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ELECTRICAL AND MAGNETIC PROPERTIES

Magnetic Nanoparticles as a Strong Contributor to the Biocompatibility of Ferrogels

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Abstract—Biomedical engineering is the most promising field for the application of ferrogels as scaffolds for cell culturing in regenerative medicine, targeted drug delivery, and biosensorics. This study is focused on the contribution of ferric-oxide magnetic nanoparticles (MNPs) to the biocompatibility of ferrogels with human dermal fibroblasts. The results of experiments with polyacrylamide gels filled with MNPs are presented. These experiments demonstrate that, regardless of the mechanical and electrical characteristics of ferrogels, MNPs have a significant effect on the biological activity of cells.

Keywords: magnetic nanoparticles, ferrogels, cells, tissue engineering

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INTRODUCTION

A ferrogel (FG) is a composite material based on a polymer that swells in a solvent and has magnetic particles embedded into its three-dimensional network. The sensitivity of an FG to the external magnetic field makes it useful in various engineering applications. Biomedical engineering is the most promising field for the application of FGs as magnetically controlled structures in regenerative medicine, targeted drug delivery, and biosensorics [1, 2]. Ferrogels may also serve as fine model materials for the study of fundamental properties of magnetic composites with small concentrations of magnetic nanoparticles (MNPs) [3, 4].

The compatibility of various cells with polyacrylamide (PAA) gels filled with iron-oxide MNPs, which were produced using electrophysical techniques, was demonstrated in our previous studies [5, 6]. Specifically, it was found that the gradual increase (from 0 to 1.0%) in the weight fraction of MNPs in the polymer gel network is accompanied by a substantial increase in the adhesion index both for human dermal fibroblasts and for human peripheral blood leukocytes. The obtained result was impossible to interpret, since the increase in the concentration of MNPs in the PAA gel was followed by a significant shift in the electrical potential and the stiffness of the FG. Both these factors (especially the elastic properties of the scaffold material for cell technologies) exert a considerable influence on the biological activity of cells [7–10].

It was demonstrated at the same time that with the Young moduli of cell culturing platforms being equal, the adhesive and proliferative activities of human dermal fibroblasts on the surface of the PAA gel are significantly lower than those on the surface of the FG based on this gel [11]. Therefore, regardless of the effect of MNPs on the FG stiffness, magnetic particles directly enhance the biological activity of cells.

It is known that the production of pharmaceuticals containing nanoparticles is limited by the size of the batch produced in a single fabrication cycle [12]. Although chemical methods for the synthesis of iron-oxide MNPs are used widely, most of them are not comparable to electrophysical methods (such as the exploding wire method [13] and laser target evaporation [14]) in terms of production rate. MNPs produced by laser target evaporation were used in [5, 6, 11]. With the synthesis conditions observed strictly and MNPs graded thoroughly, this method allows one to legitimately compare the results of different studies.

This study is focused on the search for additional arguments in favor of the positive effect of iron-oxide MNPs on the FG biocompatibility. A series of PAA ferrogels with MNPs, which are very close in their properties to the ones used earlier, was synthesized for

this purpose, and the specifics of adhesion and proliferation of human fibroblasts on these scaffolds were examined. It will be demonstrated below that the compatibility of FGs with cells increases significantly with MNP concentration in the region of MNP weight fractions (1.0–2.0%) where the electrical and mechanical properties of the material are certain to remain unchanged.

MATERIALS AND METHODS

Maghemite (γ-Fe_2O_3) nanoparticles were prepared by laser target evaporation [14], and their structure and magnetic properties were examined in detail using standard methods [3, 5, 11]. Specifically, transmission electronic microscopy (JEOL JEM2100) revealed that the particles were not agglomerated and spherical in shape. Their size distribution (plotted based on the results of graphical processing of 2160 images) was lognormal with a median of 11.4 nm and a variance of 0.423. The number-average and weighted average particle sizes were 11.7 and 18.5 nm, respectively. The BET surface area of nanoparticles determined based on the data on low-temperature nitrogen sorption (Micromeritics TriStar3000) was 78.1 m^2/g. The surface-average particle diameter was 16.7 nm. X-ray phase analysis (Bruker D8 Discover) revealed that the crystal structure of particles corresponded to an inverse spinel: space group *Fd3m*, which is characteristic of magnetite and maghemite. The chemical composition of particles was determined by potentiometric titration (Schott Titriline) and corresponded to maghemite (γ-Fe_2O_3).

