Magnetically responsive nanofibrous ceramic scaffolds for on-demand motion and drug delivery

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ABSTRACT

Smart biomaterials, featuring not only bioactivity, but also dynamic responsiveness to external stimuli, are desired for biomedical applications, such as regenerative medicine, and hold great potential to expand the boundaries of the modern clinical practice. Herein, a magnetically responsive three-dimensional scaffold with sandwich structure is developed by using hydroxyapatite (HA) nanowires and ferrosoferric oxide (Fe₃O₄) nanoparticles as building blocks. The magnetic HA/Fe₃O₄ scaffold is fully inorganic in nature, but shows polymeric hydrogel-like characteristics including a 3D fibrous network that is highly porous (>99.7% free volume), deformable (50% deformation) and elastic, and tunable stiffness. The magnetic HA/Fe₃O₄ scaffold has been shown to execute multimodal motion upon exposure to an external magnetic field including shape transformation, rolling and somersault. In addition, we have demonstrated that the magnetic scaffold can serve as a smart carrier for remotely controlled, on-demand delivery of compounds including an organic dye and a protein. Finally, the magnetic scaffold has exhibited good biocompatibility, supporting the attachment and proliferation of human mesenchymal stromal cells, thereby showing great potential as smart biomaterials for a variety of biomedical applications.

1. Introduction

Biomaterials have played an indispensable role in improving human life quality and extending life expectancy since ancient times. Historically, the role of biomaterials and the requirements they need to meet has undergone a continuous change. Untreated, nature-derived objects were the earliest biomaterials used in humans. For example, ancient Egyptians used natural cotton fibers and horsehair to suture wounds [1]. With the development of natural sciences and technology, untreated natural materials were gradually replaced by synthetic bioinert biomaterials. Examples of these are polymethylmethacrylate, polyethylene, polyesters and stainless steel, used in the middle of the 20th century as tooth, blood vessel and bone replacements [2]. By the end of the 20th century, a variety of bioactive materials that feature adaptiveness to the biological environment, has been developed. Bioactive biomaterials, designed to stimulate and/or direct appropriate cellular and tissue responses, have significantly promoted the development of modern medicine, including pharmacology, diagnosis, and regenerative medicine, among others [3]. In the past twenty years, the advances in micro- and nanotechnology and fabrication, along with an ever-growing interest in precision medicine, have triggered the emergence of next-generation “smart” biomaterials [4,5], which are not only bioactive, but also dynamically responsive to a variety of external stimuli, such as pH [6,7], acoustic signal [8,9], light [10–13], temperature [14,15], stress [16,17] and magnetism [18,19]. In particular, the use of magnetic fields has been suggested to be a safe and effective tool in biomedical applications for bioimaging and tissue engineering, since they can deeply penetrate the human body without attenuation [20,21]. To date, a variety of magnetically responsive three dimensional (3D) materials have been developed for various biomedical applications including on-demand drug delivery [22], mechanical stimulation of cells [23], as shape-memory [24] and shape-programmable scaffolds [25], and for targeted cargo transportation [20,26]. Despite these advancements that clearly show the potential of magnetically responsive...
In the past few years, a family of advanced inorganic nanofibrous aerogels and inorganic nanofibrous sponges with exciting, untraditional properties including ultrahigh porosity (>99%), highly interconnected porous architecture with open cell geometry, large surface area, and elasticity have drawn significant attention as promising candidates for applications in many fields including tissue engineering, catalysis, protein separation and air purification [27-29]. Examples include SiO$_2$ nanofibrous aerogels [28], yttrium-stabilized ZrO$_2$ nanofibrous sponges [27], BaTiO$_3$ sponges [29], and hydroxyapatite (HA)/beta-tricalcium phosphate (β-TCP) nanofibrous sponges [30]. HA, more accurately, poorly crystalline substituted apatite is the main inorganic component of bone. Naturally derived and synthetic HA has been extensively investigated in the fields of bone defect repair and drug delivery, owing to its excellent biocompatibility and bioactivity [31-34]. Iron oxide nanoparticles, such as magnetite (Fe$_3$O$_4$), maghemite (γ-Fe$_2$O$_3$) and mixed ferrites have received attention due to their unique properties of being biodegradable and biocompatible, and have been employed for diagnostic and therapeutic purposes including magnetic resonance imaging, targeted delivery of drugs, biosensing and tissue regeneration [35].

