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Onion-like carbon-encapsulated Co, Ni, and Fe magnetic nanoparticles with low cytotoxicity synthesized by a pulsed plasma in a liquid

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Abstract

We synthesized onion-like carbon-encapsulated Co, Ni, and Fe (Co-C, Ni-C, and Fe-C) magnetic nanoparticles with low cytotoxicity using pulsed plasma in a liquid. The pulsed plasma is induced by a low-voltage spark discharge submerged in a dielectric liquid. The face-centered cubic Co and Ni, and 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. Vibrating-sample magnetometer measurements revealed the ferromagnetic properties of as-synthesized samples at room temperature (Co-C=360 Oe, Fe-C=380 Oe, and Ni-C=211 Oe). Raman-spectroscopy analysis found onion-like carbon shells composed of well-organized graphitic structures. Thermal gravimetric analysis showed a high

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stability of the as-synthesized samples under thermal treatment and oxidation. Cytotoxicity measurements showed higher cancer cell viability than samples synthesized by different methods.

1. Introduction

Carbon-encapsulated magnetic metal nanoparticles are of great interest due to their unique properties [1] and wide range of practical and clinical applications [2]. Among the various coating materials for Co, Ni, and Fe metal nanoparticles such as silica, organic substances and polymers, carbon is more stable against oxidation, physical and chemical degradation. Carbon can provide good biocompatibility while protecting from agglomeration [3]. Due to their ability to function at the cellular and molecular levels, magnetic nanoparticles can be functionalized to deliver anti-cancer drugs to human cancer tissues [4, 5]. Cobalt, nickel, and iron nanoparticles encapsulated in carbon shells (Co-C, Ni-C, and Fe-C) are applicable in medicine as localized radio frequency absorbers in cancer therapy [6], bio-engineering applications [7], and drug delivery [8]. They also have physical applications such as magnetic data storage, electromagnetic-wave absorption and ferrofluids [9, 10].

One key reason to study the cytotoxic effects of magnetic nanoparticles encapsulated in carbon is that they have great potential to contribute to cancer therapy. Recently, magnetic nanoparticles have played a notable role in magnetic hyperthermia, one of the most promising cancer therapies with minimum side effects. *In vivo* cancer tissues can be exposed to controllable alternating magnetic fields. When heated to 42–46 °C, cancer tissues become more vulnerable and sensitive to anti-cancer drugs and radiation. However, normal cells can survive at this temperature. After the penetration of magnetic particles, membrane blebbing of the cytoplasm can occur, leading to apoptosis and death of the cancer cell [11-13].

Several methods have been used for synthesis of carbon-encapsulated magnetic metal
nanoparticles (Me-C), such as chemical vapor deposition (CVD) [14], conventional arc discharge [15], magnetron and ion-beam co-sputtering [16], laser irradiation [17, 18], spray pyrolysis [19] and explosions [20]. 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. Plasma methods are favorable and conducive to large amount synthesis of carbon encapsulated metal nanoparticles with well-defined crystalline structures [21].

In our study, we report the synthesis of cobalt, nickel, and iron nanoparticles encapsulated in onion-like carbon shells (Co-C, Ni-C, and Fe-C) using a pulsed plasma in a liquid [22]. The aims of our study are to synthesize magnetic nanoparticles with low toxicity and to compare the toxicity of our samples with the toxicity of nanoparticles prepared by aforementioned methods. Although cytotoxic properties of nanoparticles are still in the experimental stage, they have great potential in clinical applications.

2. Experimental

2.1. Synthesis of Co-C, Ni-C, and Fe-C nanoparticles

The experimental procedure is described in [22]. Here, we briefly give the process. Figure 1 shows the experimental setup of the pulsed plasma in liquid method. Pulsed electrical discharge plasma generated by the low voltage spark discharge driven by the capacitor energy. Electrical current duration between pulsed plasma discharges was equal to 10 microseconds (μs). In order to set the optimal conditions for synthesis of Co-C, Ni-C, and Fe-C nanoparticles, different electrical conditions were performed for each experiment (voltage 150-170V, frequency 60 Hz-30 kHz, current 1.5-3 A).

