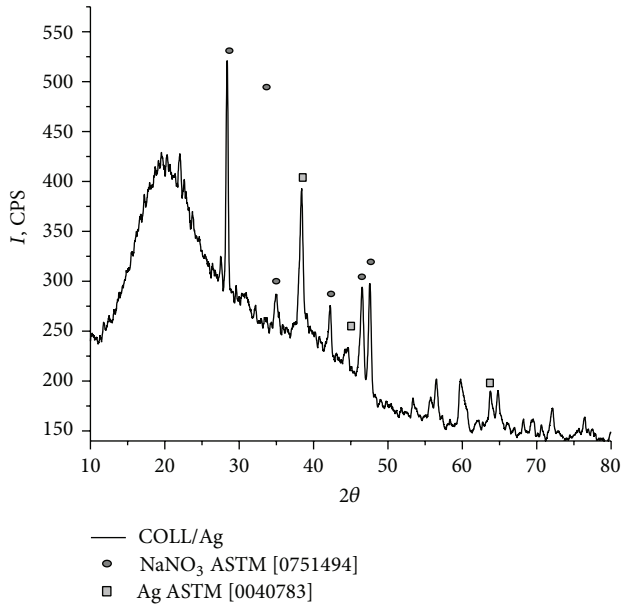

Figure 2: XRD pattern of COLL/Ag antiseptic composite materials.

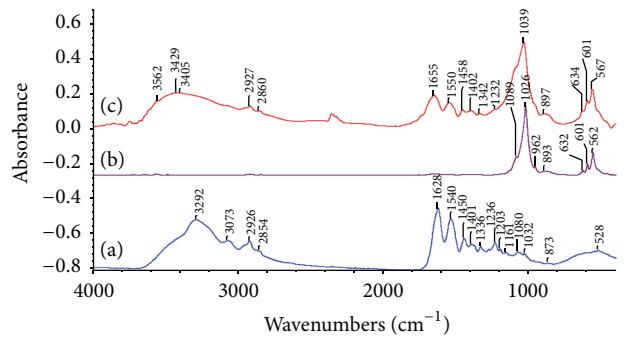

Figure 3: FTIR spectra of (a) COLL/Ag, (b) HA-Ag, and (c) COLL/HA-Ag antiseptic materials.

3. Results and Discussion

The antiseptic materials were characterized by appropriate methods.

3.1. X-Ray Diffraction. X-ray diffraction pattern was used to prove the formation of the AgNPs regardless of the synthesis method as we presented in Figure 2. Silver was identified based on the ASTM file number 0040783. Sodium nitrate was identified as a secondary crystalline phase, its presence being explained based on the collagen extraction technology.

3.2. Infrared Spectroscopy. The three FTIR spectra reveal the absorption bands of the components except the AgNPs which appear far below the lower limit of wavelength of the spectrophotometer as Figure 3 showed.

The main absorption band of HA appears as follows, a triple degenerate band associated with the O-P-O band at 560, 600, and 630 cm^{-1}; a triple degenerate band at 1030, 1090, and 1110 cm^{-1}; and a band associated with a symmetric stretch of P-O band at 960 cm^{-1}, while the main absorption bands of collagen appear at 1628 (amide I), 1540 (amide II), 1236 (amide III), 2854 (CH_2 asymmetric stretching), 2926 (CH_2 symmetric stretching), and 2957 (CH_3 symmetric stretching). The wide band from 3000 to 3600 cm^{-1} corresponds to the associated hydroxyl groups from collagen, hydroxyapatite, and water.

3.3. Scanning Electron Microscopy. The silver particles cannot be identified by SEM images because of their low content and nanosize into the collagen composites (Figure 4). At 2000 and 3,500x magnification, the COLL/HA-Ag sample presents agglomerations which can be easily assigned to the inorganic, hydroxyapatite phase [24]. This observation is also supported by the comparison with the COLL/Ag sample (Figures 4(a′)–4(c′)) where no agglomerations can be identified. Silver visualization will be possible at higher magnification using TEM or HRTEM. That is why only in the case of COLL/HA-Ag composite material agglomerates can be visualised on the collagenic matrices, these agglomerates being clearly identified on the collagenic matrix at a magnification of 2000x while in the case of COLL/Ag material no agglomerations can be identified at this magnification.