In this work, we have developed a biocompatible, bioactive, magnetically responsive inorganic nanofibrous scaffold with sandwich structure which consists of a self-supporting network of seamlessly interwoven HA nanowires and a relatively small amount of ferrosilicoferric oxide (Fe$_5$O$_4$) nanoparticles. Previous studies reported inorganic magnetic scaffolds, such as akermanite-Fe$_5$O$_4$-Ca$_2$O scaffolds [36], magnetic mesoporous bioactive glass scaffolds [37], magnetic hardystonite scaffold [38], and magnetic calcium-zirconia scaffold [39]. In contrast to these materials, which were brittle and fragile, the magnetic scaffold developed here showed outstanding elasticity, despite being fully inorganic. In addition, despite its inorganic nature, the magnetic scaffold showed great similarity to polymeric hydrogel materials including 3D nanofibrous network, high porosity, elasticity, and tunable stiffness. The magnetic HA/Fe$_5$O$_4$ scaffold was fabricated with a controllable amount and spatial distribution of Fe$_5$O$_4$ particles. The existence of relatively small amount of Fe$_5$O$_4$ particles endowed the scaffold with excellent magnetically responsive properties, without compromising its biocompatibility. For example, the scaffold displayed on-demand motion such as shape transformation, rolling and domino-effect upon exposure to an external magnetic field, which could not be achieved with previously reported magnetic hydrogels and magnetic polymeric scaffolds, such as magnetic poly(ethylene glycol) hydrogel [40], magnetic silk fibroin hydrogel/scaffold [41,42], magnetic polyglycolic acid scaffold [43], and magnetic poly(lactic acid) scaffold [44]. Although a few hydrogels and polymeric scaffolds with similar magnetically responsive property were reported, these studies have also shown room for further improvement of their biocompatibility [20,22,23,26]. The magnetic field induced deformation and volume variation allowed the magnetic HA/Fe$_5$O$_4$ scaffolds to be used as a carrier for various compounds including organic dye and protein, the release of which can be controlled on demand. The scaffold exhibited excellent biocompatibility supporting the attachment and proliferation of human mesenchymal stromal cells (hMSCs). Previous studies have illustrated positive effects of magnetically responsive scaffolds/gels on the preosteoblast proliferation [45], osteoblastic and vasculogenesis potentials of tissue-engineered bone grafts [40], and osteogenic differentiation of mesenchymal stromal cells [46]. Therefore, we anticipate that the inorganic magnetic HA/Fe$_5$O$_4$ scaffold developed here with ultrahigh porosity, interconnected hierarchical pores with open cell geometry, extracellular matrix like nanofibrous 3D network, on-demand drug release property, as well as on-demand motion, might serve as a promising multifunctional platform for targeted drug delivery and bone regeneration.

2. Experimental section

Materials: Anhydrous calcium chloride (96%, Thermos Fisher), sodium oleate (>92% fatty acids), Sigma-Aldrich), ammonium phosphate dibasic (>99%, Sigma Aldrich), iron (II,III) oxide (50–100 nm particle size, Sigma Aldrich), hemoglobin (Sigma Aldrich), methylene blue (VWR), sodium hydroxide (VWR).

