JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 10, No. 3, March 2008, p. 693 - 696

Study of iron oxide nanoparticles coated with dextrin obtained by coprecipitation

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In this paper we present the preparation and biological tests of iron oxide nanoparticles coated with dextrin. Iron oxide was also prepared by co-precipitation. The samples were characterized by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Biocompatibility test of the iron oxide nanoparticles coated with dextrin substrate has been done using primary osteoblast cell line. After osteoblast culture achievement, the cells were treated with trypsin 0.05% and splitted in 35/35 mm Petri dish. The influence in vitro was assessed using primary osteoblast cell line and various techniques to observe cell-particles interactions. The results of these analyses are presented in this work.

(Received September 25, 2007; accepted March 12, 2008)

Keywords: Magnetic nanoparticles, Dextrin, Cell culture, Cell viability, TEM, SEM

1. Introduction

Magnetic iron oxide nanoparticles have been used for many years because of their usefulness as contrast agent for magnetic resonance imaging (MRI) contrast agent [1,2], diagnostics, immunoassays, magnetic targeting (drugs, genes, radiopharmaceuticals), cell separation and purification as well as hyperthermia generation [3]. Studies on different contrast agents have shown that the biodistributions depends on the size, change and thickness of the coating of the particles [4].

The arrangement of the particles within clusters plays a major role in determining the observed magnetic properties [5]. On the other hand, the very small crystal size and surface effects have a strong influence on the magnetic behavior of these materials [6]. Numerous attempts have been made to establish new methods for the efficient, rapid and reliable immobilization of small molecules utilizing physical adsorption to surface [7].

In this paper, iron oxide nanoparticles coated with dextrin were synthesized according to Bunn's method [8]. The aim of this work is to investigate of iron oxide nanoparticles coated with dextrin. Their synthesis and their characterization by IR spectroscopy, transmission electron microscopy (TEM) are described and discussed in this article. This paper therefore aims to investigate the influence of such iron oxide nanoparticles coated with dextrin on cells in vitro for up to 72h.

2. Materials and method

2.1. Synthesis of iron-oxide-dextrin nanoparticles

Iron oxide nanoparticles coated with dextrin (IOD) were synthesized by on one step process: to aqueous mixture of ferrite solution (30 ml) containing stoichiometric ratio of 1:2 ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$) and ferric chloride hexahydratate ($FeCl_3 \cdot 6H_2O$) was added dropwise to the 40 ml of 5M NaOH containing 10% dextrin. This method was based on the work of Bunn et al [8] and which prepared magnetite dextran complexes using aqueous solutions of ferric and ferrous salts and mixtures of dextran with ammonia. The suspension was incubated for 1h at 90^0C for 1h with gentle stirring. The 5M NaOH was added dropwise to obtain a pH of 11. The precipitate were centrifuged and washed with deionized water. The product was separated by centrifugation (10000 rpm) and dried at 40^0C [9].

2.2. Transmission electron microscopy

A transmission electron microscopy (TEM) was carried out on a JEOL 200 CX. The specimen for TEM imaging was prepared from the particles suspension in deionized water. A drop of well-dispersed supernatant was placed on a carbon – coated 200 mesh copper grid, followed by drying the sample at ambient conditions before it is attached to the sample holder on the microscope.

2.3. Scanning electron microscopy

The scanning electron microscopy (SEM) of nanoparticles and cell morphology were investigated by a XL-30-ESEM TMP device.

The cells were fixed with 1,5% gluteraldehyde buffered in 0,1 M sodium cacodilate (4^0C, 1h) after 48 h incubation in the particles. The cells were then post-fixed in 1% osmium tetroxide for 1h and 1% tannic acid was used as a mordant. Samples were dehydrated through a series og alcohol concentration stained in 0,5 % uranyl acetate, followed by further dehydratation [10]. The final dehydratation was in hexamethyl-disilazane, followed by air-drying. Once dray, the samples were sputter coated with silver before examination.

2.4. Cell culture

Osteoblasts were grown in Dulbecco Modified Eagle's Medium (DMEM) supplied with 10% fetal bovine serum, DMEM sodium pyruvate, 2% glutamine and antibiotic mix. Medium compounds were purchased from Gibco (UK). The cells were incubated at 37°C, 5% CO_2 and the split was performed using tripsin-EDTA solution 1x (Sigma-Aldrich) and phosphate-buffered saline (PBS) from Gibco.

