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Dynamic and biocompatible thermo-responsive magnetic hydrogels that respond to an alternating magnetic field

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ABSTRACT

Magnetic thermo-responsive hydrogels are a new class of materials that have recently attracted interest in biomedicine due to their ability to change phase upon magnetic stimulation. They have been used for drug release, magnetic hyperthermia treatment, and can potentially be engineered as stimuli-responsive substrates for cell mechanobiology. In this regard, we propose a series of magnetic thermo-responsive nanocomposite substrates that undergo cyclical swelling and de-swelling phases when actuated by an alternating magnetic field in aqueous environment. The synthetized substrates are obtained with a facile and reproducible method from poly-N-isopropylacrylamide and superparamagnetic iron oxide nanoparticles. Their conformation and the temperature-related, magnetic, and biological behaviors were characterized via scanning electron microscopy, swelling ratio analysis, vibrating sample magnetometry, alternating magnetic field stimulation and indirect viability assays. The nanocomposites showed no cytotoxicity with fibroblast cells, and exhibited swelling/de-swelling behavior near physiological temperatures (around 34 °C). Therefore these magnetic thermo-responsive hydrogels are promising materials as stimuli-responsive substrates allowing the study of cell-behavior by changing the hydrogel properties in situ.

1. Introduction

Hydrogels, hydrophilic polymer networks which can absorb > 10–30% of their dry weight in water, are attracting considerable interest in biomedicine and as biomaterials due to their intrinsic properties such as biocompatibility, structure, and chemical and mechanical behavior [1–4]. Moreover, these materials can be tailored to possess properties similar to that of native biological tissues. Recently a novel class of hydrogels, which offer additional features, has been proposed: magnetic hydrogels that are composite materials comprised of magnetic nano- or microparticles incorporated within a polymeric matrix [5]. In biomedicine, these magnetic hydrogels have been investigated for a variety of applications. For example, magnetic actuators that combine high elasticity and magnetic behavior have been developed to mimic skeletal muscles behavior [6], and magnetic bio-hybrid scaffolds with improved physico-chemical and mechanical properties have been engineered for bone regeneration [7]. Furthermore an emerging application of magnetic hydrogels is to implement superparamagnetic iron oxide nanoparticles (SPIONs) as a functional, stimuli-responsive component within thermo-responsive hydrogels [8,9]. Thermo-responsive hydrogels are materials that swell or collapse upon temperature changes, and by incorporating SPIONs it is possible to magnetically trigger this transition.

SPIONs with a core diameter between 5 and 28 nm [10], when exposed to an alternating magnetic field (AMF), dissipate heat though Néel and Brownian relaxation phenomena [11,12]. When incorporated into a thermo-responsive hydrogel, the heat is then transferred to the surrounding polymeric matrix thereby inducing conformational change and polymer collapse [13]. These magnetic hydrogels have a reversible behavior and therefore can re-acquire the initial conformation when the AMF is off [14]. Several groups have thus developed remotely triggered drug delivery systems with micro-gels [15,16], injectable hydrogels [17] and solid patches that can release drug when exposed to an AMF [14,18]. Others have proposed hydrogels that potentially can target and heat a tumor for hyperthermia treatment [19].

However hyperthermia treatments and drug delivery technologies are not the only fields that benefit from magneto thermo-hydrogels. In tissue engineering, and specifically mechanobiology, the temporal

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Table 1
Summary of synthetic parameters.

	NIPAM (mg)	BIS (mg)	TEMED (μL)	Milli-Q Water (ml)	SPIONs (ml) conc.=8.46 mg/ml	APS 10% w/v (μL)
PNIPAM 0%	318	4.5	1.88	3	0	18.75
PNIPAM-SPIONs 1%	318	4.5	1.88	2.62	0.38	18.75
PNIPAM-SPIONs 2.5%	318	4.5	1.88	2.05	0.95	18.75
PNIPAM-SPIONs 5%	318	4.5	1.88	1.1	1.9	18.75

variations of a substrate surface are thought to be fundamental in governing cell adhesion, morphology and differentiation [20–22]. Hence to mimic the natural cells' environment that undergoes remodeling in physiological or pathological conditions [23,24], a classical petri dish or a static cell substrate with defined mechanical properties is not sufficient. To overcome this limitation and study the cellular reaction to mechanical environmental changes, several dynamic cell-culture substrates, in which stiffness and topography can be tuned in situ, have been developed [20,25,26].

In the growing field of dynamic, stimuli-responsive cell substrates there are only a few which involve the use of magnetic hydrogels [27–29] and none rely on AMF stimulation. In particular, acrylamide gels loaded with nickel micro-wires were shown to alter their surface roughness upon the application of a static magnetic field and to induce changes in the adhesion area of vascular smooth cells [27]. Another study focused on the development of a magneto-active elastomer that changes stiffness and topography after application of static and oscillating field [29]. This substrate was used to study migration and morphology of human fibroblasts. Moreover, cylindrical micro-pillars made of iron oxide nanoparticles-loaded poly(caprolactone)-based polymer substrates were bent and extended with the use of an AMF. However in the presence of cells, the morphology of the substrate was triggered only by a direct change in the temperature of the cell culture medium and not by the AMF [28]. Thus, by including SPIONs directly into a thermo-responsive hydrogel it is possible to trigger mechanical changes in a hydrogel substrate via an AMF.

