Synthesis of Nanomaterials by the Pulsed Plasma in Liquid and their Bio-medical Applications

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Abstract. Pulsed plasma in liquid is a simple, ecologically friendly, cost-efficient method based on electrical discharge between two metal electrodes submerged into a dielectric liquid. We synthesized carbon-encapsulated Fe (Fe@C) magnetic nanoparticles with low cytotoxicity using pulsed plasma in a liquid. Body-centered cubic Fe core nanoparticles showed good crystalline structures with an average size between 20 and 30 nm were encapsulated in onion-like carbon coatings with a thickness of 2–10 nm. Thermal gravimetric analysis showed a high stability of the as-synthesized samples under thermal treatment and oxidation. Cytotoxicity measurements showed higher cancer cell viability than samples synthesized by different methods. Carbon coated ZnO nanorods with about 20 nm thickness and 150 nm length were synthesized by this method using different surfactant materials such as cetyl trimethylammonium bromide (CTAB) and sodium dodecyl sulphate (SDS). Cu and Ag nanoparticles of about 10 nm in size were also synthesized by the pulsed plasma in aquatic solution of 0.2 % gelatine as surfactant material. These nanoparticles showed high antibacterial activity for Erwinia amylovora and Escherichia coli.

1. Introduction

Nowadays, metal nanoparticles are gaining more interest as a new class of alternative antimicrobial agents because of the increasing of antibiotic resistance in microorganisms [1] presenting grave hazard for public healthcare [2]. But pure metal nanoparticles without any coating or treatment are toxic and cause health concerns in even trace amounts. Carbon has been used as biocompatible material and less affected in physiological conditions. So, carbon coated metal or semiconductor are therefore biocompatible materials. Iron nanoparticles have optimum magnetic response. Carbon coated iron nanoparticles are consequently best candidate to be used for hyperthermia, drug delivery, magnetic resonance imaging contrasts, biological labels etc.
Zinc oxide (ZnO), a semiconductor with unique electrical, optoelectronic, and luminescent properties. Because of these attractive properties, nanostructured ZnO have many important practical applications in catalysis, photoluminescence, and functional devices. Coating of ZnO with carbon can extend its above-mentioned applications even to more fields, particularly, bio-medical applications: as the luminescent probes of tissues for imaging by fluorescence microscope.

There are a number of synthesis methods for carbon coated Fe and ZnO nanoparticles have been reported such as chemical vapor deposition (CVD), arc discharge and so on. However, most of these methods lead to economic disadvantages including the need to maintain high temperatures, high pressures, and vacuum systems as well as use of expensive equipment. In addition, produced by these methods nanomaterials have quite high toxicity.

We have reported a simple, ecologically friendly, cost-efficient method based on the electrical discharge between two metal electrodes submerged into a dielectric liquid [3]. This is a versatile and easily configurable method for synthesis of metals, oxides, sulfides, carbides, etc, by changing electrodes materials and the dielectric liquid. By choosing the electrical and experimental conditions, physical and chemical properties can be tuned [4]. In this paper, we report synthesis of biocompatible metal nanoparticles (Fe, Cu, Ag), carbon coated oxide nanostructure (ZnO) and their antibacterial activities.

2. Materials and Methods

All materials for electrodes and dielectric liquids were of high purity grade and obtained from Kojundo Co., Rare Metallic Co. Experiments were conducted using the pulsed plasma apparatus, which is schematically illustrated in figure 1. For synthesis of metal nanostructures, electrodes made of metal rods of about 5 mm in diameter and 12 mm in length and 200 ml dielectric liquid (toluene or ethanol) were used. And for zinc oxide nanostructures, electrodes were made of zinc and it was submerged into water. Surfactant materials with different polarities were added to water in order to achieve nanostructures resistance to aggregation and oxidation. Production yield of the samples differed according to the melting temperature of the being synthesized metals, for instance, low melting temperature metals such as Zn, can be produced up to few g/h.

X-Ray diffraction (XRD) pattern of the samples were taken using Cu Kα radiation, Rigaku RINT-2500VHF. Field Emission-Scanning Electron Microscope (FE-SEM) images of the products were taken by a JEM-2000FX.