Preparation of HA nanowire slurry: HA nanowire slurry was prepared by a hydrothermal method. Calcium chloride aqueous solution (0.16 M, 70 mL) was added into sodium oleate aqueous solution (0.58 M, 70 mL) and stirred for 1 h at room temperature, followed by the addition of ammonium phosphate dibasic aqueous solution (0.34 M, 56 mL). After 30 min, the resulting solution was evenly distributed over four 100 mL Teflon-lined stainless steel autoclaves. The autoclaves were sealed, heated in an oven to 200 °C and maintained at 200 °C for 24 h. Upon cooling of the autoclaves to room temperature, HA nanowire slurry was collected at the bottom of the Teflon autoclaves and kept in a plastic bottle for subsequent preparation of magnetic HA/Fe$_5$O$_4$ scaffolds. To determine the concentration of HA nanowires in the slurry, 5 mL of HA nanowire slurry was washed with ethanol and milliQ water, and dried. The concentration of HA nanowires in the slurry was calculated by dividing the volume of the HA nanowire slurry by the weight of HA nanowires in the slurry.

Preparation of regular magnetic HA/Fe$_5$O$_4$ scaffolds with different amount of Fe$_5$O$_4$: Desired amount of Fe$_5$O$_4$ nanoparticles was added into 10 mL of HA nanowire slurry with a concentration of 3 mg/mL, resulting in suspensions with the mass ratio of Fe$_5$O$_4$/HA being 2:1, 1:1 and 1:2, respectively. After treatment in ultrasonic bath for 1 h, the suspensions were frozen at -20 °C in molds with desired shape and lyophilized. Then, the lyophilized samples were rinsed in ethanol (EtOH), water and treated in a NaOH–H$_2$O–EtOH solution (95 vol% ethanol and 5 vol% 10 M NaOH in water) at 90 °C for 72 h. The NaOH–H$_2$O–EtOH solution was refreshed every 24 h. Finally, the Fe$_5$O$_4$/HA scaffolds were washed with water and kept in water until further use.

Preparation of magnetic HA/Fe$_5$O$_4$ scaffolds with sandwich structure: First, desired amount of Fe$_5$O$_4$/HA slurry with the mass ratio of 2:1 was added into a cylindrical mold and kept at -20 °C in a freezer for 30 min. Second, the mold was removed from the freezer and the same amount of Fe$_5$O$_4$/HA slurry with the mass ratio of 1:1 was added into the mold and immediately returned into the freezer. 30 min later, the same process as described for the second step was repeated with the Fe$_5$O$_4$/HA slurry with a ratio of 1:2. Finally, the samples were frozen at -20 °C overnight and lyophilized and washed by using the above-mentioned method for regular HA/Fe$_5$O$_4$ scaffolds.

X-ray diffraction (XRD) analysis of HA, Fe$_5$O$_4$ and HA/Fe$_5$O$_4$ scaffolds: The XRD analysis of HA, Fe$_5$O$_4$ and HA/Fe$_5$O$_4$ scaffolds was performed using X-ray powder diffraction (Bruker D2 Phaser diffractometer, Cu Kα radiation, λ = 1.5406 Å) in the range of 10° ≤ 2θ ≤ 60° in increments of 0.02° and an integration time of 0.5 s.

Cyclic compression test: Cyclic compression test was performed on an
ElectroForce 3200 machine (ElectroForce 3200, USA), with a 45 N load cell. Cylindrical regular magnetic HA/Fe₃O₄ scaffolds and cylindrical magnetic HA/Fe₃O₄ scaffolds with sandwich structure with dimensions of Ø 11 × 11 mm were cyclically exposed to 50% compressive strain at a strain rate of 1 mm/s in water for 10 cycles. Three scaffolds were used for each sample type. The modulus of elasticity was defined as the slope of the linear region of the stress-strain curve after linear fit by using the Origin software.

Specific surface area and porosity analysis: The porosity of the regular magnetic HA/Fe₃O₄ scaffolds was calculated using the following equation:

$$\text{Porosity} = (1 - \rho_{m}/\rho_{o}) \times 100\%$$

(1)

$$\rho_1 = \frac{M_1}{V_1}$$

(2)

$$\rho_2 = \frac{(\rho_{HA} \times \rho_{Fe3O4})(W_2 \times \rho_{HA} + W_1 \times \rho_{Fe3O4})}{W}$$

(3)

where $M_1$ and $V_1$ are the mass and volume of the regular magnetic HA/Fe₃O₄ scaffolds, respectively. $\rho_{HA}$ represents the theoretical density of HA, which is 3.16 g cm⁻³, and $\rho_{Fe3O4}$ represents the theoretical density of Fe₃O₄, which is 5.18 g cm⁻³. $W_1$ and $W_2$ are the mass fraction of HA and Fe₃O₄ in the regular magnetic HA/Fe₃O₄ scaffolds, respectively.

