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Study of Galfenol direct cytotoxicity and remote microactuation in cells

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Remote microactuators are of great interest in biology and medicine as minimally-invasive tools for cellular stimulation. Remote actuation can be achieved by active magnetostrictive transducers which are capable of changing shape in response to external magnetic fields thereby creating controlled displacements. Among the magnetostrictive materials, Galfenol, the multifaceted iron-based smart material, offers high magnetostriction with robust mechanical properties. In order to explore these capabilities for biomedical applications, it is necessary to study the feasibility of material miniaturization in standard fabrication processes as well as evaluate the biocompatibility. Here we develop a technology to fabricate, release, and suspend Galfenol-based microparticles, without affecting the integrity of the material. The morphology, composition and magnetic properties of the material itself are characterized. The direct cytotoxicity of Galfenol is evaluated *in vitro* using human macrophages, osteoblast and osteosarcoma cells. In addition, cytotoxicity and actuation of Galfenol microparticles in suspension are evaluated using human macrophages. The biological parameters analyzed indicate that Galfenol is not cytotoxic, even after internalization of some of the particles by macrophages. The microparticles were remotely actuated forming intra- and extracellular chains that did not impact the integrity of the cells. The results propose Galfenol as a suitable material to develop remote microactuators for cell biology studies and intracellular applications.

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1. Introduction

The development of biological or biomedical microelectromechanical systems (BioMEMS) has created multipurpose tools able to individually address biochemical [1,2] or mechanical [3,4] processes carried out by cells. Besides their sensing abilities, there has been a growing interest in the actuation features offered by these devices through electric [5], mechanical [6] or magnetic [7] forces. With the introduction of smart materials into these devices, it has been possible to achieve externally addressable control, avoid tethering problems, facilitate targeting, and localize actuation [8]. Among these materials, large magnetostrictive solutions have emerged as effective tools to create remotely controlled deformations using external magnetic fields [9], to act by themselves [10] or in combination with other smart materials in tandem to create more complex capabilities [11]. In fact the integration of magnetoelastic materials into MEMS has brought an outstanding performance, as they give properties of “self-test, self-calibration and remote sensing and actuation” already stated by Gibbs [12], which certainly offers the perfect tool for biomedical less invasive tools.

One of the magnetostrictive materials with the most flexible and compatible properties with microtechnologies is Galfenol [13,14],

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an alloy made of iron and gallium, able to transduce magnetic energy into mechanical deformation with strains that go up to hundreds ppm at low saturating magnetic fields [15]. Its mechanical strength overcomes the mechanical problems of its famous counterpart Terfenol-D which has a higher magnetostriction (~2000 ppm) [16], but is very brittle at room temperature [17]. On the contrary, Galfenol has high ductility and great durability under tensile, bending and compressive forces [18], which are preserved at micro- and nanoscale [19]. It can also be sputtered from a fixed composition alloy target, creating high quality and compositionally consistent thin films [20], with the correct proportion of gallium and iron to ensure its magnetostriction [20]. The versatility of this material has opened the path to multiple applications from microactuators [14] to micro- and nanorobots [21]. Although the introduction of Galfenol to BioMEMs is only exploratory to date [22], it still is necessary to ensure its biocompatibility, as a first step in the development of medical applications. The first attempt to evaluate the biocompatibility of Galfenol [23] was performed through indirect cytotoxicity analyses using millimetric blocks in contact with the culture media. Once the blocks were removed, the media was used for fibroblast incubation with no adverse results in the cell survival. Additionally the biodegradable properties of Galfenol were also found to be negligible [23], showing a great future for the alloy in bioapplications. Going down in scale, Galfenol nanowires were also internalized by cells, showing cell viability in a preliminary qualitative toxicity assay [24].

The present study evaluates the integration of Galfenol to suspended microdevices for cell biology studies and intracellular applications. As a first stage, thin films of the alloy were fabricated to characterize the properties of the material and to perform an initial *in vitro* test, growing cells (human macrophages, osteoblast and osteosarcoma cells) directly on the Galfenol surface. Then, to evaluate the reaction of macrophages to the internalization of Galfenol, 3 μm × 3 μm × 1 μm silicon oxide microparticles were fabricated, covered with a thin layer of the alloy, and added to cell cultures. Finally, by means of an external magnetic field, the interactions between actuated microparticles and cells were analyzed.

2. Materials and methods

2.1. Fabrication of Galfenol test surfaces and Galfenol-based microparticles

Uniform Galfenol films, 160 nm thick, were deposited by radio frequency (RF) magnetron sputter deposition onto polysilicon substrates. The parameters of the deposition were 200 W for 45 min using a target with 18.4 at% Ga nominal (Etrema Inc., USA) and an Ar pressure of 2.8 mTorr (30 sccm).

