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MAGNETICALLY MODIFIED ELECTROSPUN NANOFIBERS FOR HYPERTHERMIA TREATMENT

Several methodologies for the preparation of nanofibrous materials exist. Electrospinning is currently the most popular technique due to its versatility and simplicity. Nanofibrous materials prepared in such a way are widely studied in medicine and material engineering. Polyvinyl butyral (PVB) nanofibers were generated by a rod-shaped spinning-electrode. Nanofibers were modified by a magnetic fluid (MF) added into the PVB solution. These magnetic nanofibers can be considered as a material for magnetic hyperthermia applications, either as implants or for the surface heating. The samples with various magnetic particle concentrations were tested in the alternating magnetic field. An immediate increase in the temperature after the field application was observed. The nature of the temperature rise is interesting: a non-linear increase could be seen, which is in contrast to the rising temperature for pure magnetic fluids.

K e y w o r d s: electrospinning, magnetic fluid, polyvinyl butyral, alternating magnetic field, hyperthermia.

1. Introduction

Electrospinning is an intensively studied technology which can be used to prepare nanofibers or nanofiber mats from polymer solutions or melts, with possible incorporation of magnetic nanoparticles. There are several electrospinning procedures for electrospinning processes, including the needle-based and needleless techniques. Electrospun nano- and microfibers can be prepared from various (bio)polymers, including polyurethanes, polymethacrylates, polyvinylpyrrolidone, polyacrylonitrile, polyvinyl alcohol, degradable polyesters such as poly(L-lactide), poly(L-lactide)-co-poly(glycolide) and polycaprolactone (PCL), as well as from polysaccharides like chitosan. Blending different polymers enables to prepare fibers with improved properties. In many cases, it is also possible to prepare magnetically modified nanofibers [1, 2].

Different strategies can be employed to generate magnetically responsive nano- and microfibers. The main one is based on the direct mixing of magnetic nanoparticles with a polymer solution or blend followed by the electrospinning. Of course, the standard

experimental methods have to be used for the structural characterization of liquid systems containing nanoparticles. Especially, the application of neutron and X-ray scattering techniques may yield rich information on the morphology, size, concentration, and interaction between the dispersed nanoparticles [3–5].

To obtain homogeneous nanoparticle dispersions in a mixture, the mechanical stirring and ultrasonication are usually employed. However, magnetic nanoparticles exhibit a high tendency to agglomerate, thus being unevenly distributed in the produced fibers. Appropriate stabilizing agents such as surfactants or specific polymers are added together with the magnetic nanoparticles to the polymer solution prior to the electrospinning process [1]. In another approach (*in situ* synthesis), the poly(ethylene oxide) polymer solution containing $FeCl_3$ and $FeSO_4$ was employed; electrospinning was carried out in an ammonia atmosphere to convert iron compounds to iron oxide nanoparticles. TEM showed the polymer nanofibers to be 200–400 nm in diameter, and it also proved the presence of the nanoparticles on polymer electrospun nanofibers [6]. Electrospun nanotextile can also be magnetically modified using the simple spray procedure employing appropriate magnetic fluids. The magnetic modification led to the deposition of magnetic iron oxide nanoparticles on the surface of textile nanofibers; magnetic modification has not substantially changed the structure of the modified nanotextile [7]. In addition the simple post-magnetization process based on the chemical coprecipitation, via immersing the produced fibrous mats in an aqueous solution containing Fe(II) and Fe(III) salts at appropriate molar ratios, followed by the addition of a weak base to yield magnetic iron oxide nanoparticles, can be employed [8, 9].

Recently, a solvent-free, biofriendly melt electrospinning technology has been used for the preparation of fibers for magnetic hyperthermia. The procedure enabling a homogeneous distribution of magnetic nanoparticles in the melt of polycaprolactone was developed. In addition, a hand-held melt e-spinning device was designed [10].