Cytotoxicity of encapsulated metallic samples was evaluated by using the A549 cell line (Humanlung adenocarcinoma epithelial cells). Cell viability was measured by (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT and (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) XTT assay kits purchased from Roche Diagnostics (Japan).

Antimicrobial activity of silver nanoparticle were conducted using the E-coli (ATCC 35218) bacteria in Plate Count Agar (Merck-M105463.0500) and Violet Red Bile Agar (Merck-M101406.0500) media by filter paper and hole methods.
Figure 1. Schematic illustration of the pulsed plasma in liquid method and the Transmission Electron Microscopy images of the synthesized iron nanoparticles by this method.

3. Results and Discussion
Fe core nanoparticles with good crystalline structures of an average size between 20 and 30 nm were encapsulated in carbon coatings of 2-4 nm thickness, as shown in Fig.1. The synthesized samples showed good ferromagnetic properties at room temperature. The samples showed very high thermal stability (up to 700 °C) and high biocompatibility: more than 95 % of cells remained alive for carbon iron nanoparticles (see table 1). Such magnetic nanoparticles are perfect for using in hyperthermic cure of cancer cells, where external magnetic field fluctuates delivered to the target magnetic nanoparticles and by this generates heat. This heat is aimed to kill the cancer cells. Coating of metallic magnetic nanoparticles is critical for hyperthermia applications [5]. Table 1 represents a comparison of the cell viability of different samples. As we can see our samples showed highest cell viability among similar production methods and was equal to 95 %. In other words, only 5 % of the normal cells located in the vicinity of the cancer cell might be damaged due to targeted delivery by injection or other methods. We can conclude that magnetic nanoparticles by the pulsed plasma in liquid method are highly appropriate for hyperthermia treatments.

Table 1. Comparison of cell viability of the samples produced by different methods.

| Method                | Sample | Cell viability, % | Reference                  |
|-----------------------|--------|-------------------|----------------------------|
| Pulsed plasma in liquid | Fe@C   | 95                | Our work                   |
| Catalytic CVD          | Fe@C   | 2                 | Int. J. Nanomed. 5, 187 (2010) |
| Arc discharge          | Fe@C   | 80                | Cell Biol. Int. 32. 1001 (2008) |
| Aerosol particles      | Fe     | 6.5               | Int. J. Nanomed. 6, 187 (2011) |

Carbon coated ZnO nanorods with about 20 nm thickness and 150 nm length were synthesized by this method. When different surfactant materials (CTAB, SDS) were used, ZnO nanorods with different morphologies were produced: CTAB induced formation of longer nanorods (average length of 118 nm and diameter of 28 nm) with smooth surfaces, while SDS resulted in longer nanorods (246 nm in length and 55 nm in diameter) with rougher surfaces (Figure 2).
Closer look at the nanorod structure formed under SDS and CTAB conditions revealed that in both cases the surface of the nanorods were coated with carbon layers with different thickness: 2 nm in case of SDS and 5 nm for CTAB. Carbon is a best candidate for making materials biocompatible. Thus, we hope that our carbon-coated ZnO nanorods can be applied for biomedical applications, such as biomedical imaging (which includes fluorescence, magnetic resonance, positron emission tomography, as well as dual-modality imaging), drug delivery, gene delivery, and biosensing of a wide array of molecules of interest [6].

Silver nanoparticles were prepared by both chemical reduction and pulsed plasma (electrical discharge) in water methods. In addition, various surfactant materials were tested for keeping the nanoparticles’ stability longer. Synthesis of Ag nanoparticles were based on chemical reduction of ions by hydrazine:

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4\text{Ag}^+ + \text{N}_2\text{H}_4 + 4\text{OH}^- = 4\text{Ag} + \text{N}_2 + 4\text{H}_2\text{O}
\] (1)

XRD analyses showed no effect of gelatin to Ag nanoparticles formation during Ag ions reduction to metallic Ag. Similar results were obtained in case of pulsed plasma synthesis of Ag. Effect of stabilizing agent SDS was also conducted and showed good result, where no reflections of metallic particles can be seen. However, small content of silver oxide was appeared. Particle size of the synthesized samples was small, about 10 nm in diameter.