For synthesis of Co-C nanoparticles, we purchased electrodes made of carbon rods doped with 9 % Co and 1 % Ni, with a diameter of 6 mm and length of 100 mm, from Toyo Tanso. Ethanol of
99.5% purity, purchased from Kanto Chemical Co, was used as dielectric liquid and source of carbon. We applied pulsed plasma with a voltage of 150 V, current of 3 A, frequency of 60 Hz and single discharge duration of 10 μs.

For Ni-C nanoparticle synthesis, Ni metallic rod electrodes with a diameter of 5 mm and length of 150 mm, purchased from Rare Metallic Co, were immersed in 200 ml ethanol (99.5% purity). The power source was applied with a frequency of 30 kHz, current of 1.5 A, voltage of 160 V and discharge duration of 10 μs.

For Fe-C nanoparticles preparation, metallic Fe rod electrodes 6 mm in diameter and 140 mm in length, purchased from Rare Metallic Co, were submerged in 200 ml ethanol (99.5% purity). Electrical conditions were as follow: frequency 30 kHz, current 1.5 A, voltage 170 V and single discharge duration 10 μs.

One of the electrodes was kept vibrating to hold the discharge process stable. After one hour of continuously applying the pulsed plasma discharge, obtained powder samples were separated from ethanol by centrifuge and evaporation. The sample production rate was 3.5-4.5 g/h. Separated samples of Co-C, Ni-C, and Fe-C were treated by 5 M HCl for 12 hours and washed in water to remove the amorphous carbon and uncoated metallic Co, Ni, and Fe nanoparticles. Then, treated and washed samples were dried at 120° C for 2 hours.

For synthesis of Co-C, Ni-C, and Fe-C magnetic nanoparticles, we used different types of electrodes (carbon electrode doped with Co and Ni for Co-C synthesis; and pure Ni, and Fe electrodes for Ni-C, and Fe-C synthesis, respectively) to proceed encapsulation of magnetic metal nanoparticles in carbon.

Atomic emission spectra of the plasma discharge during the synthesis were collected by an optical spectrometer SEC2000 UV-VIS installed close to the plasma discharge zone outside the
quartz beaker. The light coming from outside sources was removed by covering the plasma
generation zone with light protective material. Identification of emission spectrum peaks was done
according to the NIST1 database.

2.2. Sample characterization

High-resolution transmission-electron microscopy (HRTEM) analysis was carried out on a Philips
Tecnai F20 S-Twin microscope at 200 keV with a point resolution of 0.18 nm. X-ray diffraction
(XRD) patterns of samples were taken on a Rigaku RINT-2500VHF diffractometer using Cu Kα
radiation. Raman spectra of the samples were recorded at room temperature on a HORIBA Jobin
Yvon HR800 spectrometer, using the argon-ion laser beam as the excitation source at wavelength
of 514.53 nm. We measured the magnetic properties of Co, Ni, and Fe encapsulated in onion-like
carbon at room temperature by using the Vibrating Sample Magnetometer (VSM), (Riken Denshi,
Co., Ltd. Japan). Thermal gravimetric analyses (TGA) were conducted on the TG-DTA6300
thermogravimeter, (SEIKO Ins. Co. Japan) by using a stainless steel pan in ambient air with a
heating temperature rate of 5°C min⁻¹.

