Biocompatibility Studies of Iron-oxide-dextrin Thin Films

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Dextrin coated magnetic nanoparticles have been obtained by co-precipitation. As a second step biocompatible magnetic thin film has been synthesized from the iron oxide coated with dextrin powders by pulsed laser deposition technique. Biocompatibility tests consisting of osteoblast cells growth, viability and proliferation were monitored on the surface of the thin films. The cells morphology was characterized by scanning electron microscopy (SEM) after 48h.

Keywords: iron oxide-dextrin, nanoparticles, thin films, osteoblast cells

In the last years an increasing interest in using magnetic nanoparticles for biological and medical applications developed [1-8]. The size of the particles can range from a few nanometers to several micrometers and thus is compatible with biological entities ranging from proteins to cells and bacteria. The design and synthesis of magnetic nanostructures with controlled size and uniform dispersion is an important subject of current materials research [9-12].

Iron oxide nanoparticles exhibit an excellent magnetic properties making them a potential clinical reagent applicable to magnetic resonance imaging (MRI) [13], hyperthermia [14] and drug delivery control [15].

Magnetite (Fe_3O_4) is one of the phases of iron oxide that find numerous applications due to their high saturation magnetization, high magnetic susceptibility [16-18] and optical properties [19-20]. These small particles are ideal candidates for incorporation into ultrathin films of polymers [21, 9-12]. To prevent the possible air oxidation as well as agglomeration, the Fe_3O_4 nanoparticles are coated with a surfactant [22-23] or a polymer [24-25]. Dextran is water-soluble polymer which has been clinically used for more than half a century [26].

In this paper we synthesized dextrin stabilized magnetite nanoparticles by the coprecipitation method. The obtained powders were pressed and used as targets for thin films synthesis by pulsed laser deposition (PLD).

The biological response of osteoblastic cells includes cell attachment, cell growth and functional activity. Concerning osteoblastic differentiation and metabolism, the results reported in the literature are somewhat controversial. While some papers show that increasing surface roughness enhances *in vitro* osteoblastic differentiation and inhibits cell proliferation, others indicate that proliferation can be improved on specimens submitted to higher surface roughness. These results suggest that there are other aspects that also modulate proliferation, differentiation and extracellular matrix production of osteoblastic cells *in vitro*.

In this study, the behavior of human osteoblastic cells treated with iron oxide nanoparticles coated with dextrin was evaluated in terms of cell proliferation (MTT assay).

Experimetal part

Materials

Ferrous chloride tetrahydratate ($FeCl_2 \times 4H_2O$), ferric chloride hexahydratate ($FeCl_3 \times 6H_2O$), natriun hydratate (NaOH) and dextrin $(C_6H_{10}O_5)_n$ where purchased from Merck, HCl these reagents were used directely as received. De-ionized water was used in the synthesis of nanoparticles, and in the rinsing of clusters.

Synthesis of dextrin coated iron-oxide nanoparticles

Dextrin solution (8.5 g in 85 mL of water) was heated at 90 ^{0}C for 1h with continuous agitation (200 rot/min). Then 40 mL of 5M NaOH was added to the solution. 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 solution [27-28]. The suspension was incubated for 1h at 90^{0}C for 1h with gentle stirring. The 5M NaOH was added dropwise to obtain a *p*H of 11 [29-30]. The precipitate were centrifuged and washed with deionized water. The product was separated by centrifugation and dried at 40^{0}C.

Growth of iron oxide dextrin thin film

The thin film deposition experiments were conducted inside a stainless steel reaction chamber. A frequency quadrupled Nd:YAG laser source (λ = 266 nm, $\tau_{FWHM} \cong 5$ ns, λ=10 Hz) was used for the irradiations. Prior to every deposition the reaction chamber was evacuated with the aid of a high vacuum installation down to a residual pressure of 10^{-4} Pa. The laser beam incidence angle onto the target was chosen of about 45°. The incident laser fluence was set at values in the range of (0.5-2) J/cm^2. For the deposition of each film we applied 12000 subsequent laser pulses. In table 1 is presented the sample identification as a function of incident laser fluence.

The targets were prepared by conventional pressing at 3 MPa of base dextrin coated iron oxide nano-powder material. The Si (100) substrates were placed parallel to the target at a separation distance of 4 cm and maintained at room temperature during the deposition process.