The specific surface area of the magnetic HA/Fe₃O₄ scaffolds with sandwich structure was characterized by a specific surface area analyzer (Micromeritics ASAP 2040, Germany).

**On-demand dye release:** A magnetic HA/Fe₃O₄ scaffold (Ø 11 × 5 mm) with sandwich structure was removed from water by using tweezers. A piece of tissue was gently placed on the surface of the scaffold to remove most of the water from the scaffold. The scaffold was allowed to absorb 100 µL of methylene blue aqueous solution with a concentration of 1 mg/mL by adding the solution dropwise on the top of the scaffold, and then placed into a cell culture dish with a diameter of 100 mm which was filled with 40 mL water, with the side having a Fe₃O₄/HA mass ratio of 1:2 facing the bottom. An electromagnet (1900 N, Conrad) was used to apply alternating magnetic field with a frequency of 0.25 Hz to the scaffold from the bottom. A scaffold to which no magnetic field was applied was used as a control. At 1, 2, 3 and 4 h, the scaffolds (n = 4) were washed with 700 µL PBS. Then, PBS was removed and 700 µL PrestoBlue™ solution (10% in basic cell culture medium) was added to each well. After incubation in the dark at 37 °C for 30 min, 100 µL of incubated solution was transferred to a new black 96-well plate. The fluorescence was measured on the microplate reader (BMG Labtech CLARIOstar, Germany) at 590 nm.

Cell morphology: At day 7 and 14, scaffolds (n = 2) with cells were washed with PBS twice and fixed in 4% w/v formaldehyde (Sigma). After treatment with 0.1% v/v Triton X-100 (WVR), the scaffolds were washed with PBS three times, and then the nonspecific binding sites were blocked with 1 w% bovine serum albumin (BSA, WVR). The cells were stained using Alexa Fluor 488 phallolidin (Fisher Scientific) and DAPI (Sigma-Aldrich). Finally, the morphology of cells on scaffolds was observed with a fluorescence microscopy (Nikon Eclipse Ti-S, Japan).

**Statistical analysis:** The data were presented as mean ± standard deviation and analyzed using GraphPad Prism 9 (GraphPad software, San Diego, USA). Unpaired Student’s t-test was used to determine the statistical significance of the results. Significant differences were accepted when p<0.05 (indicated with *) on the graphs.

