Grow of stem cells on chemical-laser generated titanium cobalt shape memory alloy nanoparticles

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Abstract:

In this research nanoparticles are fabricated using a method that is a combination of chemical and femtosecond laser ablation methods. The resulting nanoparticles are used to coat glass substrates in order to test the biocompatibility of nanoparticles with human adipose-tissue derived mesenchymal stem cells.

Nanoactuators made from nanoparticulate Co-Ti shape memory alloy show potential in mechanical stimulation of bone tissue formation from stem cells. We have represented the fabrication of Co, Ti and Co-Ti shape memory alloy nanoparticles and their biocompatibility to human adipose derived stem cells. The stoichiometry and phase transformation property of the bulk alloy is protected during attrition by femtosecond laser ablation in liquid, giving access to colloidal nanoactuators. No unfavorable effect on cell growth and attachment is observed in proliferation assay and environmental electron scanning microscopy, making this material attractive for mechanical stimulation of stem cells.

Keywords: n Nanoactuators, titanium, cobalt, stem cell, biocompatibility

INTRODUCTION

Micro- and nanoactuators made of shape memory alloys (SMA) show potential to stimulate human bone tissue formation from adipose tissue-derived mesenchymal stem cells (adMSC)[1]. Consequently, a manufacturing technology which provides SMA nanoparticles suitable for surface coatings has to be qualified. Moreover, the biocompatibility of SMA nanoparticles is a prerequisite for its application in stem cell and tissue stimulation. Nanocrystalline SMA material has become increasingly interesting in recent years because of its unique properties [2-5].

Titanium and its alloys are used in the field of stem cell research and tissue engineering . They also are used for the reparation or equivalent substitution of bone cells [6, 7].Not only the super elastic behavior of SMAs like NiTi, but also the one way effect are of interest, particularly for medical applications. study, the most important properties of nanoparticles are investigated: (i) the size of the nanoparticles, (ii) the processability for surface coating, and (iii) the stoichiometry.

Compared to the conventional methods, fabrication of nanoparticles using laser ablation promise much higher flexibility, since no chemical precursors are required [8-12]. The possibility of stoichiometric conversion of a bulk material into nanoparticles (e.g., alloys) using ultra short pulsed lasers allows nearly unlimited nanomaterial design [11,12].

In this study, we decided to investigate about cobalt, a nickel's neighbor in period table, so e at first the particles of CoTi were synthesize via a chemical-combustion method .Then they were formed as a cylinder .Finally, the colloidal synthesized nonoparticles was using femtosecond laser ablation in liquid. The resulting colloids are used to coat glass substrates, in order to test the biocompatibility of nanoparticles with human adMSCs.

EXPERIMENTAL

Biocomposite materials from titanium— cobalt alloys were obtained by the method of self propagating high-temperature synthesis (SHS). This method is based on the exothermic interaction between two or several chemical elements or compounds, which occurs under conditions of direct combustion[13,14]. Published data show that SHS may be used to prepare the porous material from titanium-cobalt alloy with the specified structure and properties [15].

For the reaction of SHS, metal powders were mixed at a stoichiometric ratio of titanium and cobalt . Calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$,HAP (10 wt %) was added to the reaction mixture. Titanium hydride served as a gasifying additive to produce the porous structure of materials. The mixture was pressed to obtain cylindrical samples (diameter 12 mm, height 15-16 mm). The samples were put vertically in an argon-filled reaction chamber. The initial temperature varied from 300 to 600°C. The local reaction was initiated by a hot wolfram spiral. After the induction of this reaction, a steady-state combustion wave spread over the sample (continuation of the process under conditions of SHS). The synthesized materials were sawed into targets(plates) with thickness of 35-70μm and used in further experiments.

The target was then placed in the center of a liquid reservoir (deionized water, Methyl acetate, and ethylene glycol)with a liquid level height of 10 mm. The sample was moved in an x–y direction, so that the femtosecond laser beam performed a meander contour on the surface. The laser beam is focused by a lens with a focal length from 10 to 250mm in order to get sufficient laser flounce for the ablation. The typical diameter of the laser spot on a bulk target changes from 0.05 to 2.0mm and was focused exactly at the CoTi foil surface, or slightly below. The movement of the laser relative to the surface was necessary to minimize the disturbance of the radiation absorbance by micro bubbles at the surface. A drop of the nanoparticle dispersion was placed on a polished alumina target, carefully dried, and investigated by a variety of analysis techniques such as scanning electron microscopy (SEM),transmission electron microscope (TEM), field emission environmental scanning electron microscope(ESEM), , energy Dispersive X-Ray(EDX) and Differential scanning calorimetry (DSC).