Here we report a magnetic, thermo-responsive model substrate based on poly-N-isopropylacrylamide (poly-NIPAM) embedded with SPIONs that reversibly changes phase upon AMF stimulation. The hydrogels were prepared as films covalently crosslinked to a glass coverslip, each containing different concentrations of nanoparticles. These materials combine good thermal behavior (change swelling and de-swelling behavior over a narrow temperature range), superparamagnetic properties, and have a high degree of biocompatibility. When excited with AMF they show a reversible de-swelling/ swelling process at physiological temperatures. Moreover, variations of the substrate mechanical properties (e.g. hydrogel elasticity and topography) can be related to the hydrogel phase change [25,30]. The hydrogels were biocompatible in both the swollen and de-swollen states as shown by a biocompatibility test for murine fibroblasts in vitro. Hence the proposed materials have the potential to be used as stimuli-responsive substrates for cell culture.

2. Material and methods

2.1. Materials

2.1.1. Chemicals

All chemicals were of analytical reagent grade unless further specified and were used without any purification except for the monomer N-isopropylacrylamide (NIPAM) which was purified prior to use. For NIPAM purification, 20 g was pre-purified by recrystallization with hexane (300 ml). Then the precipitate was filtered and washed with ice-cold hexane to give colorless crystals (19 g); the final monomer was recollected and stored at 4 °C. Iron(III) chloride hexahydrate (99%), oleic acid (technical grade, 90%), citric acid (99.5%), N,N-dimethylformamide (DMF, 99.8%), 1,2-dichlorobenzene (DCB, 99%), N,N'-Methylenebis(acrylamide) (BIS, 99%, MW=154.17), ammonium persulfate (APS, 98%) hydrochloric acid (HCl, 37%), nitric acid (HNO_3, 65%), potassium hexacyanoferrate(II) trihydrate (98.5%) and diethyl ether (99%) were supplied by Sigma-Aldrich. Sodium oleate (97%) and tri-*n*-octylamine (97%) were purchased from TCI Europe N.V. Absolute ethanol, acetone and 2-propanol were purchased from VWR Chemicals. 3-(trimethoxysilyl)propylmethacrylate (TPM) was purchased from ABCR GmbH and acetic acid (glacial, 100%) from Merck. N,N,N,N'-tetramethylethylenediamine (TEMED) was obtained from Bio-Rad and N-Isopropylacrylamide (NIPAM, 99%, MW=113.16) from ACROS Organics. All aqueous solutions were prepared with deionized water obtained from a Milli-Q system (resistivity=18.2 Ω, Millipore AG).

2.1.2. Cell culture

NIH-3T3 murine fibroblasts were obtained from American Type Culture Collection (ATCC). Cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% v/v calf bovine serum (ATCC® 30–2030™), 2 mM L-glutamine and 1% v/v Penicillin-Streptomycin at 37 °C and 5% CO_2 in a humidified incubator.

2.2. SPIONs synthesis

SPIONs with a core diameter of 21.8 ± 0.9 nm (average ± SD, n =1258) were synthetized by thermal decomposition according to a modified procedure reported by Park, et al. [31]. In brief, an iron oleate complex was prepared reacting iron chloride ($FeCl_3{\cdot}6H_2O$) with sodium oleate. Then the complex was thermally decomposed in presence of oleic acid at 320 °C in tri-*n*-octylamine for 1 h (details in Supplementary Information). The solution was rapidly cooled down, and the nanoparticles were separated and washed by sequential centrifugations. Resulting oleic acid coated nanoparticles were re-dispersed in hexane and stored at 4 °C.

2.3. SPIONs functionalization

A ligand exchange procedure was performed in order to exchange oleic acid on the SPION surface with citric acid, thereby yielding nanoparticles colloidally stable in polar (i.e. aqueous) solvents and suitable for incorporation in the polymer [32]. For this, the SPIONs were dispersed in a solution of DCB and DMF (ratio 1:1). Citric acid was then added (0.8 mg citric acid/mg Fe) and the solution was stirred for 24 h at 100 °C. Following this, the nanoparticles were precipitated in 200 ml of diethyl ether and recovered with a magnet (Nickel-plated NdFeB, Supermagnete). The resulting citric acid coated SPIONs were washed in acetone and finally re-dispersed in Milli-Q water.

2.4. Transmission electron microscopy

The size and morphology of citric acid SPIONs was investigated by transmission electron microscopy (TEM). Samples were prepared drying the nanoparticle suspensions on copper carbon-coated mesh grids. Micrographs were acquired with a Tecnai Spirit transmission electron microscope (FEI) operating at 120 kV and equipped with a Veleta CCD camera (Olympus). The core diameters of the nanoparticles