To avoid piercing the targets were rotated during the multipulse laser irradiation with a frequency of 0.4 Hz. The

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Table 1
SAMPLE IDENTIFICATION AS A FUNCTION OF INCIDENT LASER FLUENCE

Sample	Laser fluence [J/cm^2]
DF01	1.5
DF02	0.5
DF03	2
DF04	1

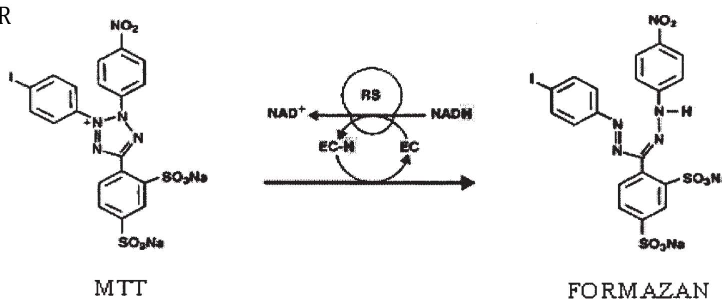

Fig. 1. MTT is reduced to formazan in mitochondria of the cells

target cleaning was performed with the application of 1000 preliminary laser pulses. During this process a shutter was interposed at the mid-distance between the target and the substrate, parallel to them. Previous experience demonstrated that this proves essential for the elimination of contaminants and impurities present on the target surface.

Scanning electron microscopy (SEM) of cells morphology

The cells were fixed with 1.5% gluteral delyde (Sigma U.K.) buffered in 0.1 M sodium cacodylate (Agar U.K.) (4 ^{0}C,1h) after 24h incubation in the particles. The cells were then post-fixed in 2% osmium tetroxide for 1h (Agar U.K.) and 1% tannic acid (Agar UK) was used as a mordant. Samples were dehydrated through a series of alcohol concentrations, stained in 0.5% uranyl acetate followed by air- drying. Once dry, the samples were sputter coated with gold before examination with a Hitachi S 800 field emission SEM.

Biocompatibility studies

Primary human osteoblast cells used to determine proliferation, viability and citotoxicity interaction with dextrin - iron oxide nanoparticles and thin films have been obtained from the upper part of patient femur. These patients were submitted to surgery intervention in arthritis affection when the haunch articulation was removed. The primary osteoblast culture from bone explants was designed according to [31-32]. The pieces from bone tissue, detached from soft conjunctive tissue of the external bone area, are transferred into a sterile recipient with PBS. 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.

Osteoblast cells were further 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.

As a next step the cells were placed 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 osteoblast cells arises after 7-10 days of incubation (5% CO_2 atmosphere, T=37°C) 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 x 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.

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 on thin film for 48 h. In contrast, osteoblast cells were seeded at a same density of 10^5 cells/mL in Petri dish and incubated with dextrin coated iron-oxide nanoparticles for 2, 4, 12 and 24 h. The cell viability was determined by MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction test (fig. 1). The cells were incubated (5% CO_2 atmosphere, T=37°C) for 4h with MTT (0.1 mg/mL).

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 with TECAN spectrophotometer.

Results and discussions

A SEM image at 48 h time points provides further information on cell morphology in response to particle incubation. The main problem associated with most studies appears to be that although the particles may localize to a cell membrane receptor, most are still internalized into the cells via receptor-mediated

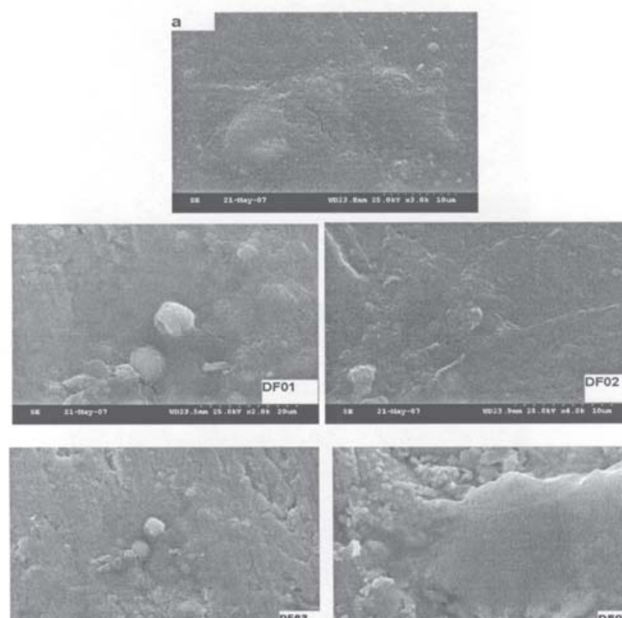

Fig. 2. SEM images of control cells (a), iron oxide nanoparticles coated with dextrin (DF01, DF02, DF03, DF04)