Osteoblasts used to determine the cell proliferation, viability and citotoxicity interaction with thin film and iron oxide coated with dextrin has been obtained from the upper part of the patient's femur. These patients undergo the surgery intervention in arthritis disease when the haunch articulation is removing.

Primary osteoblast culture from bone explants was designed according to Gallagher et al (1996) protocol [11-13]. The pieces from bone tissue are transferred into a sterile recipient with PBS. Obtained tissue is detached from soft conjunctive tissue of the external bone area. The tissue is rinsed in sterile PBS and removed in Petri dishes which contain a small volume of sterile PBS proportionally to the size of the pieces.

Next step was to place the explant fragments in DMEM with antibiotics supply, washing successively with antibody solutions, cultivate in DMEM medium supplied with 15% Bovine Serum Albumin (BSA), 2% glutamine and buffered with natrium bicarbonate.

The first osteoblasts from explants arises after 7-10 days of incubation (5% CO_2 atmosphere, T=37 ^oC) and were suitable for split after 4-6 weeks; after the second passage, the culture contains strictly osteoblast cells. Subsequent splits were performed at confluence (2×10^6 cells/plate) in about 10 days, with a 1:3 ratio. Confluent cultures have been treated with trypsin for 2-3 min and then centrifuged at 1.500 rpm for 10 min. Cells were re-suspended in minimal DMEM volume, counted with Burker-Turk chamber and evenly distributed on sterile supports, previously treated with polylisine.

2.5. Cell viability

Biocompatibility test of the iron oxide coated with dextrin (IOD) has been done using primary osteoblast cell line. After osteoblast culture achievement, the cells were treated with trypsin 0.05% and spited in 35/35 mm Petri dish.

Cells were seeded at a density of 10^5 cells/ml in Petri dish and incubated with IOD at 1% concentration for 2, 4, 12 and 24 hours. The cell viability was determined by MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction test. The cells were incubated (5% CO_2 atmosphere, T=37^oC) for 4h with MTT (0.1 mg/ml).

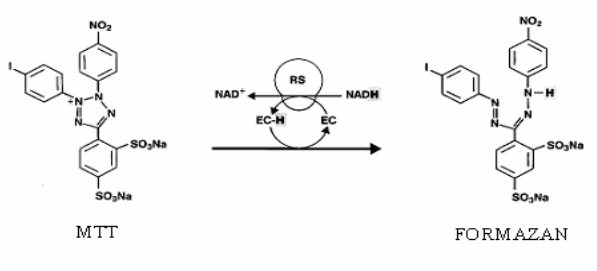

Fig. 1. MTT is reduced to formazan in mitochondria of the cells.

The viability cell number is directly proportional to the production of formazan. The isopropanol was added to dissolve the insoluble purple formazan product into a colored solution. The absorbance was quantified by measuring the wavelength at 595 nm by TECAN spectrophotometer.

3. Results and discussion

Fig. 2 shows TEM images and grain size distributions from TEM of iron oxide nanoparticles coated with dextrin.

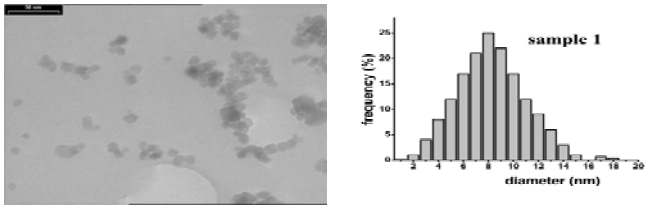

Fig. 2. Transmission electron microscopy images of iron oxide nanoparticles coated with dextrin and grain size distribution from TEM.

The TEM image of the iron oxide nanoparticles coated with dextrin was used to determine the shape, size and uniformity of the particles. As can be seen in Figure 2 the iron oxide nanoparticles are nearly spherical and monodispersed with an average diameter of about 8.0 nm.

Results obtained by scanning electron microscopy analysis for the iron oxide nanoparticles coated dextrin powder is shown in Fig. 3.