Scanning electron microscopy was also used for the characterization of HA-Ag nanopowder (Figure 5). Based on the micrographs, it can be seen that nanometric particles were obtained. The size and shape are difficult to determine based on the SEM images and consequently TEM will be further used to evaluate the size and to determine the shape of these nanoparticles.

3.4. Transmission Electron Microscopy. TEM analysis was performed on pure silver nanoparticles obtained by plasma sputtering (Figure 6), COLL/Ag sample obtained by plasma sputtering (Figure 7), and HA-Ag nanopowder obtained by HA precipitation and chemical reduction of Ag^+ (Figure 8). In the case of pure silver nanoparticles obtained by plasma sputtering nanoAg agglomerates can be identified. From the point of view of particle size distribution very small particles with 1-2 nm as well as oversized particles with about 10–20 nm diameter can be visualized. The characteristic silver bands can be identified in SAED as well as silver oxide which means that during the deposition silver is partially oxidized to silver oxide.

In the case of COLL/Ag antiseptic sample, due to the collagen harsh matrix the silver is more uniformly deposited, the particles having generally 2–4 nm diameter.

Analyzing Figure 7, it can be seen that practically the particles are independent which means that collagen matrix acts as a dispersing agent and does not allow the silver nanoparticles to form agglomerates.

In the case of HA-Ag sample both HA and Ag can be identified based on their different contrast or based on their interplanar distances. At low magnification a severe agglomeration of the silver nanoparticles (darker nanoparticles) is noticed while, at high resolution characteristic planes of HA and Ag demonstrate their presence. Comparing with the COLL/Ag sample, the HA-Ag is less homogeneous because,