The magnetic properties of γ-Fe_2O_3 MNPs were studied at room and cryogenic temperatures with a SQUID magnetometer (Quantum Design MPMS-7).

PAA hydrogels were synthesized by free-radical polymerization in a 1.6 M aqueous solution of a monomer (acrylamide) with the use of *N*,*N*'-methylenediacrylamide (MDAA) as a crosslinking agent. The MDAA/AA ratio was set so as to achieve a crosslink density of 1 : 100 in the polymer network of hydrogels. The process of polymerization was initiated by 3 mM ammonium persulphate, and 5 mM *N*,*N*,*N*',*N*'-tetramethylethylenediamine served as a catalyst. All of these reagents were produced by Merck (Germany).

The reaction mixture for FG synthesis was prepared with the use of an aqueous suspension of nanoparticles stabilized with 5 mM sodium citrate. The mixture was disaggregated by ultrasonic treatment (30 min, 250 W) using a CPX-750 processor (Cole-Parmer Instruments Corp., United States) under continued cooling. The remaining aggregates were removed by centrifuging at 8000 rpm for 5 min. The ferrofluid was then diluted with 5 mM sodium citrate so as to obtain ferrogels with an MNP weight fraction of 1.0 or 2.0%.

Gels and FGs were polymerized for 60 min at 25°C in specially prepared frames to produce samples of the needed size. Specifically, cylinders ~10 mm in diameter and ~8 mm in height were fabricated for electrical and mechanical tests. Disks with a thickness of ~1 mm and a diameter of ~13 mm were prepared for experiments with cell cultures. Immediately before use, these disks were sterilized in an autoclave for 20 min at 121°C.

The elastic properties of gels and FGs were determined using a laboratory setup for mechanical tests [5]. Cylindrical samples were placed between two plates. One was connected rigidly to the actuator of a linear electromagnetic motor, and the other was connected to a precision strain-gage sensor. The motor induced compression strain with a magnitude up to 20% in steps of 2% of the initial gel length. Stress–strain dependences were plotted as a result of these tests, and their linear sections were used to determine the Young modulus for the tested materials.

The electrochemical potential of gels was determined using the standard patch-clamp technique, which is routinely applied to living cells. Specifically, two identical silver-chloride electrodes in glass micropipettes (World Precision Instruments, Inc., United States) with a tip diameter of ~1 μm filled with a 3 M KCl solution were used. One electrode was placed in the solution surrounding the gel, and the other one was introduced into the studied sample. The potential difference was measured with an INA 129 instrumentation amplifier (Burr-Brown, United States).

Human dermal fibroblasts were chosen to estimate the compatibility of cells with gels. The primary cell culture was prepared from a ~0.5 × 1 cm piece of human skin cut out during a routine surgical operation. Written permission was obtained from the patient in advance. Experiments with biological material were approved by the ethics committee of the Institute of Medical Cell Technologies.

The procedure of fibroblast culture preparation was detailed in [5, 6, 11]. Gel discs were introduced into the wells of 24-well plates (Techno Plastic Products, Switzerland), and the growth medium suspension with a density of 3000 cells/cm^2 was then added. For reference, cells were also introduced into gel-free wells. The plates were held in a CO_2 incubator up to the moment of measurement. The biocompatibility of materials was estimated by the density of the cell monolayer (i.e., the number of fibroblasts per 1 cm^2 of the sample surface after 12 h and 4 d of incubation).

Following incubation, cells on gel discs and on the culture plastic were washed with phosphate buffer, fixed, and stained with DAPI (nuclei) and pyrazolone yellow (cytoplasm). An Axio Lab.A1 FL fluorescence microscope (Carl Zeiss, Germany) was used for imaging. ImageJ (Wayne Rasband, NIH, United States) was used to count the number of cells in images in each well in nine fields of view.

The data were processed statistically in STATISTICA 6.0. The two-tailed Mann–Whitney U test was used for comparative analysis of two independent groups; the statistical hypothesis was assumed to be verified at $P \le 0.05$.