To analyze the morphology and crystallite size, SEM analysis has been conducted. As confirmed by SEM micrograph the attachment of the dextrin on the iron oxide nanoparticles surface. The iron oxide nanoparticles seemed to be mostly incorporated in the dextrin spheres and no free magnetic particles were discernable in SEM.

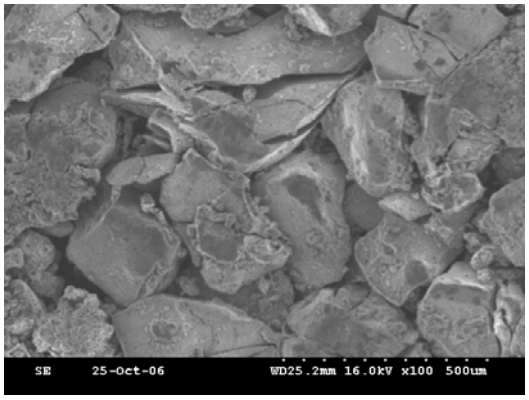

Fig. 3. Typical SEM image of iron oxide nanoparticles coated dextrin.

SEM observation of cell morphology for control cells demonstrated flattened spread cells with osteoblast (Fig. 4).The IOD nanoparticles did appear to be internalized by the cell surface, seemed to have bumped cell morphology.

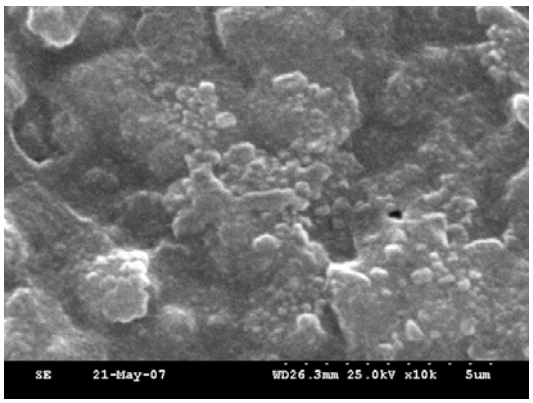

Fig. 4. SEM images of cells incubated in iron oxide nanoparticles coated with dextrin for 48 h.

Cell membrane is not disrupted in response to iron oxide nanoparticles coated with dextrin producing a large cell extension.

MTT assay is a laboratory test and a standard colorimetric assay (an assay which measures changes in colour) for measuring cellular proliferation (cell growth). It is used to determine cytotoxicity of potential medicinal agents and other toxic materials.

Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. A solubilization solution (isopropanol) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600 nm) by a spectrophotometer.

This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion is directly related to the number of viable cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced, through the production of a dose-response curve.

Table 1. Absorbance values at 595 nm.

Samples	DO_{595nm}	Viability (%)
Control	0.3000025	100
IOD – 2 hours	0.16845	56.14532
IOD – 4 hours	0.201667	67.2166
IOD – 12 hours	0.3037	101.2249
IOD – 24 hours	0.379	126.3228

Osteoblast cells were permanent monitored to detect any possible influence due to IOD bioceramic that might modify the cell growth, viability and proliferation. This study represents one of the key-step in cell biology, mitochondrial dehydrogenases being essential.

The results obtained after MTT assay have revealed (Table 1) as we expected, the fact that control sample has one of the greatest value (0.30). This value is also established by the high intensity of the color (deep purple) in control due to the amount of formazan produced by cells.

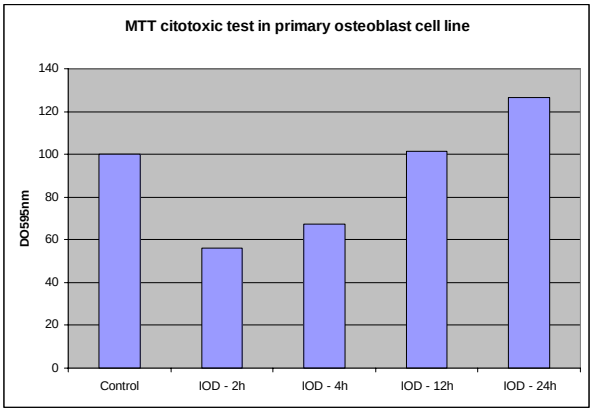

Fig. 5. MTT assay in osteoblast cell's growing with IOD bioceramic.