2.3. Cytotoxicity measurements

Cytotoxicity of samples was evaluated by using the A549 cell line (Human lung adenocarcinoma
epithelial cells) seeded onto 96-well plate one day before measurement. These cells were
maintained as monolayer cultures in Dulbecco’s modified eagle medium (DMEM) solution
supplemented with 10 % Fetal Calf Serum (Gibco BRL, USA) and 1 % concentration of Penicillin
and Streptomycin antibiotics (100 X stock, Sigma). The cells were incubated at 37 °C in a 5 %

1NIST Atomic Spectra Database: http://physics.nist.gov/PhysRefData/ASD/lines_form.html
CO₂ humidified incubator. 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). Cells
were treated with predetermined concentrations of the Co-C, Ni-C, and Fe-C nanoparticles
synthesized by a pulsed plasma in a liquid and allowed to grow up for 24 hours. After the
treatment completed, the mediums were removed to avoid the interference of nanoparticles and
replaced with new mediums containing MTT and XTT. MTT reagent with cells was incubated for
4 hours, and XTT reagent for 2 h at 37 °C, 6.5 % CO₂. Cell viability results were plotted
according to optical density values measured at a wavelength of 540 nm using Enzyme-linked
immunosorbent assay (ELISA) reader.

3. Results and discussion

3.1. High-resolution transmission-electron microscopy (HRTEM) and energy dispersion X-ray
(EDX) analyses

Fig. 2 shows a) the HRTEM image of the Co-C nanoparticle, b) the line profile of the image
contrast along the line shown in the inset, c) the magnified image of the area drawn in (a), inset is
the FFT of the inner Co core, suggesting that the particle is a cobalt single crystal with cubic
structure. (d) An EDX pattern of particle shown in (a); e) HRTEM image of the Co-C
nanoparticles; f) Diameter distribution chart of the Co-C fitted by a Gaussian curve. A cobalt
particle with a size of 20 nm was encapsulated in onion-like carbon shells with a thickness of
about 5 nm (Fig. 2a). Diameters of the spherical Co-C particles ranged from 5-50 nm. The
interplanar d-spacings of the inner Co core were determined to be 2.04 nm (Fig. 2b), which are in
a good agreement with the face-centered cubic (fcc) Co (111) planes. The interlayer distance of
the outer onion-like carbon shells found to be 0.32 nm, slightly smaller than the (002) planes of
graphitic carbon [23]. This shows that interlayer spacings of the onion-like carbon coatings were reduced by 5.8 % in the Co-C nanoparticles synthesized by pulsed plasma in liquid compared to the 0.34 nm spacings of graphite. The interlayer distance of onion-like carbon shells fluctuated in the range of 0.31-0.4 nm, depending on the synthesis method [24-26]. We analyzed the Co-C nanoparticle using EDX and showed its composition to be Co (55.06%), C (28.79%) and Cu (16.14%). Cu peak is from the TEM grid and C from the onion-like carbon shells. The average diameter of Co particles in Co-C estimated as 8.5 nm, and thickness of graphitic coatings as 3-7 nm.

Fig. 3 shows a) the HRTEM image of the Ni-C nanoparticle, b) the line profile of the image contrast along the line shown in the inset, c) the magnified image of the area drawn in (a), inset is the FFT of the inner Ni core, showing single crystal nickel particle with cubic structure. (d) An EDX pattern taken from the Ni-C particle shown in (a); e) HRTEM image of the Ni-C nanoparticles; f) Diameter distribution chart of the Ni-C fitted by a Gaussian curve.

The size of the Ni core is about 26 nm. It was encapsulated in onion-like carbon with a shell thickness of about 10 nm. We estimated the interplanar d-spacing of the inner Ni core to be 2.04 nm, which corresponds to (111) planes of fcc Ni. The interlayer spacings of the outer onion-like graphite shells were estimated to be 0.315 nm, while the graphite had a d-spacing of 0.34 nm. Therefore, this spacing was reduced by 7 % in the Ni-C nanoparticles synthesized by pulsed plasma in liquid. EDX analysis done on a Ni-C particle showed the elemental composition to be Ni (56.53%), C (31.05%) and Cu (12.4 %). A Gaussian fit to the data showed an average diameter of Ni particles in Ni-C to be 15.1 nm with a size distribution in the range 10-100 nm. The thickness of graphitic coatings in the Ni-C measured as 5-10 nm.