RESULTS

The magnetic measurements (Fig. 1) provided data on the magnetic response of MNPs, which are needed to analyze the magnetic and magnetomechanical FG response [15]. It can be seen that the curve for particles cooled in advance in zero field (ZFC) has no maximum (Fig. 1a). It is also evident that this curve deviates from the curve (FC) for particles cooled in advance in a 100 Oe field. These results suggest that MNPs are in the blocked state. To estimate the magnetic parameters of MNPs, the curves of approaching magnetization to saturation were fitted with the following relation [14]:

$$M(H) = M_s\left(1 - \frac{1}{15}\frac{H_a^2}{H^{1/2}\left(H^{3/2} + H_R^{3/2}\right)}\right) + \chi H, \quad (1)$$

where M_s is the saturation magnetization, H_a is the anisotropy field, H_R is the exchange field in the core–shell system, and χ is the magnetic susceptibility.

This approximation characterizes well the magnetization curves in strong fields (Fig. 1b) and allows one to estimate the saturation magnetization of particles.

The saturation magnetization of particles is crucial to further discussion (Table 1).

Note that the temperature gradient of magnetization M_s near room temperature is marginal. The saturation magnetization of particles in the above experiments with living cells may therefore be assumed constant and equal to 319 G.

The results of measurements of the electrical potential and the Young modulus in the samples of PAA gels and FGs based on them with different MNP concentrations are listed in Table 2. These data are presented in the form of $X \pm \sigma$, where X is the mean parameter value ($n = 6$) and σ is the standard deviation. It can be seen that the introduction of MNPs (1% by weight) into the PAA gel leads to a considerable increase in the electronegativity and the elasticity of samples ($p < 0.01$). At the same time, ferrogels had close parameters characterizing the electrical and mechanical properties of samples in the indicated range of MNP concentrations.

The results of quantitative assessment of fibroblasts on the surface of the studied materials after 12 hours and 4 days of incubation in plate wells are listed in Table 3. The fibroblast monolayer density was analyzed in 54 images per material type. The data are presented in the form of $X \pm m$, where m is the standard error of the mean (X).

According to the obtained data, the density of the cell monolayer on the surface of FGs was higher than that on PAA gels. Conspicuous is the fact that the

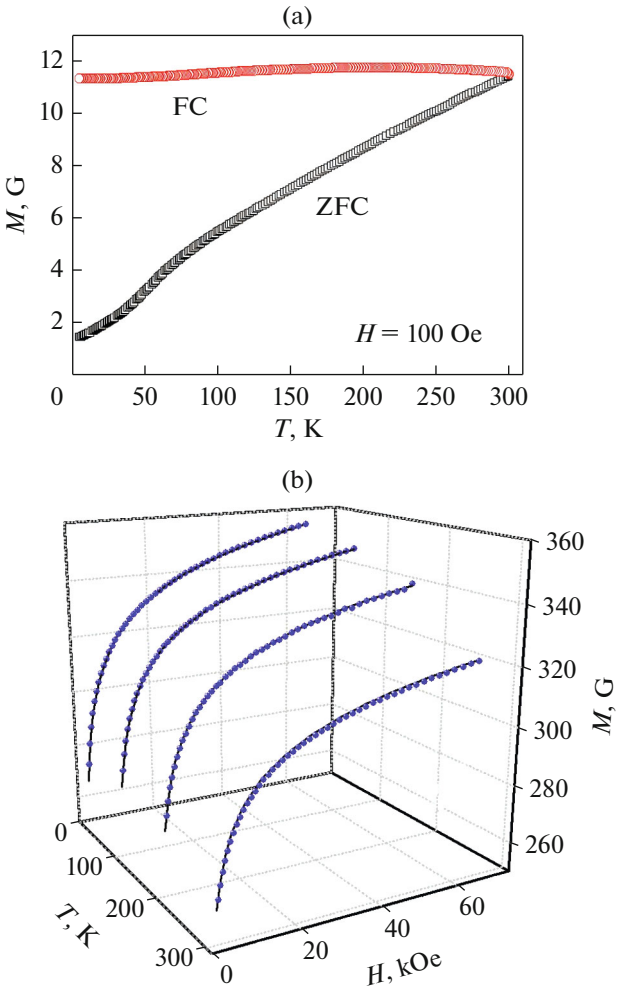

Fig. 1. (a) Thermomagnetic curves ZFC–FC, $H = 100$ Oe. (b) Magnetization curves in the near-saturation region for several temperatures.