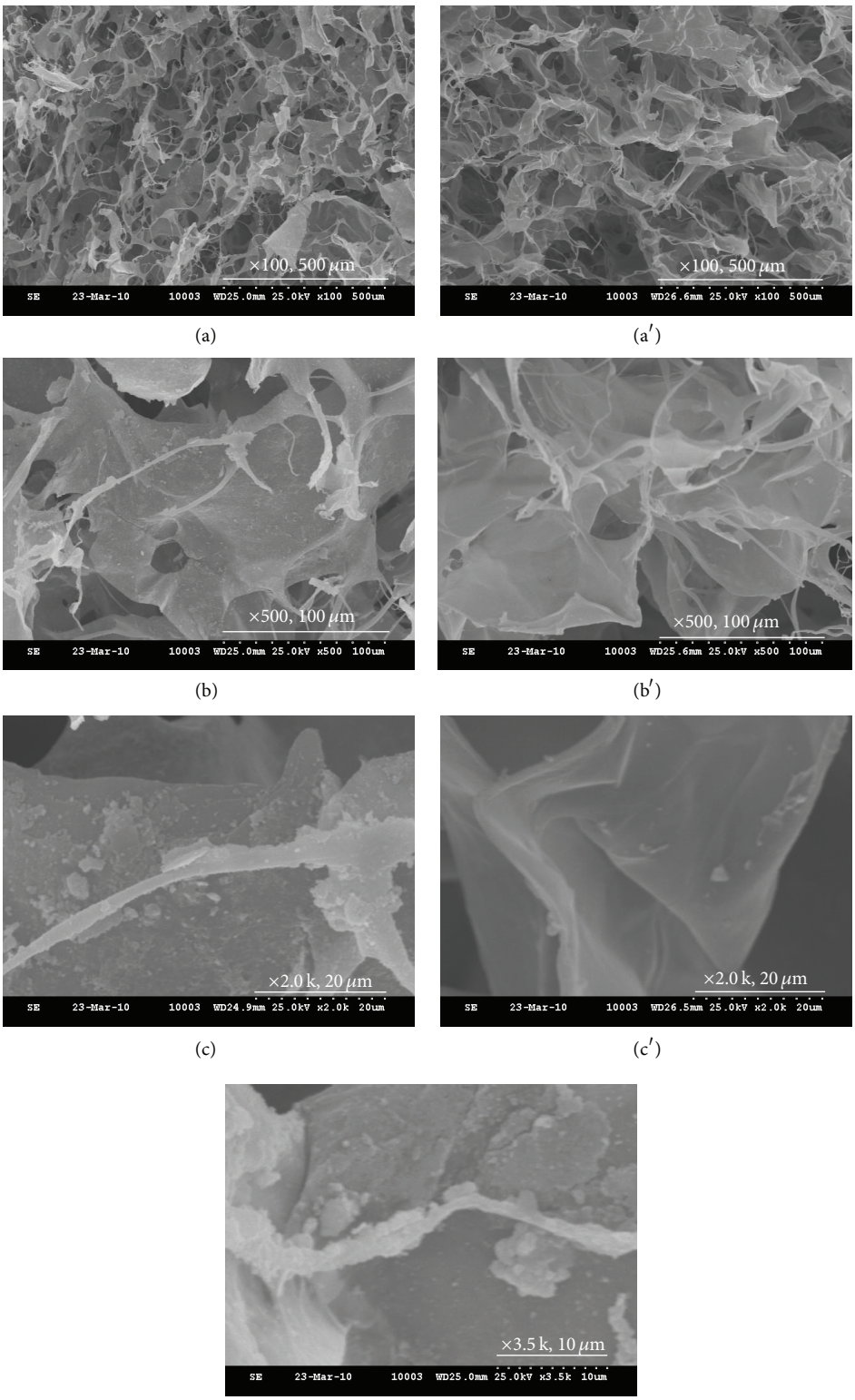

Figure 4: SEM images of (a)–(d) COLL/HA-Ag nanocomposite and (a′)–(c′) COLL/Ag.

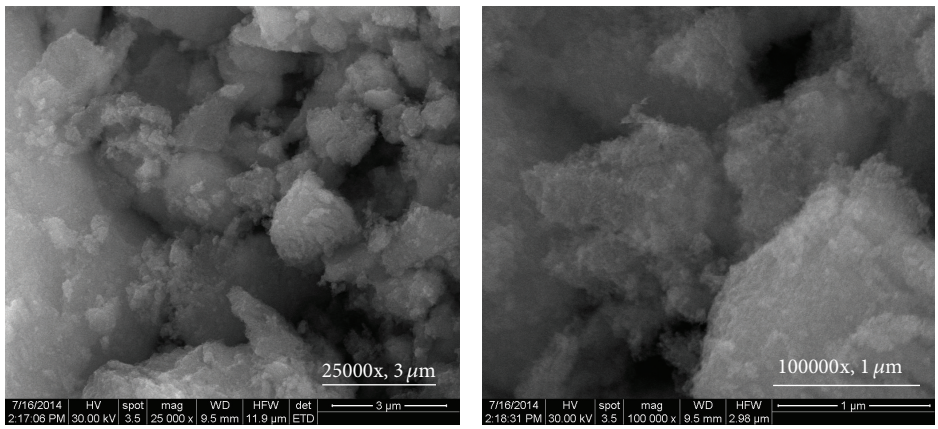

Figure 5: SEM image of HA-Ag nanopowder.