We found by HRTEM analysis that Fe nanoparticles synthesized by the pulsed plasma in ethanol were encapsulated in onion-like carbon. Fig. 4 shows a) the HRTEM image of the Fe-C
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nanoparticle, b) the line profile of the image contrast along the line shown in the inset, c) the
magnified image of the area drawn in (a), inset is the FFT of the inner Fe core, suggesting that the
particle is an iron single crystal with body centered cubic structure. (d) An EDX pattern of particle
shown in (a); e) HRTEM image of the Fe-C nanoparticles; f) Diameter distribution chart of the
Fe-C fitted by a Gaussian curve. The diameter of the inner Fe core was 35 nm, while the outer
onion-like carbon shells consisted of 11 layers with a thickness of about 3.69 nm. The interplanar
d-spacings of the inner Fe core found to be 0.205 nm, which are in a good agreement with the
(110) planes of body-centered cubic Fe. We calculated the interlayer spacings of the outer onion-
like carbon shells to be 0.335 nm, close to the (002) planes of graphitic carbon. EDX analysis of
the Fe-C nanoparticle showed its elemental composition to be Fe (54.18 %), C (32.05 %) and Cu
(13.76 %). A Gaussian curve fitted to the diameter distribution chart of Fe-C, revealed an average
diameter of Fe particles to be 7.9 nm. The thickness of graphitic coatings in the Fe-C measured as
3-7 nm.

3.2. X-ray diffraction (XRD), Atomic emission and Raman spectroscopy analyses

Fig. 5 shows the XRD patterns of Ni-C, Co-C, and Fe-C nanoparticles synthesized by a pulsed
plasma in a liquid. The diffraction pattern for Ni-C nanoparticles, given in Fig. 5a, shows
reflections at 2θ=44.34˚, 51.67˚ and 76.09˚, which are identified as the (111), (200) and (220)
planes of fcc Ni, (JCPDS file No 65-0380). The diffraction pattern of the sample, prepared by the pulsed plasma between carbon electrodes
doped with Co and Ni (Fig. 5b), displays peaks at 2θ=44.21˚, 51.52˚ and 75.85˚, corresponding to
the (111), (200) and (220) planes of fcc Co, (JCPDS file No 15-0806). The broad peak centered at
a diffraction angle 26.42˚ corresponds to the hexagonal graphite (002) planes; we suppose that it
was appeared because the main content of the electrode used for synthesis of Co-C nanoparticles
was carbon, doped with Co and Ni. However, we did not observe Ni peaks, which may be caused by its insignificant amount (1%) in the electrode.

For the Fe-C nanoparticles (Fig. 5c), XRD revealed diffraction peaks at $2\theta=44.14^\circ$ and $65.18^\circ$ which are characterized by (110) and (200) planes of the bcc Fe, respectively (JCPDS file No 01-1252). Absence of (002) reflection in XRD patterns of the Ni-C, and Fe-C samples caused by using pure metallic Ni, and pure metallic Fe electrodes during the plasma discharge.

The width of diffraction peaks allow us to estimate average particle sizes using Scherrer formula [27], which is applicable to particles with single crystal structure:

$$D= \frac{0.93\lambda}{\beta \cos \theta}$$  \hspace{1cm} (1)

Here, $D$ is an average particle size, $\lambda$ is the wavelength of the applied radiation (CuK$\alpha$, $\lambda=1.5406$ Å), $\beta$ is the broadening of the diffraction peak, and $\theta$ is the Bragg angle. According to this formula, average sizes of Co-C, Ni-C, and Fe-C particles were calculated to be 17 nm, 29 nm, and 16 nm, respectively. These values are close to the high resolution TEM results.

Atomic emission spectra collected from the plasma discharge zone are given in Fig. 6. From the atomic emission spectrum, we identified peaks of C I, C II, C III, Co I, II, Ni I, II, and Fe I, II. These active atoms and ions interacted with each other to form Co-C, Ni-C, and Fe-C nanoparticles.