Table 1. Saturation magnetization of particles

T, K	5	100	200	300
M_s, G	359	348	337	319

Table 2. Electrical and mechanical characteristics of the polyacrylamide gel and ferrogels based on it (see text for details)

Material	Potential, mV	Young modulus, kPa
PAA-100	−11 ± 2	22 ± 5
PAA-100 + 1% Fe	−38 ± 6	42 ± 4
PAA-100 + 2% Fe	−39 ± 4	47 ± 6

Table 3. Density of fibroblasts per 1cm^2 of the studied surface. $P \le 0.05$ relative to the reference sample (*) and to PAA-100+2% Fe (†)

Material	12 h	4 d
PAA-100	480 ± 70*†	1300 ± 200*†
PAA-100 + 1% Fe	2400 ± 200*†	3200 ± 400*†
PAA-100 + 2% Fe	2900 ± 200*	6800 ± 800*
Plastic (reference)	3900 ± 400	18 000 ± 1500

number of cells per 1 cm^2 on the surface of the FG with an MNP weight fraction of 2% was meaningfully higher than that on the surface of the FG with 1% MNPs after both 12 hours and 4 days of incubation. Figure 2 is a qualitative illustration of this pattern.

It should be added that the fibroblast monolayer density on the culture plastic (reference) was meaningfully higher than the densities of monolayers on all the other materials. This confirms the viability of the used cell culture. It is also evident (see Table 3) that the fibroblast monolayer density on ferrogels increases with MNP concentration in the PAA gel. In other words, FGs with a higher MNP concentration are more biocompatible.

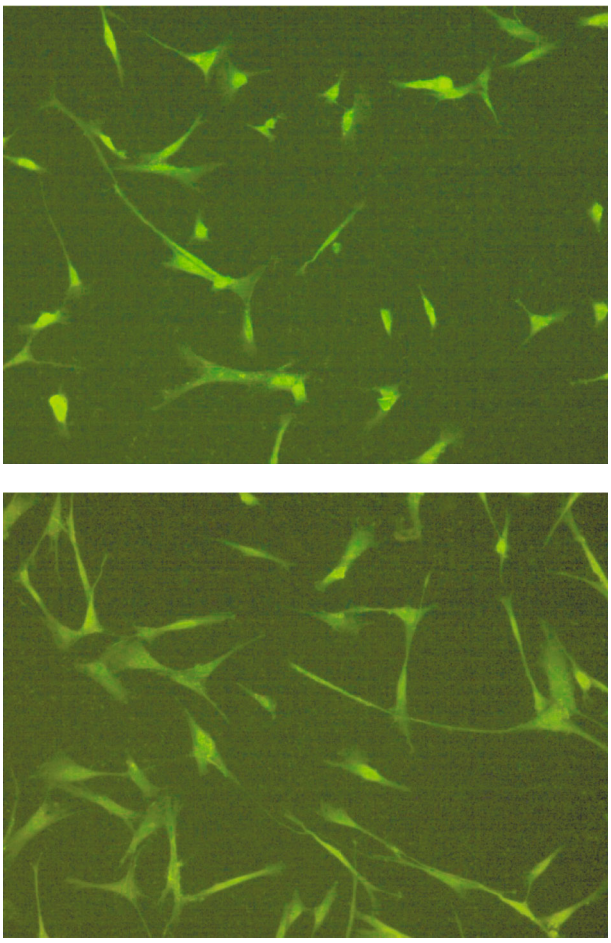

Fig. 2. Example visualizations of human dermal fibroblasts on the surface of FGs with an MNP weight fraction of 1 (upper panel) and 2% (lower panel) after 4 d of incubation. Magnification: ×100; the cytoplasm is stained with pyrazolone yellow.

DISCUSSION

A certain amount of knowledge and expertise in the use of composite materials as scaffolds for cell culturing in tissue engineering and regenerative medicine has already been accumulated. Ferrogels are considered among the most promising materials of this type, since their physical properties may be altered by the external magnetic field. At the same time, several unresolved issues hinder the construction of new FG-based scaffolds. The contribution of MNPs to the biocompatibility of magnetic composites is one of these issues.

This study is largely theoretical in nature. It is a logical continuation of the series of earlier studies focused on the contribution of MNPs to the regulation of compatibility of living cells with FGs [5, 6, 11]. It was demonstrated in all these studies that MNPs have an overall positive effect on the biological activity of cells on FG surfaces. However, the key factors behind this effect remain unknown.

MNPs may influence the biological processes in cells directly and indirectly. The effect of MNPs on the elasticity, the electrical potential, and the surface roughness of FG scaffolds and on the magnetic field around cells are the factors of indirect influence. The contribution of most of them to the biological activity of cells was confirmed experimentally in [7–10].