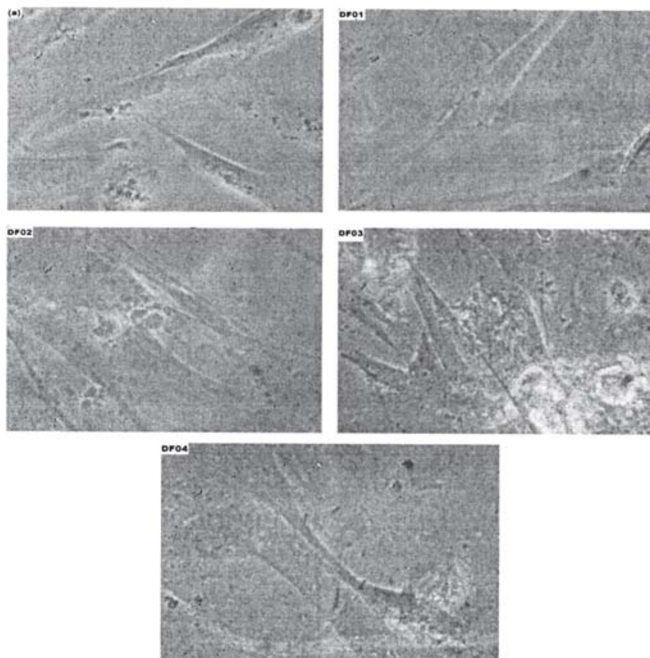

Fig. 3. SEM images of control (a), DF01, DF02, DF03 and DF04 thin films

endocytosis, resulting in cell morphology as shown in figure 2 (DF01, DF02, DF03, DF04)[33-36].

Osteoblast cells were permanently monitored to detect any possible influence due to iron oxide coated with dextrin that might modify the cell growth, viability and proliferation. Electronic microscopy was used, but we observed no evident alteration in DF01, DF02, DF03 and DF04 thin films relating to control (fig. 3).

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. This study represents one of the key-step in cell biology, mitochondrial dehydrogenases being essential.

Cells incubation in the presence of DF01, DF02, DF03 and DF04 thin films shown a decrease of viability (69.2%, 81.3%, 61.6% and 92.1%), compared to control (100%). This effect might be due to cells adaptation at interaction with samples (table 2).

The results obtained after MTT assay have revealed (fig. 4) as we expected [37-38], the fact that viability for control sample has the greatest value (0.34), comparing to thin films DF01, DF02, DF03 and DF04. This value is also established by the high intensity of the colour (deep purple) in control due to the amount of formazan produced by cells.

Table 2
ABSORBANCE VALUES AT 595 nm

Samples	DO_{595nm}	Viability (%)
Control	0.34	100
DF01	0.23	69.2
DF02	0.27	81.3
DF03	0.21	61.6
DF04	0.31	92.1

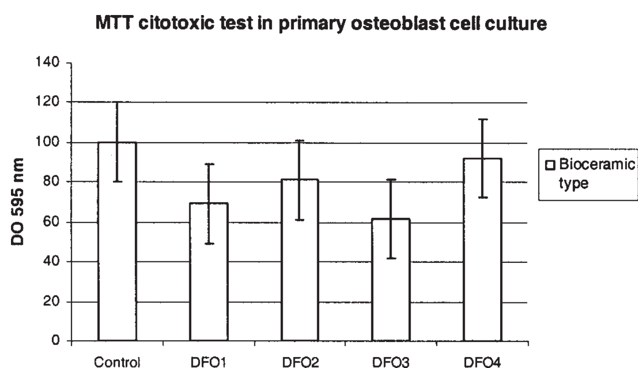

Fig. 4. MTT assay in osteoblast cells growing on DF01, DF02, DF03 and DF04 thin films

Conclusions

Iron oxide nanoparticles coated with the dextrin were synthesized by coprecipitation of two main solutions $FeCl_2 \times 4H_2O$ and $FeCl_3 \times 6H_2O$ in a stoichiometric ratio 1:2 in dextrin solution adding 5M NaOH.

The micro cells configurations made by thin films could be suitable supports for osteoblast cells adhesion and proliferation without any modification of their structure and function. The results we obtained using MTT test demonstrate that cells growing on DF01, DF02, DF03 and DF04 thin films can modify growth parameters, leading to a decrease of proliferation and viability comparing to control. DF04 and DF02 thin films are the most suitable substrates for adherence and cell proliferation regarding to DF01 and DF03. It is important to conclude that DF01, DF02, DF03 and DF04 thin films have micro cells configurations that allow them to be utilized for obtaining medical biocompatible supports.

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