A potential new strategy has been suggested recently for the magnetic hyperthermia treatment, based on the use of biocompatible electrospun fiber webs, heavily loaded with magnetic iron oxide nanoparticles. The emerging potential of electrospun nanofibers in cancer researches has been reviewed recently [11, 12]. The high loading capacity of the magnetically modified fibers and a very high surface to volume ratio enables their efficient interactions with tumors and surrounding cancer cells and a significant heating of the environment when an alternating magnetic field is applied. The surface of polymer magnetic fibers can be usually functionalized for specific applications. Well-prepared magnetic fibers can be repeatedly heated without loss of the heating capacity or release of magnetic nanoparticles. Because the fiber webs can be modified with a well-controlled amount of magnetic nanoparticles and can be localized in the body by Magnetic Resonance Imaging, magnetically modified electrospun fibers can become promising materials for a highly reproducible and repeated heating of cancer tissues *in vivo*. The magnetically modified fibers can be surgically located at the tumor site. A modification of the fiber surface can enable the binding of circulating cancer cells which can be subsequently destroyed by the magnetic heating [13].

On the contrary to the standard hyperthermia treatment with free magnetic particles, where usually two distinct heating mechanisms are considered (namely Brownian motion and Néel relaxation), the iron oxide particles in magnetically modified fibers are strongly anchored onto the polymer phase [14–16]. That's why the Brownian motion is probably very limited, while the Néel relaxation and hysteresis losses (for relatively large nanoparticles) are the main mechanisms of magnetic energy dissipation. Polymer fiber webs containing magnetic iron oxide nanoparticles can be usually repeatedly heated without loss of the heating capacity or release of particles [17].

The prepared smart hyperthermia nanofibers enabled the simultaneous heat generation and drug release; the nanofiber is composed of a temperature-responsive polymer with an appropriate anticancer drug and magnetic nanoparticles, which serve as a trigger of the drug release and a source of heat, respectively [18, 19].

The precise deposition of magnetically modified fibers on a specific surface including human skin and potentially tumors by portable electrostatic spinners has been described recently. These techniques enable one to deposit nanostructured fibers on complex, irregular tumor tissue to form continuous, compact, flexible membranes with excellent integrity, which act as a powerful source for the local heating of the tumor

region to the desired temperature without overheating the surrounding healthy tissues [10, 20].

Currently, most of the *in vitro* studies are performed on cancer cells, employing magnetically modified fibers for hyperthermia. The experiments confirmed the efficient cell death of cancer cells bound to magnetic fibers. The cell death occurred at short exposure times, which indicates a high cell killing efficiency of the magnetic fiber webs. However, the further inspiration can be found in *in vivo* experiments with drug-loaded electrospun nanofibrous systems designed for local anticancer therapy, as reviewed recently [21].

2. Materials and Methods

The magnetic nanofibrous samples were formed from the mixture of a mineral oil based magnetic fluid (MF) containing Fe_3O_4 nanoparticles and polyvinyl butyral (PVB) polymer which is often used for the electrospinning process at high voltages [22, 23]. Fe_3O_4 nanoparticles were prepared by the coprecipitation method using $FeCl_3 \times 6H_2O$ and $FeSO_4 \times \times 7H_2O$, in the presence of NH_4OH; the mineral oil-based MF was prepared using the well-known procedure [24, 25]. The prepared MF had a magnetite concentration of 277 mg/ml and the mean particle diameter of 10.55 nm. The magnetite concentration was determined from magnetization measurements as the ratio of the magnetization saturations of a magnetic fluid and magnetite nanoparticles [26]. The mean particle size was determined by transmission electron microscopy; the subsequent number-based particle size distribution led to a standard deviation of 2.17 nm [27]. The 10% (w/w) solution of PVB was prepared in a ethanol-isopropyl alcohol mixture (4 : 1). This polymer solution (45 ml) was mixed at room temperature with 4 ml (sample: PVB + MF concentrated) and 2 ml (sample: PVB + MF) of the magnetic fluid. Thus, the resulting mixtures of PVB and MF with Fe_3O_4 concentrations of 22.6 mg/ml and 11.3 mg/ml were formed. The prepared magnetic colloid was then electrospun following the standard electrospinning approach; for the optimal spinning of the used solution, an ac high voltage of 40 kV at a frequency of 50 Hz was used.