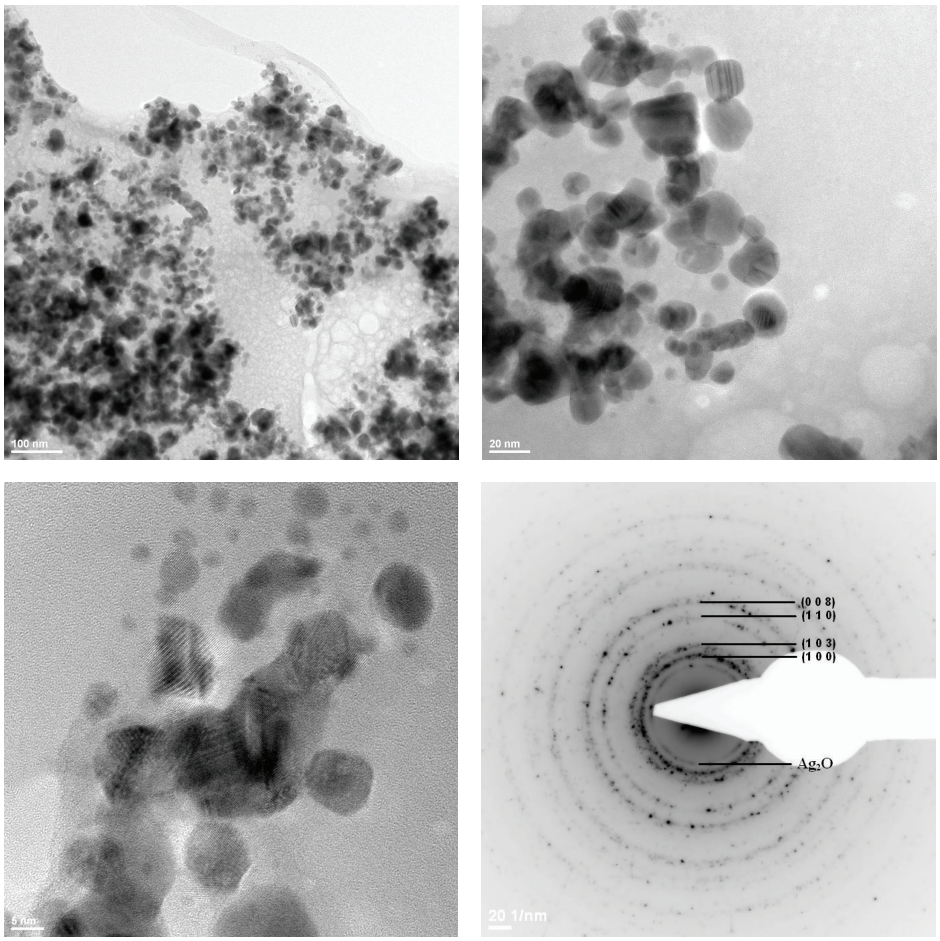

Figure 6: TEM images of plasma sputtered silver nanoparticles.

in the bulk nanopowder, it is possible to identify silver-rich areas (containing silver agglomerates) but also silver-free areas (pure HA). Based on TEM image, silver as well as HA can be considered monodisperse, silver having spherical form and a maximum diameter of less than 20 nm.

3.5. Antimicrobial Studies. As found in the literature data, the antimicrobial activity is dependent on concentration, silver size, and shape [40]. Because only for the HA-Ag sample different compositions were obtained, the antimicrobial studies will be presented only for HA/Ag samples obtained by

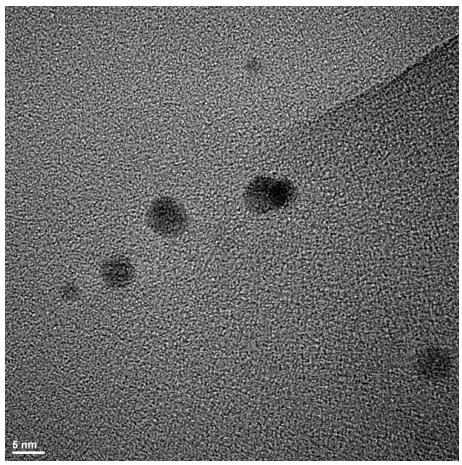

Figure 7: TEM image of the antiseptic COLL/Ag matrix.

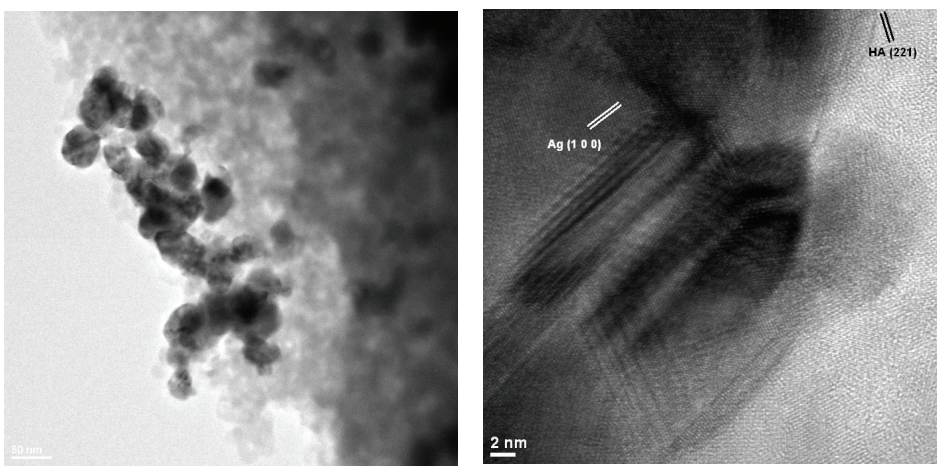

Figure 8: TEM images of HA-Ag powder.