3. Results and discussion

In this work, we have developed a magnetically-responsive fully inorganic nanofibrous scaffold, with mechanical and handling properties similar to those of polymeric (hydro)gels, by using HA nanowires and Fe₃O₄ nanoparticles as building blocks. The HA nanowires and Fe₃O₄ nanoparticles were phase-pure HA and magnetite, respectively (Fig. S1, Supporting Information). The fabrication process of the magnetic scaffolds (Fig. 1A) comprised the steps of the suspension formation between Fe₃O₄ nanoparticles and HA nanowire slurry, freezing, lyophilization, and removal of impurities. The as-prepared HA nanowire slurry comprised monodispersed HA nanowires because of the presence of oleate bilayer on the nanowire surface [47]. After adding Fe₃O₄ nanoparticles into the HA nanowire slurry and treatment in ultrasonic bath for 1 h, a uniform suspension consisting of Fe₃O₄ nanoparticles and HA nanowire was formed. During the freezing process, ice crystals formed in the suspension, inducing phase separation and self-assembly of the HA nanowires into a seamlessly interwoven highly porous network. Microscale Fe₃O₄ clusters, derived from Fe₃O₄ nanoparticles, were physically embedded in the nanofibrous network comprising HA nanowires (Figs. 1A and 2A). The amount of Fe₃O₄ nanoparticles in the magnetic HA/Fe₃O₄ scaffolds was highly tunable by changing the amount of Fe₃O₄ nanoparticles added to the HA nanowire slurry during the fabrication process, as illustrated in Fig. 1B and C. Here, we have fabricated magnetic HA/Fe₃O₄ scaffolds with the HA/Fe₃O₄ mass ratio of 1:2, 1:1 and 2:1, respectively. The amount of Fe₃O₄ nanoparticles did not have an obvious effect on the microstructure of the magnetic
HA/Fe₃O₄ scaffolds. All the magnetic HA/Fe₃O₄ scaffolds showed similar highly porous microstructure with open cell geometry and hierarchical pores ranging from hundreds of micrometers to several micrometers. The only obvious difference was a more abundant presence of Fe₃O₄ nanoparticles on the pore walls of scaffolds with a higher amount of Fe₃O₄ nanoparticles (Fig. 2, A, D and G). The intensity of the HA-specific diffraction peaks in the XRD patterns was higher in the scaffolds with lower amount of Fe₃O₄ particles (Fig. 1D). The scaffolds exhibited ultralow density and ultrahigh porosity ranging from 6.2 mg/cm³ and 99.85%, respectively for 33.3% Fe₃O₄ to 10.7 mg/cm³ and 99.75%, respectively for 66.7% Fe₃O₄ (Fig. 1E and F).

Despite the inorganic nature of HA nanowires and Fe₃O₄ nanoparticles, the as prepared magnetic HA/Fe₃O₄ scaffolds were not brittle, but instead exhibited an elastic behavior. The HA nanowires were shown to bend without collapsing/fracturing upon exposure to external loading, and to recover to their original shape when the loading was removed [30], suggesting that the unique elasticity of the magnetic HA/Fe₃O₄ scaffolds during the last five test cycles was calculated to be 3.46, 3.67, and 3.59 KPa for 66.7% Fe₃O₄, 50% Fe₃O₄, and 33.3% Fe₃O₄, respectively (Fig. 2, C, F, and I), indicating that the amount of Fe₃O₄ nanoparticles did not have an obvious effect on the stiffness of the scaffolds. A reason for this may be the fact that the Fe₃O₄ were dispersed within the HA nanowire network, thus providing a limited contribution to the mechanical properties of the HA/Fe₃O₄ scaffolds. In contrast, by increasing the concentration of HA nanowire slurry from 3 mg/mL to 5 mg/mL during the fabrication process, the stiffness of the magnetic HA/Fe₃O₄ scaffolds increased accordingly from 3.46 to 4.18 KPa, from 3.67 to 4.08 KPa, and from 3.59 to 4.16 KPa for 66.7%, 50%, and 33.3% Fe₃O₄, respectively (Fig. S2), because with a higher slurry concentration, a more dense HA network was formed. The elastic modulus of the HA/Fe₃O₄ scaffolds was lower than those of the HA nanowire aerogels and HA ceramic sponge reported earlier, because of the use of a relatively low concentration of HA nanowire slurry during the fabrication process [30,48]. Considering that the concentration of HA nanowire slurry was controllable during the synthetic process, it is plausible that the stiffness of the HA/Fe₃O₄ scaffolds developed here can further be tuned.

In addition to varying the amount of Fe₃O₄ nanoparticles among different samples, it was also possible to control the spatial distribution of Fe₃O₄ nanoparticles inside one scaffold. For example, we fabricated a magnetic HA/Fe₃O₄ scaffold with sandwich structure consisting of three different layers with the mass ratio of Fe₃O₄/HA being 2:1, 1:1 and 1:2, respectively. The fabrication process of the magnetic HA/Fe₃O₄ sandwich scaffold was similar to that of the regular scaffolds, with the exception that the middle step of suspension freezing inside a mold was divided into three substeps, with at each substep the addition of the suspension with different mass ratio of Fe₃O₄/HA (Fig. 3A). In detail, 300 µL of suspension of HA/Fe₃O₄ (mass ratio of 2:1) was added into the bottom of a cylindrical mold with a diameter of 10 mm to serve as the first layer and kept at -20 °C in a freezer for 30 min. During the freezing process, the viscosity of the suspension increased as the temperature decreased while it still maintained in liquid state. Subsequently, 300 µL

