Self-standing magnetic composite nanosheets prepared in the presence of an external magnetic field: Characterization and potential for medical applications

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Abstract
The aim of this study is to develop self-standing, ultrathin film, nanosheets with high magnetic response for use in a medical device that can be migrated to a target location in the body by using an external magnetic field. First, iron oxide nanoparticles are synthesized by either the sol-gel method or thermal decomposition. The resulting magnetic properties of the nanoparticles show that the thermal decomposition method provides a greater saturation magnetization value than the sol-gel method. Next, the nanoparticles obtained by the thermal decomposition method are embedded into nanosheets of poly(epsilon-lactide) at varying concentrations. Embedding of the nanoparticles in the composite nanosheets is achieved by the application of an external magnetic field. The composite nanosheets are then characterized. The thickness of the nanosheet increases, and the nanoparticles are well dispersed, with an increase in poly(epsilon-lactide) concentration. The NP-embedded nanosheets are imaged by transmission electron microscopy, which reveals thin, long aggregates aligned in collinear line features. X-ray diffraction results indicate that the magnetic hard axis of the nanoparticles in the nanosheets is aligned in parallel to the plane of the nanosheet by magnetic field application during nanosheet preparation. In addition, the nanosheets at high poly(epsilon-lactide) concentrations that had been subjected to a magnetic field during preparation show a slightly greater magnetic response compared with both nanosheets without magnetic field exposure and nanosheets prepared at low poly(epsilon-lactide) concentrations.

Keywords
magnetic nanoparticles, medical application, nanosheet, poly(epsilon-lactide), self-standing film

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Introduction
Self-standing, ultrathin films called nanosheets (NSs), which are fabricated with soft polymers, polysaccharides, and other functional materials, have macroscopic lateral dimensions and molecular-scale thickness. NSs have attracted attention because of their unique properties such as high flexibility, adhesiveness, and transparency.1,2 The NSs have been widely investigated for possible use in microelectromechanical devices in the form of sensors or actuators, which endure repeated elastic deformation,3 and for use in biological and medical fields. Hatanaka et al.4 developed a biocompatible NS containing betamethasone valerate, a model drug for the formulation of transdermal delivery. Takeoka and colleagues5–6 have reported that a flexible NS was able to adhere tightly to organ surfaces through van der Waals interactions and was useful as a “nanoadhesive plaster” for a wound dressing. The authors also fabricated an antimicrobial NS by embedding silver nanoparticles.7 However, the NS could not be peeled off and then repasted after adhesion to the organ surface which was a problem when the NS had been initially pasted at a slightly different location than intended. To paste such NSs in precise positions, Taccola and colleagues8,9 developed the NSs with embedded magnetic nanoparticles and evaluated their morphology and magnetic properties. The NSs include magnetic components might be used more effectively in real application such as a
“nanoadhesive plaster” for endoluminal surgery on surgical incisions because magnetic components allow the precise positioning of NSs by using an external magnetic field.

Magnetic nanoparticle dispersions can assemble along a horizontally applied external magnetic field. For example, such dispersions have been applied as magnetic responsive photonic crystals.10,11 The free-standing photonic crystal films were reportedly fabricated by using an instant radical polymerization process under an external magnetic field.12 NSs with embedded NPs that are aligned perpendicular to the plane of the NSs can be expected to exhibit a high magnetic response.

In this study, we have synthesized magnetic nanoparticles (NPs) by sol-gel and thermal decomposition methods and then compared their magnetic properties. Subsequently, the NSs embedded with NPs were prepared with various concentrations of poly(L-lactide) (PLLA) in an external magnetic field. The NSs were then characterized regarding their thickness, their dispersibility, the relative orientation of the NPs, and their magnetic response.