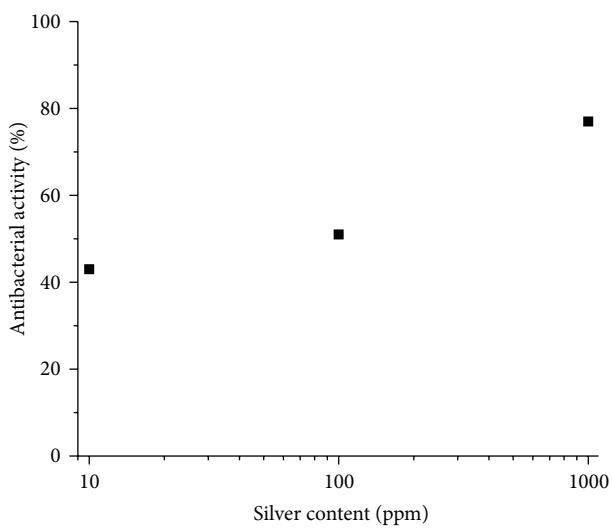

Figure 9: Antibacterial activity of the HA-Ag samples.

Table 1: Potential applications of the synthesized samples.

Materials	Synthesis method	Potential applications
Colloidal silver	Chemical reduction	Treatment of different infections (equivalent of antibiotics) or even cancer and so forth
COLL/Ag	Chemical reduction	Skin cancer or burns (not recommended in the case of infections because glucose and its derivatives could serve as a growth medium)
	PS symmetric	Skin cancer, infections associated with burns (can be used also for the people with diabetes)
	PS asymmetric	Skin cancer/infections (silver-rich face is in contact with the skin); burns (silver-rich face is not in contact with the skin; Ag nanoparticles are only for their antiseptic activity)
HA/Ag or COLL/HA-Ag	Chemical reduction	Treatment of bone defects and even for bone cancer (Ag nanoparticles have antitumoral activity and antiseptic activity, respectively)
	PS symmetric	
	PS asymmetric	Treatment of bone defects and bone cancer (silver-rich face has to be in contact with cancerous tissue due to its antitumoral and antiseptic activity)

plasma sputtering and containing 10, 100, and 1000 ppm silver nanoparticles. The bacteriological experiments performed *in vitro* demonstrated the effectiveness of these samples in inhibiting the growth of *Escherichia coli* (Figure 9), even at low silver content.

It can be concluded that even at low content of silver nanoparticles (10 ppm), the HA-Ag sample inhibits the growth of *E. coli* (43%) while increasing content of silver induces a higher level of antimicrobial activity (51% for 100 ppm and 77% for 1000 ppm of nanoAg, resp.).

Based on the presented results these materials are intended to be further tested for the following applications, as presented in Table 1.

4. Conclusions

Three types of antiseptic, multifunctional materials were obtained, each having different potential medical applications. COLL/nanoAg is potential material for skin repair and can be used especially for the injuries caused by burns or cancer. HA/nanoAg and COLL/HA-nanoAg are potential bone grafts antiseptic materials but can be also used in different kinds of bone cancer, where surgical resection is necessary. Besides the material, in the cases of infections or tumours the silver-rich face of the materials has to be in contact with these tissues. When only antiseptic activity is required both symmetric (homogenous) and asymmetric materials can be used. In the cases of skin injuries it is recommended to use asymmetric COLL/Ag scaffolds and the silver-rich face does not have to be in contact with the skin, having only protective role for potential infections.

Conflict of Interests

The authors declare no conflict of interests.

Acknowledgments

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