The nanofibrous magnetization was measured by a vibrating sample magnetometer (VSM) installed on a cryogen free superconducting magnet from Cryogenic Ltd. The measurements were performed at room temperature in a magnetic field up to 5 T.

An alternating magnetic field (AMF) was generated by a home-made setup. A high-frequency sinusoidal signal was amplified by an AL-600-HF-A power amplifier (Amp-Line; West Nyack, NY, USA) connected to the LC circuit. The AMF was generated in the middle of the coil cavity, where the sample was placed. An FPI-HR module equipped with the fiber optic temperature sensor (around which the magnetic nanotextile was wrapped), model FOT-L-SD-C1-F2-M2-R5-SCAI (FISO Technologies; Québec, Canada) was used for the temperature recording in time at a given frequency in an applied magnetic field. The accuracy of temperature measurements was ± 0.1 K.

3. Results

Polyvinyl butyral nanobeads are hydrophilic and biocompatible nanomaterials and are, therefore, well suited for applications in medicine and biology [28, 29]. That's why polyvinyl butyral electrospun nanofibers containing finely dispersed magnetite nanoparticles are an interesting material potentially applicable for the hyperthermia treatment of cancer tissue. Even though the used oil-based magnetic fluid is not a typical biocompatible one, it was used for the magnetic nanotextile formation, because it was completely characterized, and its properties were described [25, 27]. During the electrospinning process, the magnetic iron oxide nanoparticles were mostly embedded inside the polymer fibers and, thus, have not substantially influenced the polyvinyl butyral biocompatibility.

The prepared PVB nanotextile containing various amounts of magnetite nanoparticles was magnetically characterized and tested for the heat production. The impact of the concentration on magnetic properties is presented in Fig. 1. At 5 T, we can see well-saturated samples with magnetization saturations of 4.9 Am^2/kg and 16.8 Am^2/kg for PVB + MF and PVB + MF concentrated, respectively. Both curves exhibit the superparamagnetic behavior with zero coercivity. No potential diamagnetic contribution originating from PVB is detected.

The time dependence of the temperature for PVB + MF and PVB + MF concentrated samples in AMF was measured at various field intensities H and the constant frequency $f = 190$ kHz (Fig. 2). The

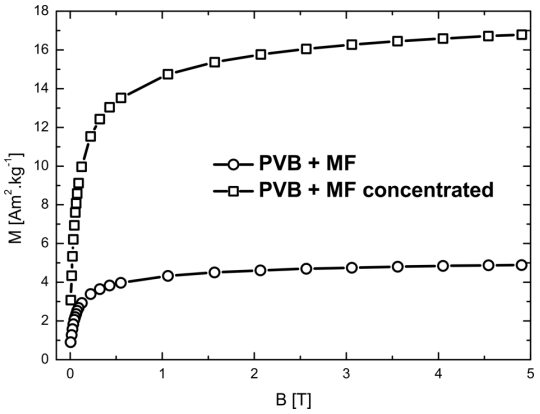

Fig. 1. Magnetization curves (in the first quadrant) of PVB + + MF and PVB + MF concentrated samples at room temperature (298 K)