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**Fig. 1.** (A,B,C) Schematic illustration of preparation process of magnetic HA/Fe₃O₄ scaffolds with different amounts of Fe₃O₄. (D,E,F) XRD patterns, density and porosity of magnetic HA/Fe₃O₄ scaffolds with the mass ratio of Fe₃O₄/HA of 2:1, 1:1 and 1:2, respectively.
of suspension of HA/Fe₃O₄ (mass ratio of 1:1) was added into the mold. Since the freshly added suspension of HA/Fe₃O₄ (mass ratio of 1:1) had a lower density and lower viscosity compared to those of the first layer, a stable second layer was formed while preserving a good structural integrity of the first layer. The same process as described for the second layer was repeated to fabricate the last layer by using the Fe₃O₄/HA slurry with a ratio of 1:2. Finally, the samples were frozen at -20 °C overnight and lyophilized. The magnetic HA/Fe₃O₄ sandwich scaffold was also highly elastic with interconnected porous structure with open cell geometry. These interconnected cellular pores could serve as a reversible water reservoir, allowing water to flow out of and into the scaffold by applying cyclic compression (Fig. 3B and C and D). The magnetic HA/Fe₃O₄ sandwich scaffold showed a similar trend of stress-strain curves during the 10-times repeated compression test. The average elastic modulus of the last five tests was 3.32 kPa, which is comparable to those of regular magnetic HA/Fe₃O₄ scaffolds (Fig. 3E and F and G).

Taken together, we have developed fully inorganic, soft and elastic magnetic scaffolds with tunable stiffness, and controlled amount and spatial distribution of magnetic nanoparticles using a simple freeze-drying method. The magnetic Fe₃O₄/HA scaffolds in water exhibited physical properties similar to those of polymeric hydrogel materials featuring 3D nanofibrous network, tunable stiffness, high porosity and good elasticity (Table S1, Supporting Information). In addition, the magnetic Fe₃O₄/HA scaffolds had a specific surface area of 24.39 m²/g, which is in the range of what was previously reported for some hydrogels [49,50].

Fe₃O₄ nanoparticles are superparamagnetic, meaning that they are magnetic when exposed to an external magnetic field, but no longer magnetic when the field is removed. As a result, materials containing superparamagnetic Fe₃O₄ nanoparticles can be guided by an external magnetic field gradient or alternating magnetic field to perform a variety of on-demand activities, including movement, accumulation, hyperthermia and drug release [35]. In this work, the presence of Fe₃O₄ nanoparticles endowed the HA/Fe₃O₄ sandwich scaffolds with the ability to execute on-demand multimodal motion, including shape change, rolling and somersault upon exposure to an external magnetic field. As is shown in Fig. 4A, a cylindrical HA/Fe₃O₄ sandwich scaffold (Ø 6 × 6 mm) was placed in water with the side with the Fe₃O₄/HA mass ratio of 1:2, thus the side with the lowest amount of magnetic nanoparticles, facing the bottom. When a cylindrical magnet (Ø 40 × 20 mm) approached the bottom of the dish containing the scaffold, the scaffold transformed from relaxed state to a compressed state automatically, and recovered to its original shape when the magnet was removed (Supplementary Movie 1). For the magnetic stimulation, the magnet was placed underneath the magnetic scaffold, thus on one side of the scaffold. As a result, the magnetic attraction force on the magnetic particles and the resulting compression force on the scaffold were not balanced over the entire height of the scaffold. To compensate for this, a sandwich structure was used, with the layer containing the lowest amount Fe₃O₄ particles serving as the bottom layer, closest to the magnet. Besides this shape transformation, the HA/Fe₃O₄ sandwich scaffold was able to make more complex movements like somersault (Fig. 4B, Supplementary Movie 2) and rolling (Fig. 4C, Supplementary Movie 3) and move in the desired direction by placing a mobile magnet at a certain distance from the scaffold.