In this study, we tried to exclude the possible influence of two key indirect factors (elasticity and electrical potential) on the results of FG biocompatibility tests. Ferrogels with an MNP weight fraction of 1% and 2% were synthesized for this purpose. This choice was dictated by the results presented in [5, 6], where the dependences of the Young modulus and the electrical potential on the MNP weight fraction in PAA ferrogels were obtained.

Specifically, it was found that the introduction of a minimum amount of MNPs (0.25%) into the PAA gel results in a considerable stiffness enhancement. This stiffening trend persisted through to an MNP weight fraction of 0.75%. When the concentration was raised further to 1.0%, the FG elasticity did not change in a significant way. The dependence of the electrical potential in ferrogels on the MNP weight fraction was qualitatively similar: the potential shifted abruptly toward negative values at the minimum concentration (0.25%) and varied smoothly at higher concentrations, leveling off at 0.75%.

Thus, it was hypothesized that the mechanical and electrical properties of magnetic scaffolds should not

be affected in a significant way if the MNP weight fraction in FGs increases to 1% or goes even higher. This is the reason why ferrogels with an MNP weight fraction of 1 and 2% were synthesized. It should be added that the synthesis of FGs with an MNP weight fraction higher than 2% was proven to be technologically infeasible at the present time.

At the same time, the obtained results of measurements of the Young modulus and the electrical potential in FGs with two different MNP concentrations demonstrate clearly that they have no significant differences in parameters (see Table 2). It is important that all the tested parameters in ferrogels were meaningfully better than the corresponding parameters in the PAA gel used to synthesize these FGs. This agrees completely with the available data [5, 6, 11].

It was also found that the number of cells per unit surface area of scaffolds increases considerably as the MNP weight fraction in gels changes from 0 to 2% (see Table 3). It is fundamentally important that the density of the fibroblast monolayer on ferrogels with an MNP weight fraction of 1% was markedly higher (specifically, ~2 times higher after 4 days of incubation) than that on FGs with 1% MNPs. Therefore, regardless of the elastic and electrical characteristics of FG-based scaffolds, the compatibility of cells with magnetic composites improves as the MNP concentration increases: MNPs contribute strongly to the biocompatibility of scaffolds used for cell culturing.

The potential role of MNPs in shaping the parameters of the FG surface was recently examined in [11]. It was demonstrated that PAA gels with a crosslink density of 1 : 50 and ferrogels based on PAA-1:100 with an MNP weight fraction of 0.34% had similar Young moduli, but differed fundamentally in their compatibility with human dermal fibroblasts. In addition, the high biocompatibility of FGs was associated with higher surface roughness of the magnetic composite.

The surface roughness of the PAA gel and FGs was estimated using an electron microscope. The studied samples had to be desiccated prior to these studies. The lack of a solvent in gels forbids one from treating this result as direct evidence of the contribution of MNPs to the FG surface modification. However, this fact may be regarded as indirect proof of the validity of the hypothesis stating that the specifics of the FG surface structure and the FG biocompatibility are related.

Note that the magnetic field produced by the MNP system inside and on the surface of an FG should become stronger as the particle concentrations increases from 1 to 2%. Since the fields produced by magnetic particles decrease rapidly with distance, it can be inferred that the strength of these fields from particles with a saturation induction of ~4000 G should not be higher than several (or several tens of) oersted at almost any point in the bulk of an FG. According to [16, 17], such MNP concentrations and such magnetic fields do not induce any significant surface deformation. However, the magnitude of irreversible deformation associated with the displacement of particles in an FG may increase with time. In contrast to [16, 17], the incubation times in the present study were very long (12 hours and 4 days). The magnetomechanical effect may therefore be significant.

The direct influence of MNPs on the biological activity of fibroblasts should not also be overlooked when one considers the compatibility of cells with FGs. A considerable number of papers dealing with the influence of iron-oxide particles on the properties of cells and tissues have already been published, but the results presented are controversial. This suggests that the indirect influence of MNPs effected by altering the physical properties and the structure of the material is the most probable justification for the fine biocompatibility of FGs.

Thus, regardless of the specific direct and/or indirect factors of influence of MNPs on the biological activity of fibroblasts, iron-oxide nanoparticles contribute beneficially to the biocompatibility of ferrogels.

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