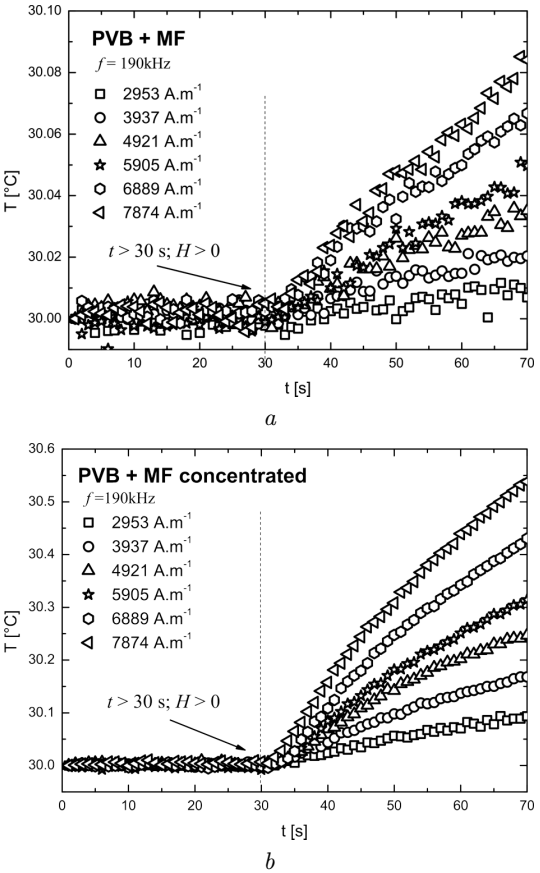

Fig. 2. Measured temperature increase versus the time for various magnetic field intensities at 190 kHz. The samples differed in MF concentration. Sample PVB + MF (*a*) contained 11.3 mg/ml of Fe_3O_4 nanoparticles, while sample PVB + MF concentrated (*b*) contained 22.6 mg/ml of Fe_3O_4 nanoparticles

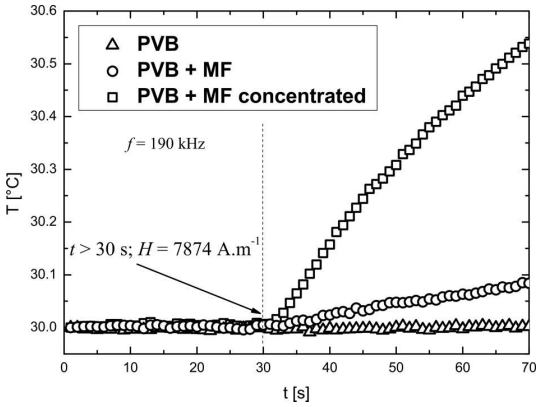

Fig. 3. Temperature increase versus the time at the highest applied field, $H = 7.8$ kA/m and $f = 190$ kHz

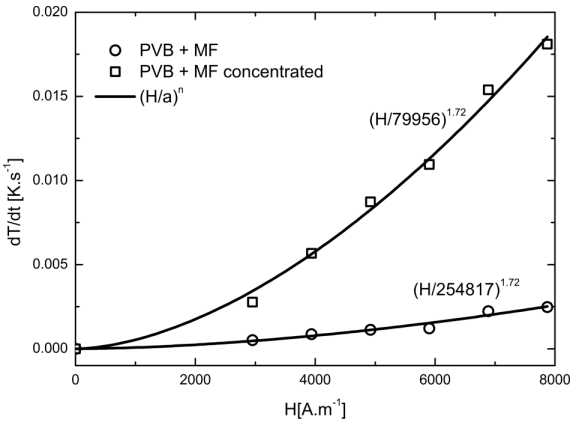

Fig. 4. Dependence $(dT/dt)_{t=0}$ as a function of the alternating magnetic field with intensity H

linear character of the dependence, with very small temperature oscillations around 30 °C during the first 30 s, provided information about the sample stability at the starting temperature. After 30 s of the measurement, the magnetic field H was applied for 40 s, and an increase in the temperature was observed. As H increased, the heating rate $\Delta T/\Delta t$ increased as well.

