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Early adhesion of cells to ferromagnetic shape memory alloys functionalized with plasma assembled biomolecules – a single cell force spectroscopy study

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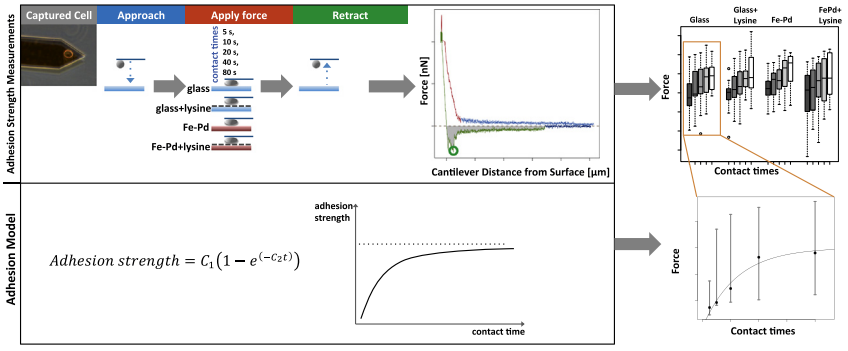
HIGHLIGHTS

- Fe-Pd based magnetic shape memory alloy functionalized in an inert gas plasma is a smart material with improved bioactivity.
- Single cell force spectroscopy in combination with a time-dependent bond model quantifies cell-surface interaction.
- Functionalization of Fe-Pd with plasma-polymerized L-lysine is a flexible, ultra-durable coating for improved cell adhesion.

GRAPHICAL ABSTRACT



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ABSTRACT

Biomaterial performance and integration of prostheses in vivo strongly depend on the ability of cells to adhere. Plasma-assisted functionalization of smart metals with biopolymers, including plasma polymerized L-lysine (PPLL), constitutes a recently-developed promising approach to synthesize highly flexible, yet robust and strongly adherent protein coatings that support cell-biomaterial interaction. In the present study we employ single cell force spectroscopy to demonstrate that PPLL coatings promote early adhesion of fibroblast cells on the ferromagnetic shape memory alloy Fe—Pd – a promising magnetically switchable biomaterial. By varying the contact time of a cell with the substrate surface, we show that the forces and work needed to fully detach a cell increase with time and quantify bioactivity of the material. In contrast to glass and PPLL-coated glass, cell detachment from Fe—Pd requires much larger work, while a PPLL biofunctionalization further improves cell adhesion and binding affinity by an increased detachment work on short time scales. Together with a time-dependent bond model we postulate a transition from unspecific to specific cell adhesion on Fe—Pd and PPLL-coated Fe—Pd, while on glass detachment forces are lower and level off to a saturation regime on short times prior to the expected time necessary for specific integrin-based bond formation.

1. Introduction

Cell adhesion to the extracellular matrix (ECM) or any other scaffold is a prerequisite for survival, proliferation and many other cellular

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processes. However, at a tissue-implant interface ECM molecules that support specific cell adhesion are lacking, particularly when composed of metals employed e.g. for joint replacements [1]. Bioactive coatings can enhance biomaterial performance and cell attachment [2], while alternatively cells deposit ECM molecules themselves to which they then bind [3]. The first adhesion event between the cell and a biomaterial results in the formation of focal complexes and can be mediated by hyaluronic acid, which occurs on time scales of seconds [4]. After this first soft-binding step, maturation to focal adhesion takes place that strong and specific adhesion sites form via the interaction of adhesion receptors with ECM molecules [5,6].

Within the last decade more and more artificial materials have arisen for biomedical and biotechnological applications. In contact with living cells, tissues and organs, new challenges have come up regarding the material - cell interface and coupling of the biomaterial to the implant. The use of biomaterials include high-end applications such as organ replacement [7], tissue engineering [8,9], dental [10], orthopedic [11] and cochlear implants [12], as well as cardiovascular [13], diagnostic [14] and pharmaceutical applications [15]. New approaches aim to design biomaterials which are not solely replacing damaged tissue by a passive implant that interfere with the surrounding tissue as little as possible, but active implants that interact with desirable stimuli [16,17]. Nevertheless, functioning of medical devices and implants relies on good integration and cellular adhesion in vivo. However, many widely used implant materials naturally do not promote adequate cell adhesion for the integration of the implant, and weak mechanical coupling, e.g. for bone prosthesis, can result in tissue degeneration and implant loosening due to stress shielding [18]. One difficulty is that the elastic moduli of widely used implant materials composed of metals have elastic moduli decades larger than that of tissues. Moreover, proteins that support cell adhesion such as fibronectin hardly adsorb on stainless steel and titanium dioxide surfaces [19,20]. Coatings that promote adhesion, such as hydroxyapatite for improved bone attachment, are frequently brittle and can rupture from the underlying implant.