In Fig. 7, we illustrate the proposed formation mechanism of Co, Ni, and Fe nanoparticles encapsulated in onion-like carbon by pulsed plasma. Considering the temperature of the plasma discharge zone to be around 2500-3000 K, we assumed that C I atoms, C II and C III ions were evaporated from ethanol. In Co-C nanoparticle formation, carbon ions might be released either from ethanol or carbon electrodes; we observed peaks for C2 radicals in the region of 513-516 nm of emission spectrum [28]. Metal I and II ions evaporated from Ni and Fe metal electrodes and Co atoms evaporated from carbon-doped Co electrodes then undergo ionization to form metal-carbon
vapor. C2 radicals generated from ethanol in our experiments formed graphite network, and then onion-like structures due to the catalytic effect of the metal atoms (Co, Ni, and Fe). Segregation of metal and carbon particles occurred in the cooling zone. We conclude that metal particles may act as catalysts in onion-like carbon structure formation and become trapped inside them, forming the Me-C nanoparticles.

Fig. 8 presents the Raman spectra for (a) Ni-C, (b) Co-C, and (c) Fe-C nanoparticles synthesized by a pulsed plasma in a liquid. D band associated with the disordered carbon (at 1300-1350 cm$^{-1}$ locations) is shifted to 1354 cm$^{-1}$, 1353 cm$^{-1}$, and 1352 cm$^{-1}$ in the Raman region for Ni-C, Co-C, and Fe-C nanoparticles, respectively. The G band for Ni-C was detected at 1577 cm$^{-1}$, for Co-C at the 1573 cm$^{-1}$, and at the 1572 cm$^{-1}$ for Fe-C nanoparticles, associated with the G band for the onion-like carbons, which has been shifted downward slightly (1569–1577 cm$^{-1}$ at various locations) [29].

The level of graphitization can be expressed as the intensity ratio of the D-band to G-band ($I_D/I_G$), which in our case is equal to 0.65 for Ni-C, 0.6 for Co-C, and 0.5 for Fe-C nanoparticles. Such intensity ratio values characterize the sp$^2$ bonded graphitic carbon, and according to the relationship between the crystallite width and the Raman intensity, $L_a=4.4(I_D/I_G)^{-1}$ (in nm) [30, 31], onion-like carbon coatings of the Co, Ni, and Fe nanoparticles produced by pulsed plasma have a crystallite size of 2-3 nm. High intensity of the G band shows that the amount of graphitized and well-organized carbon exceeds the amount of amorphous and disordered carbon in our samples, enabling their functionalization and use in medicine.

3.3 Magnetic properties and Thermal gravimetric (TGA) analyses

Magnetic properties of the Co-C, Ni-C, and Fe-C nanoparticles synthesized by pulsed plasma in liquid were measured at room temperature and are shown by magnetization hysteresis loops in Fig.
9. The nanoparticles showed high coercivity: Co-C (460 Oe) and Fe-C (480 Oe). The ratio of remanence to saturation magnetization \( M_r/M_s \) indicated ferromagnetic behaviors of the Co-C (\( M_r/M_s=0.322 \)), Ni-C (\( M_r/M_s=0.151 \)), and Fe-C (\( M_r/M_s=0.335 \)) nanoparticles at the room temperature. The Ni-C showed coercivity of 211 Oe, which is lower than the coercivities of Co-C and Fe-C. This can be explained by the particle size effect. As mentioned in [32, 33], the coercivity and particle size of Ni are inversely proportional. These magnetic data show the suitability of our samples for use as magnetic recording materials, for cancer treatment applications, drug delivery and magnetic resonance imaging (MRI).