Surfactant materials were used for increasing stability of silver nanoparticles. SDS surfactant was added to water in 0.4 % concentration and then the pulsed electrical discharge was conducted inside it. Obtained samples showed better stability and UV/visible absorption spectrometer confirmed that the nanoparticles aggregation over a time period of a week showed better stability than that of without surfactant. These samples were tested for antibacterial activity for some bacteria as described below.

Biological activity tests were conducted for the synthesized Ag nanoparticles produced by both chemical reduction and pulsed plasma method using E-coli bacteria. Antibacterial activity of Ag nanoparticles prepared by chemical reduction method were determined for E-coli bacteria by filter paper method, where a round shaped special filter paper was submerged into a solution of Ag nanoparticles of certain concentration. Then this filter paper was placed to the bacteria cell culture in Petri dish. Figure 3 shows the E-coli bacteria culture with Ag nanoparticles lysis zones, which shows the area of the bacteria cell growth suppression zone. It is the part where bacteria growth was not possible due to the antibacterial effect of nanoparticles. Lysis zone for the Ag nanoparticles by chemical method was determined to be 5-7 mm, which is in the usual range value for such particles [7].
Figure 3. Effect of antibacterial properties of Ag nanoparticles produced by chemical reduction method to the *E-coli* bacteria cell culture.

Antibacterial activity of Ag nanoparticles prepared by electrical discharge method were also determined for *E-coli* bacteria by filter paper and hole method, which can be seen from the Figure 4. These are results for the Ag nanoparticles + SDS surfactant. Among surfactant materials SDS showed no antibacterial effect to *E-coli* bacteria, while CTAB had inhibited suppression zone when tested for antibacterial activity in pure state without any other particles. This urged us to use SDS surfactant material as stabilizing agent for nanoparticles and protects nanoparticles from aggregation, oxidation and other outer influences. Red oval line shows the effect of our samples. We can see that the bacteria around the nano Ag + SDS samples could not grow and growth suppression zones are clearly visible. In the hole method (Fig. 4b), where nano Ag + SDS sample was dropped to the holes had extraordinary effect to bacteria cell growth and almost totally killed them. Both filter paper method and hole method displayed that nano Ag + SDS sample exhibits excellent antibacterial property to the *E-coli* bacteria.

Figure 4. Effect of antibacterial properties of Ag nanoparticles produced by electrical discharge method in presence of SDS surfactant to the *E-coli* bacteria cell culture by the a) filter paper and b) hole methods
Table 2 shows results of the antibacterial test of our samples for the E-coli bacteria cells. As we mentioned above, CTAB surfactant material exhibits antibacterial property, thus, antibacterial activity of the Ag nanoparticles produced in presence of CTAB surfactant could not be clearly determined. However, SDS surfactant had no effect to bacteria. In addition, its stabilizing effect to Ag nanoparticles was excellent and nano Ag particles could be stable to external influence over time. Nano Ag + SDS sample produced by the electrical method showed better antibacterial property than the nano Ag produced by the chemical reduction method. This shows that the newly formed nanoparticles in plasma conditions can be quickly stabilized in-situ by SDS surfactant during formation process.

### Table 2. Values of growth suppression zone of samples on E-coli bacteria culture.

| Sample name                          | Lisys zone, mm |
|--------------------------------------|----------------|
|                                      | Filter paper method | Hole method |
| CTAB itself                          | 1               | 7-10        |
| SDS itself                           | 0               | 0           |
| Nano Ag by chemical method           | 5-7             | -           |
| Nano Ag + SDS by electrical discharge| 7-9             | 15          |

4. Conclusions
We have described the synthesis of carbon encapsulated Fe nanoparticles and ZnO nanorods by simple and low-energy pulsed plasma in liquid method. Carbon coated Fe magnetic nanoparticles showed high biocompatibility: more than 95% of cells remained alive for carbon iron nanoparticles. Carbon coating of ZnO nanorods can be achieved by using surfactant materials, which served as carbon source.

Nanopowders of Ag synthesized both by chemical and physical methods exhibited good antibacterial activity for E-coli and the suppression zone size was between 5 to 9 mm. Nano Ag + SDS sample produced by the electrical discharge method showed higher antibacterial activity than the nano Ag produced by the chemical reduction method.

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