Results and discussion

Characterization of the NPs obtained by thermal decomposition and sol-gel methods

NPs were synthesized by thermal decomposition and sol-gel methods. For thermal decomposition, faceted particles with an average diameter of 17 nm were obtained (Figure 1(a)). An example of the resulting X-ray diffraction (XRD) pattern shows peaks associated with the synthesized iron oxide particles (Figure 1(c)). Because the peaks of γ-Fe₂O₃ are similar to those of Fe₃O₄,13 we estimated the composition from the saturation magnetization value (Ms) of the NPs. Fe₃O₄ NPs with diameters ranging from several nanometers to several tens of nanometers obtained by thermal decomposition have a Ms value of approximately 80–85 emu/g based on vibrating sample magnetometer (VSM) measurements, which is a similar value to that of bulk magnetite (Ms ~ 90 emu/g), and this is almost size independent.14,15 In contrast, the Ms values of γ-Fe₂O₃ NPs with a diameter of approximately 20 nm prepared by thermal decomposition of the trinuclear complex [Fe₂CrO(CH₃COO)₆(H₂O)₃]NO₃ and thermal decomposition of Fe₃O₄ have been reported to have Ms values of 20–24 emu/g16 and 31 emu/g17 respectively. In our case, the Ms value of the synthesized product was 55.5 emu/g, which was lower than the reported value of the single-phase Fe₃O₄ NPs. Therefore, in our system, the synthesized NPs appeared to consist of two phases, Fe₃O₄ and γ-Fe₂O₃.

When prepared by the sol-gel method, nanorods with an average diameter × length of 7 nm × 17 nm were obtained (Figure 1(b)), and the associated XRD pattern indicated the presence of a mixed phase of α-Fe₂O₃ and γ-Fe₂O₃. The Ms value was significantly lower than that of the NPs prepared by thermal decomposition (Figure 2) because the α-type is antiferromagnetic, whereas the γ-type is ferromagnetic.18 Therefore, we used the NPs prepared by thermal decomposition, which had the larger Ms value, for the subsequent NS fabrication steps.

Characterization of the NSs

NSs embedded with NPs were prepared by casting using a chloroform solution at three concentrations of PLLA (1, 5, 10 mg/mL) and a fixed concentration of NPs (1 mg/mL).
See the Supplemental material for a graphic detailing the NS preparation method. After casting the mixed solution of PLLA and NPs, a neodymium magnet was used to apply a magnetic field to the NSs. Hereafter, the fabricated NSs are named according to the concentration of PLLA chloroform solution whether or not a magnetic field was applied during preparation. For example, NS samples prepared at 5 mg/mL PLLA with or without magnetic field application were 5-NS+mag and 5-NS, respectively.

The NS thickness increased with increasing PLLA concentration (Figure 3), reaching 169 nm for 10-NS+mag. NS samples prepared in the presence of the magnetic field tended to have a slightly greater thickness compared with those without magnetic field exposure during preparation. Because the central portion of the neodymium magnet had a greater magnetic flux density than its peripheral region, NPs would have been easily concentrated over the central portion of the magnet, where the magnetic field was stronger. As a result, the NP-containing polymer matrix was also concentrated in the center. NSs prepared in the presence of the magnetic field appeared brown at the center and transparent at their periphery. Reportedly, the adhesive property of NSs is significantly increased at thicknesses less than 200 nm. Therefore, all NSs prepared with a thickness less than 200 nm can be expected to exhibit a high degree of adhesiveness.

Subsequently, we investigated the dispersibility of the NPs in the NSs by transmission electron microscopy (TEM) (Figure 4). As a result, a significant quantity of aggregates was observed in 1-NS, while the number of aggregates was reduced in 5-NS. Taccola et al. prepared a nanofilm embedded with NPs by spin coating and showed that the presence of aggregates in the nanofilm at lower polymer concentrations was more evident than at higher polymer concentrations. Our observations are consistent with their result. The phenomenon of NP aggregate formation in the NSs prepared by casting was likely influenced by the short-range van der Waals dispersive interactions between NPs, which would have induced the formation of the aggregates during solvent evaporating. In our work, for the NSs prepared in the presence of the magnetic field, elongated aggregates were observed in a collinear fashion (Figure 4(d) and (h)). This fact indicates that the NPs were aligned along the line of magnetic force. The shape of the aggregates in the 5-NS+mag samples was thinner and longer than 1-NS+mag. Because the NPs in 5-NS were easier to monodisperse compared with those in 1-NS, the monodispersed NPs were induced along the external magnetic field. For 10-NS and 10-NS+mag, we were not able to perform TEM observations because the NS would not adhere to the TEM grid after vacuum-drying. That is, both 10-NS and 10-NS+mag exhibited poor adhesion due to their comparatively high NS thickness.