Thermal gravimetric analysis was meaningful in detection of oxidation resistance and thermal stability of our samples. TGA analyses of the Co, Ni, and Fe nanoparticles encapsulated in onion-like carbon were recorded from room temperature to 900 °C in air. Fig. 10 compares the thermal stability of each sample. Fe-C showed higher stability, with weight loss (60 %) starting from 513 °C. Decomposition of the Ni-C started from 505 °C, and 29 % of the sample remained after about 3 hours at 772 °C. During the heating of the Co-C sample, weight loss was observed from 495 °C, with 27 % remaining after about 3 hours at 767 °C. These results show that the amount of amorphous carbon is insignificant in the samples, which indicates their thermal and medical applications.

3.4. Cytotoxicity of the Co-C, Ni-C, and Fe-C nanoparticles

For cytotoxicity studies, human lung epithelial A549 cells were exposed to Co-C, Ni-C, and Fe-C nanoparticles synthesized by pulsed plasma for 24 h. Suspensions of Co-C, Ni-C, and Fe-C nanoparticles with concentrations of 10, 20, 40, 80 and 160 µg/ml were prepared by serial dilution. Cytotoxic effects were determined by using the MTT and XTT assays. Tetrazolium salts MTT and XTT are especially useful for assaying the quantification of viable cells. Both, MTT and XTT
work by being to a formazan dye only by metabolic active cells. MTT assay based on the
cleavage of the yellow tetrazolium salt to purple formazan crystals, while the XTT assay based on
the cleavage of the yellow tetrazolium salt to orange formazan dye. Fig. 11 shows the cytotoxic
effects of the Co, Ni, and Fe nanoparticles encapsulated in onion-like carbon shells synthesized by
pulsed plasma in a liquid. MTT assay showed that our samples did not produce significant toxicity
up to the concentration of 10 µg/ml. In case of XTT assay, cell viability started to decrease at
concentration of 40 µg/ml. As the concentration of nanoparticles increased, cell viability
decreased in a concentration-dependent manner. Our samples showed lower toxicity compared to
other magnetic nanoparticles synthesized by different methods. Compared results are given in
Table 1. Cytotoxic effects of the Co-C nanoparticles synthesized by catalytic chemical vapor
deposition (CCVD) on the HeLa cancer cells showed a cell viability of 97.7 % without radio
frequency radiation (RF) exposure [6]. Our samples showed higher cell viability results under the
same conditions, 98.6 %, 97.9%, and 95 % for the Co-C, Ni-C, and Fe-C nanoparticles,
respectively. Cell viability of the Fe-C sample in our work was 8 % higher than that of Fe-C
synthesized by the Kratschmer-Huffmann arc discharge method [16]. It was shown that toxicity of
the uncoated Co, Ni, and Fe magnetic nanoparticles is very high [34], compared to those
encapsulated in carbon. We assumed that lower toxicity of our samples might be induced by well-
graphitized carbon coatings. Graphite is a slightly hazardous material and formed during the
plasma discharge reaction from ethanol and carbon.

4. Summary

We have described the synthesis of onion-like carbon encapsulated Co, Ni, and Fe nanoparticles
by simple and low-energy pulsed plasma in liquid method. HRTEM studies revealed that Co, Ni,
and Fe nanoparticles with average crystallite sizes of 17 nm, 29 nm, and 16 nm, respectively, were
encapsulated in onion-like carbon shells. XRD analysis of samples showed the fcc-Co, fcc-Ni, and bcc-Fe structure cores. Raman spectroscopy analysis detected the highly ordered graphitic nature of the outer onion-like carbon shells. Co-C, Ni-C, and Fe-C nanoparticles synthesized by pulsed plasma in liquid exhibited high thermal and environmental stabilities. Magnetic measurements revealed high coercivities, indicating ferromagnetic and superparamagnetic properties of the samples at room temperature. Cytotoxicity effects of the Co-C, Ni-C, and Fe-C nanoparticles on cancer cells showed low toxicity, suggesting their in vivo applications in magnetic fluid hyperthermia, magnetic resonance imaging and drug delivery.

Acknowledgements

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References


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Table 1 - Cytotoxicity data of the Co-C, Ni-C and Fe-C samples synthesized by pulsed plasma in liquid compared with samples synthesized by other methods.