It is noteworthy that due to the tunable stiffness of the inorganic network and controllable spatial distribution of Fe₃O₄ nanoparticles in the scaffold, the total amount of Fe₃O₄ nanoparticles needed to achieve a good shape-transforming ability (Fe₃O₄: HA ratio 1:1) was considerably
lower than what was reported in previous studies (7:1 and 13:1) [22, 23]. This is an advantage, since it has been shown that an excess of Fe$_3$O$_4$ nanoparticles might be toxic to cells [23]. Moreover, the interwoven network of HA/Fe$_3$O$_4$ sandwich scaffold consisted of bioactive HA crystals [51], rather than bioinert silicone rubber as was used in many studies [20, 25, 26].

Taken together, the remote actuation by magnetic fields of such an inorganic, highly porous and fast shape-shifting soft materials with good biocompatibility offers new possibilities for applications in biomedical devices, materials for regenerative medicine and soft robotics. As a proof of concept, in the next section, we demonstrated that the HA/Fe$_3$O$_4$ sandwich scaffolds can be used as a smart carrier for different cargos. In the past decade, the development of bioactive, smart, and multifunctional drug carriers for integrated diagnosis and treatment approaches and on-demand drug delivery has been a topic of great interest, to address clinical challenges related to, e.g., cancer, diabetes and infections [52–55]. We hypothesized that the magnetic field-induced shape transformation and resulting water convection, associated with the shear stress, can promote dissociation of drugs from and enhance transport of unbound drugs out of the porous structure, thus accelerating the release of drugs loaded on the HA/Fe$_3$O$_4$ sandwich scaffolds (Fig. 5A). To test this hypothesis visually, we first used methylene blue dye-loaded HA/Fe$_3$O$_4$ sandwich scaffolds as a model. As shown in Fig. 5B, without magnetic field stimulation, the methylene blue dye was released slowly from the scaffold in a typical physical diffusion manner, i.e., diffusion of the dye molecules from high concentration to low concentration occurred, forming a gradient of blue color around the scaffold. In contrast, a much faster release and more uniform distribution of the methylene blue dye was observed when an alternating magnetic field was applied to the HA/Fe$_3$O$_4$ sandwich scaffold (Fig. 5C). Under the magnetic field stimulation, the HA/Fe$_3$O$_4$ sandwich scaffold analogous to a beating heart, thereby ‘pumping’ the methylene blue dye out of its porous structure. In addition, the water convection caused by the shape transforming activity of the magnetic scaffold may have accelerated the diffusion rate of the methylene blue molecules. As a consequence, the HA/Fe$_3$O$_4$ sandwich scaffold showed a much faster release of the methylene blue dye upon stimulation by the magnetic field compared to that of the static one.