Galfenol-based microparticles were fabricated using 1 μm thick thermal silicon oxide grown on a 100 mm Ø p-type silicon wafer (Fig. 1A). Positive UV photoresist was used to define an array of 3 μm × 3 μm squares separated by 3 μm (Fig. 1B). The oxide was patterned by Reactive Ion Etching (RIE; Alcatel 601E, France) with CHF_3 plasma for 11 min (Fig. 1C), followed by a strip of the photoresist, leaving the silicon oxide particles (Fig. 1D). Next, partial isotropic etching by RIE created a narrow anchor (Fig. 1E), followed by a thin layer deposition of Galfenol (160 nm) by same process used for the films above (Fig. 1F). The particles were released using mechanical peeling [2] which broke the silicon anchor without affecting the integrity of the Galfenol film (Fig. 1 G). Finally the particles were collected and suspended in ethanol for their storage (Fig. 1H).

Finally, another thin layer of Galfenol (160 nm) was sputtered on glass coverslips (Gold Seal, Portsmouth, NH, USA) with dimensions of 18 mm × 18 mm × 0.13 mm for magnetostriction measurements.

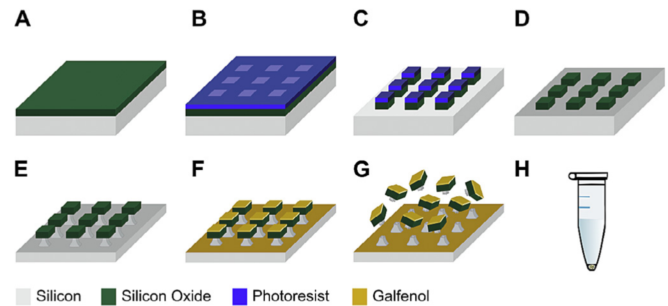

Fig. 1. Fabrication process of Galfenol microparticles. (A) Fabrication started with a silicon substrate with 1 μm thick thermal oxide growth. (B)(C) (D) Photolithography followed by a vertical etching defined the structure. (E) Anchors were made by etching the silicon isotropically. (F) 160 nm of Galfenol was deposited by sputtering. (G) The particles were mechanically released, (H) collected and suspended in ethanol.

2.2. Characterization of the alloy surfaces and the microparticles

Grain morphology of the samples was observed using a scanning electron microscope (SEM; Carl Zeiss, Auriga Series, 3 KV, Germany). The surface roughness of both samples was measured by means of an atomic force microscope (AFM; Veeco, USA) using the tapping mode. The morphology of the microparticles was observed using SEM, in order to verify the shape, size and anchor for releasing them effectively. A microparticle was cross sectioned by focused ion beam (FIB; Leo 1530 Zeiss, Germany) and observed with a Gemini SEM column, to observe the interface of the Galfenol film and the microparticle. The FIB used a Ga^+ ion beam at an accelerating voltage of 30 kV and a beam current of 30–100 pA. The composition of the Galfenol was confirmed through energy dispersive X-ray spectroscopy (EDX; Carl Zeiss, Auriga Series, 3 KV, Germany). The crystalline microstructure of the alloy was studied using an X-ray diffractometer (XRD; Bruker, AXS D8-Advance), for which the thin film specimens were cut into rectangular pieces (0.8 cm × 0.7 cm). The integrity of the microparticles after their release was evaluated by releasing a 2 μL drop of the ethanol suspension onto a clean Si substrate. Once the ethanol evaporated, the particles were observed using SEM. In terms of functional properties of the material, the magnetic properties of the samples were measured with a vibrating sample magnetometer (VSM; MicroMag 3900; Princeton Measurements Corporation, USA) at room temperature (RT), applying a maximum field of 10 kOe. The VSM samples were 160 nm Galfenol films on polysilicon substrates (6 mm × 8 mm) and a platform of the same size with unreleased Galfenol/SiO_2 microparticles. Furthermore, the magnetostriction of Galfenol on glass coverslips was measured using a capacitive bridge system described in detail in Ref. [25].

2.3. Cell lines

Three different human cell lines were used to test the effects of Galfenol films and microparticles: macrophages, osteosarcoma cells and osteoblast cells.

THP-1 monocyte cells were grown under standard conditions (37 °C and 5% CO_2) in RPMI 1670 medium (Life Technologies, UK) supplemented with 25% fetal bovine serum (FBS; Life Technologies, UK) and 5% L-glutamine (Biowest, USA). To differentiate monocytes into macrophages, 10^5 cells were seeded into 4-well plates and treated with 0.16 mM phorbol-12-myristate-13-acetate (PMA; Sigma, USA) for 48 h. The human osteosarcoma cell line Saos-2 (ATCC, USA) was cultured in Dulbecco's modified Eagle medium (DMEM; Invitrogen, USA) with 10% FBS under standard conditions.

Finally, human osteoblast cells (hOBs), isolated form trabecular

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