Figure 1 - Schematics of the pulsed plasma in a liquid method.

Figure 2 - (a) HRTEM image of the Co-C nanoparticle; (b) Interplanar spacings plot along the line shown in the inset of the Co core; (c) Magnified image of the area drawn in (a), inset is the FFT of the inner Co core, suggesting that the particle is a cobalt single crystal with cubic structure; (d) An EDX pattern of particle shown in (a), indicating that the particle composed of cobalt, Cu peaks from the TEM grid and C peak from onion-like carbon; (e) HRTEM image of the Co-C nanoparticles; (f) Diameter distribution chart of the Co-C fitted by Gaussian curve.

Figure 3 - (a) HRTEM image of the Ni-C nanoparticle; (b) interplanar spacings plot along the line shown in the inset of the Ni core; (c) magnified image of the area drawn in (a), inset is the FFT of the inner Ni core, suggesting that the particle is a nickel single crystal with cubic structure; (d) EDX pattern; (e) HRTEM image of the Ni-C nanoparticles; (f) Diameter distribution chart of the Ni-C fitted by Gaussian curve.

Figure 4 - (a) HRTEM image of the Fe-C nanoparticle; (b) interplanar spacings plot along the line shown in the inset of the Fe core; (c) magnified image of the area drawn in (a); inset is the FFT of the inner Fe core, suggesting that the particle is an iron single crystal with body centered cubic structure; (d) EDX pattern; (e) HRTEM image of the Fe-C nanoparticles; (f) Diameter distribution chart of the Fe-C fitted by Gaussian curve.

Figure 5 - XRD patterns of the Co-C, Ni-C, and Fe-C nanoparticles synthesized by a pulsed plasma in a liquid.

Figure 6 - Optical emission spectra of the Co-C, Ni-C, and Fe-C magnetic nanoparticles.

Figure 7 - Formation mechanism of Co-C, Ni-C, and Fe-C nanoparticles by pulsed plasma in liquid.
Figure 8-Raman spectra of the Co-C, Ni-C, and Fe-C magnetic nanoparticles synthesized by pulsed plasma in a liquid.

Figure 9-Magnetization curves of the Co-C, Ni-C, and Fe-C magnetic nanoparticles synthesized by pulsed plasma in a liquid.

Figure 10-Thermal gravimetric (TGA) analysis of the Co-C, Ni-C, and Fe-C magnetic nanoparticles produced by pulsed plasma in a liquid.

Figure 11-Cytotoxicity of the Co-C, Ni-C, and Fe-C magnetic nanoparticles synthesized by a pulsed plasma in a liquid determined by MTT and XTT assays.
Table 1—Comparison of cytotoxic parameters for Co-C, Ni-C, and Fe-C nanoparticles synthesized by different methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample</th>
<th>RF heating</th>
<th>NPs concentration</th>
<th>Cell viability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPL (pulsed plasma in liquid)</td>
<td>Co–C</td>
<td>No</td>
<td>10 µg/ml</td>
<td>98.6 %</td>
<td>This work</td>
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<tr>
<td></td>
<td>Ni–C</td>
<td>No</td>
<td>10 µg/ml</td>
<td>95 %</td>
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<tr>
<td></td>
<td>Fe–C</td>
<td>No</td>
<td>10 µg/ml</td>
<td>97.9 %</td>
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<tr>
<td>CCVD (catalytic chemical vapor deposition)</td>
<td>Co–C</td>
<td>No</td>
<td>10 µg/ml</td>
<td>97.7 %</td>
<td>Xu Y et al. [6]</td>
</tr>
<tr>
<td>CCVD (catalytic chemical vapor deposition)</td>
<td>Co–C</td>
<td>2 min</td>
<td>3.32 µg/ml</td>
<td>80 %</td>
<td>Xu Y et al. [15]</td>
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<tr>
<td></td>
<td>Fe–C</td>
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<td>3.32 µg/ml</td>
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<td></td>
<td>Fe/Co–C</td>
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<td>Kratschmer-Huffmann arc-discharge method</td>
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<td>Aerosol particles</td>
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<td>0.7 %</td>
<td>Machado BI et al. [34]</td>
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<td>Ni</td>
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<tr>
<td></td>
<td>Fe</td>
<td>No</td>
<td>10 µg/ml</td>
<td>0.6 %</td>
<td></td>
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Figure 1. Abdullaeva et al.