We then chose Hb as a model drug. The UV–vis absorption measurements at 405 nm of the Hb solution before and after immersion of an HA/Fe$_3$O$_4$ sandwich scaffold is shown in Fig. 5D. Due to the ultrahigh porosity and hierarchical porous architecture, the Hb loading capacity of HA/Fe$_3$O$_4$ sandwich scaffold was as high as 71.3 $\pm$ 3.6 $\mu$g/mg, which was much higher than that of HA porous ceramics and octacalcium phosphate/poly(lactic-co-glycolic acid) scaffold studied earlier [56, 57]. The Hb loaded HA/Fe$_3$O$_4$ sandwich scaffolds were then placed in a PBS solution, and one group of the scaffolds was subjected to the magnetic field with a frequency of 0.25 Hz, while no magnetic field was applied to the other group. The cumulative release of Hb from the HA/Fe$_3$O$_4$ sandwich scaffolds exposed to magnetic stimulation for 4 h was 64.2 $\pm$ 3.9 $\mu$g (13.8% of total amount), which was about twice as high as that of HA/Fe$_3$O$_4$ sandwich scaffolds without magnetic stimulation (Fig. 5E).
The average release rate of Hb from the two groups of samples at each time point during 4 h is shown in Fig. 5F, demonstrating that the release rate upon magnetic stimulation was higher than in the absence of magnetic stimulation throughout the experiment. We propose that the mechanism underlying the accelerated release of the methylene blue dye in response to magnetic stimulation. In this study, nearly 5% of human dermal fibroblasts were dead after 4 h of co-culture [22]. In our study, the metabolic activity of hMSCs cultured on HA/Fe\textsubscript{3}O\textsubscript{4} sandwich scaffolds, the attachment and proliferation of hMSCs cultured for 7 and 14 days were studied using fluorescence staining and microscopy. At day 7, the cells were abundant and homogeneously distributed on both sides and the periphery of the HA/Fe\textsubscript{3}O\textsubscript{4} sandwich scaffolds, as illustrated by the low-magnification (4X) fluorescence image (Fig. S5, Supporting Information). As shown on the higher magnification (10X) fluorescence images (Fig. 6A–C), the cells exhibited a spread morphology, with long filopodia and actin cytoskeleton taking the shape of the underlying nanofibrous material. At day 14, a similar observation was made, with more cells present and a more elongated actin cytoskeleton morphology (Fig. S6 (Magnification 4X) and Fig. S7 (Magnification 10X), Supporting Information). There fore, the HA/Fe\textsubscript{3}O\textsubscript{4} sandwich scaffolds showed good biocompatibility.

It is noteworthy that the magnetic-field-induced shape transformation and resulting water convection could generate associated compression force and shear force. Considering the fact that the HA/Fe\textsubscript{3}O\textsubscript{4} sandwich scaffolds showed good biocompatibility, supporting cell proliferation and attachment, we anticipate that the scaffolds developed here may also serve as a unique 3D model for the study of mechanical stimulation of cells in a non-invasive manner [23]. We intend to use our HA/Fe\textsubscript{3}O\textsubscript{4} sandwich scaffolds as intrinsically-deformable bioreactors to study mechanical stimulation of cells for bone tissue engineering applications.

4. Conclusion

In this work, a magnetically-responsive inorganic nanofibrous scaffold was developed by using anisotropic HA nanowires and Fe\textsubscript{3}O\textsubscript{4} nanoparticles as building blocks. The scaffold was soft and elastic, rather than brittle, and showed similarities to polymeric hydrogel materials in physical properties including porosity and tunable modulus despite its inorganic nature. The amount and distribution of the Fe\textsubscript{3}O\textsubscript{4} nanoparticles in the porous structure could easily be varied, to create...
scaffolds with a sandwich structure with layers containing different amounts of magnetic nanoparticles. A much lower amount of Fe₃O₄ nanoparticles compared to those used in other studies could efficiently endow the HA/Fe₃O₄ sandwich scaffolds with the ability to execute on-demand multimodal motion such as shape transformation (compression), rolling, somersault and displacement upon exposure to an external magnetic field. The compression and related volume variation made the HA/Fe₃O₄ sandwich scaffold suitable for carrying and releasing organic compounds upon remote control by a magnet. Finally, the use of HA crystals and small amount of Fe₃O₄ nanoparticles endowed the HA/Fe₃O₄ sandwich scaffolds with superior biocompatibility compared to that of other magnetically responsive materials made from bioinert building blocks. Taken together, the HA/Fe₃O₄ sandwich scaffolds developed here offers new possibilities for applications in biomedical devices, materials for regenerative medicine and soft robotics, especially in targeted drug delivery and bone regeneration.

**CRediT authorship contribution statement**

**Yonggang Zhang**: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Jiaping Li**: Methodology, Validation, Investigation, Writing – review & editing. **Pamela Habibovic**: Methodology, Writing – review & editing, Visualization.
Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioactmat.2022.02.028.

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Fig. 6. Actin cytoskeleton (phalloidin: green) and nuclei (DAPI: blue) staining of hMSCs cultured on HA/Fe3O4 sandwich scaffolds for 7 days. Scale bar: 250 μm.
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