Figure 3 represents the temperature evolution at the highest applied $H = 7.8$ kA/m; a big impact of the iron oxide concentration in a nanotextile at the temperature increase can be seen. The bottom line represents a pure PVB sample without Fe_3O_4 nanoparticles, and it serves as a proof that, in the sample area, there is no parasitic heat from a circuit during the experiment.

By using the linear fit of the temperature versus time dependence, the heating rate $\Delta T/\Delta t$ for the samples PVB + MF and PVB + MF concentrated was determined. In such a way, the heating rates $\Delta T/\Delta t$ of all investigated samples were calculated for each applied magnetic field. The calculated heating rates $\Delta T/\Delta t$ were then plotted as a function of the applied magnetic field (Fig. 4). The curves represent experimental data fitted by the function $\Delta T/\Delta t = (H/a)^n$, where the parameters a and n depend on many particle features, such as the permeability, conductivity, shape, and size distribution. The observed type of H^n heating rate dependences on AMF with "n" close to 2 (1.72 for both investigated samples) indicates their superparamagnetic character.

It has to be mentioned that the temperature increase was measured in the surrounding medium, and the local temperature around the particles can be substantially higher. It has been shown in the recent paper how rapidly the temperature increases within the heated zone after the switching-on of a magnetic field, and how steeply it declines outside of the spherical heat generation zone [30]. In our experiments, the heat dissipation is not decisive due to the higher local temperature on the contact zone – particle vs surrounding environment; this situation creates conditions for the thermal destruction of the target structures. The reason why the data recordings were stopped after 40 sec (when the AMF was switched-on) was to avoid a possible parasitic heating from the coil winding. To determine the heating rate (dT/dt), the slope of the temperature change at the beginning of the heating process was sufficient. Anyway, these particles are able to generate a high power release into the surrounding media, and it is not a problem to heat them more.

4. Conclusions

We have presented the fabrication and characterization of an electrospun biocompatible PVB nanotextile functionalized with superparamagnetic magnetite nanoparticles. The magnetically modified nanotextile exhibited heating properties under an alternating magnetic field. By varying the concentration of magnetite nanoparticles in a PVB nanotextile, it is possible to change the observed temperatures. The sample temperature rose by 0.5 °C in 40 sec at the highest applied field intensity ($H = 7.8$ kA/m) at $f = 190$ kHz. This result is not superior, but, on the other hand, the non-linear modest temperature increase has the advantage that it avoids the overheating of the tissues, which is problematic for magnetic particles with strong heating powers [31]. The observed H^n heating rate evolution with the exponent "n" close to 2 indicates the superparamagnetic character of samples from which the heat contribution can be deduced as a consequence of the Néel and Brown relaxation processes. The potential of magnetically modified electrospun nanofibers for magnetic hyperthermia is promising after the future standardization and synthesis technology improvement.

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МАГНІТНО МОДИФІКОВАНІ
ЕЛЕКТРОСПІНІНГОВІ НАНОВОЛОКНА
ДЛЯ ГІПЕРТЕРМІЇ

Р е з ю м е

Існує кілька методологій приготування нановолокнистих матеріалів. Наразі електроспінінг є найпопулярнішою техніко̀ю завдяки своїй універсальності та простоті. Нановолокнисті матеріали, виготовлені таким чином, широко вивчаються в медицині та матеріалознавстві. Полівінілбутиральні (PVB) нановолокна генеруються спінінговим електродом у формі стрижня. Нановолокна були модифіковані магнітною рідиною (MF), доданою в розчин PVB. Ці магнітні нановолокна можна застосовувати в магнітній гіпертермії як імплантат, так і для нагрівання поверхні. Зразки з різними концентраціями магнітних частинок були тестовані в змінному магнітному полі. Спостерігалося різке підвищення температури після увімкнення поля. Природа підвищення температури цікава: можна спостерігати нелінійне збільшення на противагу від підвищення температури у випадку чистої магнітної рідини.