Schematics of the pulsed plasma in a liquid method.
Figure 2. Abdullaeva et al.

(a) HRTEM image of the Co-C nanoparticle; (b) Interplanar spacings plot along the line shown in the inset of the Co core; (c) Magnified image of the area drawn in (a), inset is the FFT of the inner Co core, suggesting that the particle is a cobalt single crystal with face centered cubic structure; (d) An EDX pattern of particle shown in (a), indicating that the particle composed of cobalt, Cu peaks from the TEM grid and C peak from onion-like carbon; e) HRTEM image of the Co-C nanoparticles; f) Diameter distribution chart of the Co-C fitted by Gaussian curve.
Figure 3. Abdullaeva et al.
(a) HRTEM image of the Ni-C nanoparticle; (b) Interplanar spacings plot along the line shown in the inset of the Ni core; (c) Magnified image of the area drawn in (a), inset is the FFT of the inner Ni core, suggesting that the particle is a nickel single crystal with face centered cubic structure; (d) An EDX pattern of particle shown in (a), indicating that the particle composed of nickel, Cu peaks from the TEM grid and C peak from onion-like carbon; e) HRTEM image of the Ni-C nanoparticles; f) Diameter distribution chart of the Ni-C fitted by Gaussian curve.
Figure 4. Abdullaeva et al.
(a) HRTEM image of the Fe-C nanoparticle; (b) Interplanar spacings plot along the line shown in the inset of the Fe core; (c) Magnified image of the area drawn in (a), inset is the FFT of the inner Fe core, suggesting that the particle is an iron single crystal with body centered cubic structure; (d) An EDX pattern of particle shown in (a), indicating that the particle composed of iron, Cu peaks from the TEM grid and C peak from onion-like carbon; e) HRTEM image of the Fe-C nanoparticles; f) Diameter distribution chart of the Fe-C fitted by Gaussian curve.
Figure 5. Abdullaeva et al.

XRD patterns of the Co-C, Ni-C, and Fe-C nanoparticles synthesized by a pulsed plasma in a liquid.
Figure 6. Abdullaeva et al.  
Optical emission spectra of the Co-C, Ni-C, and Fe-C magnetic nanoparticles synthesized by a pulsed plasma in a liquid method.
Figure 7. Abdullaeva et al.

Formation mechanism of Co-C, Ni-C, and Fe-C nanoparticles by a pulsed plasma in a liquid.

1. Plasma discharge zone

2. T= 2000-2500K
   - Metal-carbon vapor formation
   - Graphitization of carbon
   - Encapsulation of Me atoms in onion-like carbon
   - Onion-like structures formation

3. Me@C formation

4. Cooling zone
Figure 8. Abdullaeva et al.  
Raman spectra of the Co-C, Ni-C, and Fe-C magnetic nanoparticles synthesized by a pulsed plasma in a liquid.
Figure 9. Abdullaeva et al.

Magnetization curves of the Co-C, Ni-C, and Fe-C magnetic nanoparticles synthesized by a pulsed plasma in a liquid.
Figure 10. Abdullaeva et al.  
Thermal gravimetric (TGA) analysis of the Co-C, Ni-C, and Fe-C magnetic nanoparticles produced by a pulsed plasma in a liquid.
Figure 11. Abdullaeva et al.

Cytotoxicity of the Co-C, Ni-C, and Fe-C magnetic nanoparticles synthesized by a pulsed plasma in a liquid determined by MTT and XTT assays.