Next, we investigated the orientation of the crystal plane of the NPs in 1-NS+mag and 1-NS with synchrotron wide-angle X-ray diffraction (WAXD). The X-rays were emitted in a direction perpendicular to the plane of the NS. If the magnetization easy axis (111) of magnetite or maghemite is aligned in a direction perpendicular to the plane of the NS, then the peak for the (111) plane of the NPs in the NS prepared with the magnetic field should be greater compared with the NS prepared without the magnetic field. However, the (222) plane, which is the second-order peak of the (111) plane, could not be detected clearly (Figure 5). In contrast, the (400) peak of the NPs in 1-NS+mag decreased significantly compared with that of 1-NS. The [100] direction is derived from the magnetization hard axis of magnetite or maghemite. Thus, the magnetization hard axis was aligned in the parallel direction to the plane of the 1-NS+mag sheet when exposed to the external magnetic field.

Finally, the minimum magnetic field required to move the NS was evaluated (Figure 6). A graphic detailing the measurement method is shown in the Supplemental material. The minimum magnetic field required tended to decrease with increasing PLLA concentration. NPs can be dispersed well in the NSs containing a significant quantity of PLLA, and monodispersed NPs appeared to respond more readily to the applied magnetic field. In addition, NS+mag had a slightly higher magnetic response compared with NS due to the alignment of the magnetic hard axis in NS+mag. In this result, the differences in the magnetic responses of the samples were small because the magnetic force of the neodymium magnet that was used during NS preparation was not linear, except for at the center of the magnet. In general, using a magnetic field generator during NS preparation can align more NPs within the NS, giving rise to a greater magnetic response.

**Conclusions**

Our aim in this study was to develop NSs with a high magnetic response. First, we synthesized iron oxide NPs by using sol-gel and thermal decomposition methods. The resulting magnetic properties showed that NPs synthesized by thermal decomposition exhibited greater saturation magnetization values than those synthesized by the sol-gel method. Subsequently, NSs embedded with the NPs prepared by thermal decomposition were fabricated from a PLLA solution at various concentrations with 1 mg/mL NPs by the application of an external magnetic field. The thickness of the central region of the NSs increased with increasing PLLA concentration, and the NSs fabricated with the magnetic field tended to be slightly thicker in the central region than those.
fabricated without the magnetic field. This property was observed because more NP-embedded polymer matrix was present in the center than in the periphery of the NSs because the center of the magnet was a stronger NP inductor. TEM imaging showed that the number of NP aggregates decreased with increasing PLLA concentration due to a PLLA dilution effect. The TEM results also indicated that the NPs in the NSs prepared with the application of the external magnetic force were aligned along the line of the applied magnetic force. In addition, WAXD showed that the magnetization hard axis of the NPs was parallel to the plane of the NS. The results of the magnetic response evaluation indicated that the NSs with highly disperse NPs and with the NP magnetization hard axis aligned parallel to the plane of the NS tended to have a slightly greater magnetic response.

**Experimental**

**Materials**

Iron(III) acetylacetonate, iron(III) chloride hexahydrate, dibenzyl ether, hexane, ethanol, and chloroform were purchased from FUJIFILM Wako Pure Chemicals. Oleic acid, PLLA (Mw = 85,000–160,000 g/mol), and poly(vinyl alcohol) (PVA, 98% hydrolyzed, average Mw = 160,000) were purchased from Sigma-Aldrich. Propylene oxide and tetralin were purchased from Tokyo Chemical Industry Co., Ltd.