The study reveals that osteoblast cells' growing with IOD bioceramic for 24 hours present the highest peak, while those incubated for only 2 hours has the smallest peak (Fig. 5). The absorbance value (0.30) of control is higher comparing to the cells incubated with IOD bioceramic for 2 and 4 hours, but lower relating to cell's growing with the same bioceramic for 12 and 24 hours (Table 1).

The osteoblast cells incubated with the same bioceramic for 2, 4 and 12 hours presented a growth inhibition and the decrease of viability, relating to control (100%). Interestingly, after 2 hours of exposure with IOD and diminish in proliferation of the osteoblast cells (56.14%), we observed a tendency of linear increase of viability and proliferation after 4 hours (67.21%) which is proportionally to the exposure interval (Figure 5). This effect might be due to cells adaptation at interaction with bioceramics.

5. Conclusions

Iron oxide nanoparticles coated with the dextrin were synthesized by coprecipitation of two mains solution $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$ in a stoichiometric ratio 1:2 was added dropwise to the 40 ml of 5M NaOH containing 10% dextrin. The magnetic particles seemed to be mostly incorporated in the dextrin spheres were discernable in SEM.

The micro cells configurations made by dextrin doped with nanometric magnetite could be a suitable support for osteoblast cells adhesion and proliferation without any modification of their structure and function.

MTT test demonstrate that cells' incubation in the presence of IOD bioceramic can modify parameters of cell growing causing the increase or decrease of proliferation and viability relating to control. Thus, the exposure period (2, 4, 12 or 24 hours) could be an important factor in osteoblast cell's growing.

Acknowledgements

This work was financially supported by Science and Technology Ministery of Romania (Project CEEX_24/2005 and the Project CEEX_150/2006 VIASAN Program). We gratefully acknowledge Institut de Chimie de la Matière Condensée de Bordeaux for the use of theirs laboratories in the sample characterizations.

References

[1] L. M. Lacava, Z. G. M. Lacava, F. M. Da Silva, O. Silva, S. B. Chaves, R. B. Azevedo, F. Pelegrini, C. Gansau, N. Buske, D. Sobolovik, P. C. Morais, Biophys J., **80**, 2483-24836 (2001).
[2] L. Babes, B. Denzoit, G. Tanguy, J. J. Le Jeune, P. Jallet, J. Colloids Interface Sci. **212**, 474-482 (1999).
[3] U. Häfeli, W. S, Shütt, J. Teller, M. Zborowski, Scientific and Clinical Applications of Magnetic, Plenum, New York, 1997.
[4] D. Portret, B. Denoit, E. Rump, J. J. Jalet, J. Colloid Interface, Sci. **238**, 37-42 (2000).
[5] H. Pardoe, W. Chua-Anusorn, T. G. St. Pierre, J. Depson, Journal of Magnetism and magnetic Materials **225**, 41-46 (2001).
[6] J. Frenkel, J. Dorfmon, Nature **126**, 274 (1930).
[7] J .M. Ball, N. L. Henry, R. C. Montelaro, M. J. Newman, J. Immunol. Method **37**, 171 (1994).
[8] J. P. A. Bunn, D. C. F. Chan, D. Kirpotin, Magnetic microparticles, US Patent 5411730 (1995).
[9] D. Predoi, Digest journal of Nanomaterials and Biostructures **2**(2), 169 (2007).
[10] C. C. Berry, S. Wells, S. Charles, A. S. G. Curtis, Biomaterials **24**, 4551-4557 (2003).
[11] A. J. Gallagher, R. Gundle, N. J. Beresford, Isolation and culture of bone forming cells (osteoblasts) from human bone, Human Cell Culture Protocols (Jones, E.G., eds.), 233-263 (1996).
[12] A. M. Parfitt, Osteonal and hemi-osteonal remodeling: the spatial and temporal framework for signal traffic in adult human bone, J. Cell. Biochem. **55**, 273-276 (1994).
[13] P. G. Robey, A. L. Boskey, The biochemistry of bone in osteoporosis (Marcus, R., Feldman, D., Bilezikian, J. P., Kelsey, J., eds), Academic Press, New York, 95-183, 1995.

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