Ferromagnetic shape memory alloys, including Fe—Pd, constitute an example of an upcoming smart materials class with promising properties for biomedical applications [21]. Inducing high reversible strains at moderate stresses in contact with tissues opens up the possibility of applying magnetic fields even long after insertion of the implants for readjustment, while this alloy also comprises the superelastic effect of conventional shape memory materials [22]. Basic biocompatibility of the Fe—Pd alloy with murine fibroblast cells, primary human osteoblast cells and human mammary epithelial cells has previously been investigated using immunofluorescence methods [22,23]. Additionally, cell adhesion promoting biomolecules such as fibronectin and laminin enhance cell attachment even more [22,24]. In fact, Allenstein et al. showed that plasma polymerized L-lysine (PPLL) adsorbs and strongly binds to the Fe-site of the alloy in contrast to conventional poly-L-lysine coatings resulting in improved cell adhesion and spreading [25]. PPLL is a flexible, yet ultra-durable coating which demonstrates plasma-assisted functionalization a new approach to couple proteins to a metal surface. Nevertheless, even for uncoated Fe—Pd the number of focal contacts of adherent cells is higher compared to titanium and glass substrates; however, a quantitative analysis of the acting adhesion forces is lacking [26].

Most studies addressing the interaction of cells with implant materials only focus on cell spreading behavior and the number of focal contacts that form during cell adhesion, while a quantitative analysis of adhesion forces and work needed to detach the cells is not employed. In this study we quantify early adhesion forces of murine fibroblast cells on Fe—Pd thin films, as well as samples coated with PPLL by using AFM-based single cell force spectroscopy (SCFS) initially described by Benoit et al. [27]. Results were compared with cell adhesion on pure glass and PPLL coated glass surfaces. During each SCFS cycle, immobilized cells attached to a cantilever are pushed to the substrate with a defined force and retracted again after a certain contact time. The respective detachment forces and the employed work are monitored to quantify cell-surface interaction for the different materials as a measure for bioactivity of the surface independent of the type of cell-surface binding, viz. specific and unspecific bonds. Moreover, a time-dependent bond formation model between cells and substrates is employed to analyze the detachment forces and work obtained from measurements with different contact times. Our results demonstrate that early adhesion forces and the work necessary to detach cells is much larger for Fe—Pd and PPLL coated Fe—Pd compared to glass and PPLL-coated glass which quantifies good bioactivity of this ferromagnetic shape memory alloy and makes it a promising candidate for medical applications.

2. Materials and methods

2.1. Fe-Pd film preparation and biofunctionalization

50 nm thick polycrystalline $Fe_{70}Pd_{30}$ films were sputter deposited onto soda lime glass substrates from a $Fe_{70}Pd_{30}$ alloy target (ACI Alloys, San Jose, CA); a film thickness as low as this guaranteed optical transparency, while already revealing a fully closed surface under the chosen deposition conditions. The procedure was carried out in a chamber that was evacuated and flooded with an argon flow of 3.5 sccm which provides a sputtering pressure of 5×10^{-3} mbar. Argon plasma was excited by an RF input power of 45 W. We would like to point out that previous in vitro assessments on vapor-deposited as well as sputtered single- and polycrystalline $Fe_{70}Pd_{30}$ thin films and roughness graded polycrystalline splats of the same stoichiometry revealed excellent biocompatibility and even bioactivity in contact with different cell types including NIH 3T3 fibroblasts [22,28].

Plasma polymerized L-lysine coating was employed as described previously [25]. Briefly, L-lysine monomer powder (Sigma-Aldrich, CAS No. L5501) that is diluted to a concentration of 0.009 mol/l via pure ethanol (Merck KGaA, CAS No. 64-17-5) was injected into a pulsed plasma inside a quartz tube of 3 cm inner diameter with a flow rate of 0.24 ml/min. The previously evacuated quartz tube is flooded with 200 sccm argon flow that introduces 10 mbar argon pressure. Excitation of the plasma is carried out inductively by a 13.56 MHz RF-supply attached to a self-constructed matching network. Plasma is stabilized at an input power of 15 W pulsing with 80 Hz and 8% duty cycle before adding the L-lysine solution. However, parameters are changed to 10 W and 2% duty cycle during the deposition. Films achieve a thickness of 50 nm after around 60 min. Flow of plasma and lysine is turned off after deposition while argon atmosphere is kept for additional 5 min. After the procedure residual lysine monomers that failed to cross-link to the coating are removed by rinsing with deionized water. In the following, plasma polymerized L-lysine coated Fe—Pd samples are referred to FePd + Lysine.

Control experiments were performed on pure glass substrates and plasma polymerized L-lysine coated glass (glass + Lysine).

2.2. Cells and culturing procedures

We employed NIH 3T3 embryonic mouse fibroblast cells since they have been widely used to study the biocompatibility and bioactivity of many materials including the ferromagnetic shape memory alloy Fe—Pd [22,24,26]. Cells are incubated at 37 °C in 5% CO_2 humidified atmosphere in full growth medium of Dulbecco's Modified Eagles Medium (DMEM) (Biochrom, Cat. No. F0435) complemented with 10% Calf Serum (CS) (PAA, Cat. No. B15-004) and 1% Penicillin - Streptomycin antibiotic solution (PS) (Sigma-Aldrich, Cat. No. P0781). Single cell force spectroscopy measurements were carried out in pure DMEM to avoid the presence of adhesion influencing serum proteins.