**Synthesis of magnetic nanoparticles**

*Thermal decomposition method.* Preparation of the NPs was performed based on a previously reported method with modification. First, 1.07 g (3 mmol) of iron(III)
acetylacetonate was mixed with 15 mmol of oleic acid in 150 mL of dibenzyl ether, and then the solution was heated to 200 °C. After maintaining this temperature for 2 h, the solution was refluxed for 1 h. The synthesized NPs were separated by centrifugation at 8,500 rpm, washed three times with a 1:2 mixture of hexane/ethanol, and then dried under vacuum for one day.

**Sol-gel method.** NPs were prepared by using a modified sol-gel method. Iron(III) chloride hexahydrate (1.36 g or 5 mmol) was mixed with 323 µL (17.9 mmol) of water and 33 mmol of oleic acid in 150 mL of dibenzyl ether with stirring. Then, 3.7 mL of propylene oxide was added to the solution, which was stirred constantly for 30 min. The resulting brown gel was washed with ethanol and then dried. The dried gel was dispersed in 25 mL of tetra- lin, and then the dispersion was refluxed at 219°C for 10 h. After cooling to room temperature, the NPs were separated by centrifugation at 8,500 rpm, washed three times with hexane, and dried under vacuum for 1 day.

**Fabrication of self-standing magnetic nanosheets (NS).** The NSs were fabricated by using the following casting method, as shown in the Supplemental material. First, a sacrificial thin film of PVA was deposited by spin coating (ACT-220DII, Active Co., Ltd) on a glass substrate. The PVA (10 wt%) was an aqueous solution and deposition was performed at 3000 rpm for 60 min. Subsequently, a chloroform solution containing 1, 5, or 10 mg/mL PLLA and 1 mg/mL NPs was cast onto the PVA sacrificial layer, which had been placed on a neodymium magnet (0.55 T). A magnetic field was applied for 2 min. After annealing the film at 80°C, the layered glass substrate was immersed in pure water to dissolve the PVA layer and recover the prepared thin film. For comparison, the same thin films were prepared without exposure to the magnetic field.

**Characterization of NPs and NSs**

**Magnetic measurements of NPs.** The magnetic behavior of the NPs was investigated with a vibrating sample magnetometer (TEM-WF84.5R-132, Toei Kogyo, Ltd.). The magnetization curves for 40 mg-NPs samples were recorded at room temperature in an applied magnetic field that ranged between −15 K and 15 K Oe.

**TEM and XRD studies.** For observation of the synthesized NPs, 1 mg/mL of the NP dispersion in chloroform was placed on a carbon-coated copper TEM grid. For observation of the prepared NSs, each NS sample was floated on water in a petri dish, and the TEM grid was placed underneath the nanofilm. Next, the water was removed from the petri dish using a micropipette and the NS was captured on the TEM grid. After vacuum-drying, the samples were observed by TEM (FEI Titan Themis) at an accelerating voltage of 200 kV. XRD patterns of the NPs were obtained by using a 9 KW X-ray generator (Rigaku Corporation) and Cu Kα radiation. WAXD patterns of NSs were carried out at 40B2 SPring-8 (Japan) with a 0.4 m camera length using a Pilatus detector. The wavelength of the beam was 0.071 nm, and the exposure time was 30 s.

**Evaluation of NS thickness.** The NS was placed on a mica plate and then cut along the centerline. Half of the NS was then removed. The film thickness of the NS was evaluated by atomic force microscopy (AFM) based on the height of the step produced. The data are shown as the mean ± SD (n = 30).

**Evaluation of the NS magnetic response.** The NSs were floating on the surface of a volume of water in the sample tube. Then, a 0.55 T neodymium magnet was placed at the bottom of the tube, which caused the NS to move in response. The minimum magnetic field required to move the NS was determined by a Gaussmeter, as shown in the Supplemental material. The data are shown as the mean ± SD (n = 5).

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**Supplemental material**
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