Cell isolation and culture

AdMSCs were isolated from adipose tissues using mechanical fragmentation and subsequent digestion with collagenase type XI. Then they were were cultured in DMEM/199 medium (1:1) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 U/ml streptomycin at 5% CO_2. Cells were passed in a preconfluent state. Cells from the first and second passages were used for the experiments. Unless stated otherwise, all experiments were conducted thrice in triplets each.

Live/Dead assay

AdMSCs were seeded onto glass cover slips either coated with 1 lμg CoTi nanoparticles in acetone or with acetone alone as a solvent reference. The LIVE/DEAD assay was carried out according to manufacturer's guidelines, and evaluated under a fluorescence microscope with the appropriate barrier filters after 48 h incubation.

Proliferation assay

Proliferation assays were made in 24-well-plates by incubating 50,000 adMSCs in 1 mL medium on triplicates of glass cover slips coated with Cobalt, Titanium, Cobalt–Titanium, or Silver nanoparticles (1 lμg/cm^2). Proliferation was assessed by measuring extinction after 48 h. To this end, the glass cover slips were dip washed in cold phosphate buffered saline and incubated with 30 μL RIPA-24 for 1 min. Cells were then scrubbed off the glass cover slips and transferred into photo spectrometer tubes containing 1 mL distilled water. To each tube, 1 mL of Coomassie reagent was added. The protein content was used as a sensitive measurement of cell number. The experiment was repeated at three independent time points.

RESULTS AND DISSCUTIONS

A closer look at the interface between the living cells and the CoTi nanoparticle is taken using FE-ESEM at 99.7% humidity (Fig.1).

The typical thickness of adMSCs is in the range of several 10 nm, and the diameter is about 100 lm [16]. The dark areas on the ESEM pictures spot cells, brighter areas show the heavier elements nickel and titanium; and the glass plate is shown in background. The extensions of the cell spread across the nanoparticle coating and agglomerated CoTi nanoparticle islands (Fig. 1.a).

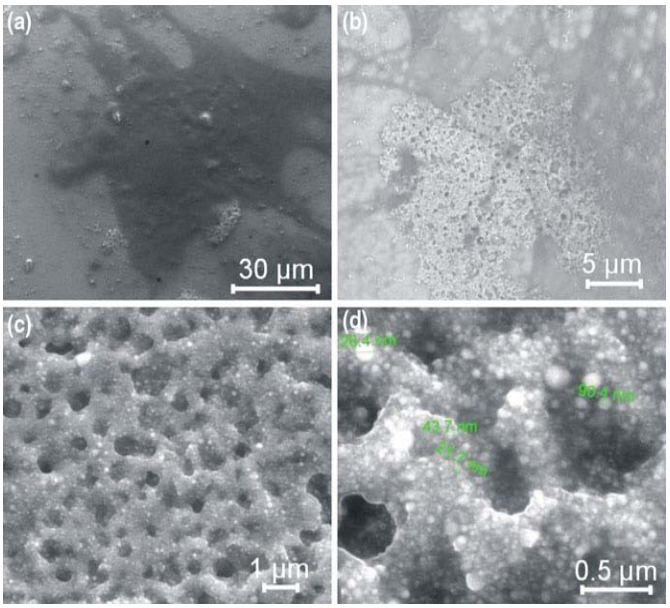

Fig. 1. Morphology of stem cell growth on CoTi nanoparticle coating. FE-ESEM images (99.7% humidity, 5–10 kV) of adMSC on glass plates coated with 1 l μg/cm2 CoTi nanoparticles.

This image indicates that cell attachment is sufficient for the growth of adMSCs. It has been reported that cell uptake of nanoparticles, especially those releasing toxic ions like silver hydroxide ions, causes apoptosis [17]. However, the adMSC show morphology of living cells. Kirchner et al. discovered that impairment of cells due to nanoparticles (CdSe or CdSe/ZnS) is not only caused by the release of ions, but also by the attachment of nanoparticles on the cell surface [18]. This scenario of nanoparticles being in direct contact with cell surfaces is comparable to our investigations. Our pictures show a cell–nanoparticle interface with no visible strong adverse effect. A closer look at the area in picture (a) is shown with higher magnitude in pictures (b)–(d). The image shows a matrix with embedded nanoparticles. Element mapping was carried out to analyze this matrix in Fig. 2.

The presence of an organic matrix becomes evident, including an embedded anoparticulate metal fraction. Cell extensions are spread over this area. The formation of this matrix might have an influence on the SM effect, and should be investigated in future studies.

Overall, the adherence and proliferation of ad-MSCs is not influenced by the presence of 1 lμg/cm^2 ^{2} NiTi nanoparticles generated using femtosecond laser ablation in liquid. In summary, it is demonstrated that laser ablation in liquids provides nanoparticulate SMA colloids. Biocompatibility of coatings derived from these colloids render future application for the nanomechanical stimulation of adMSCs possible.

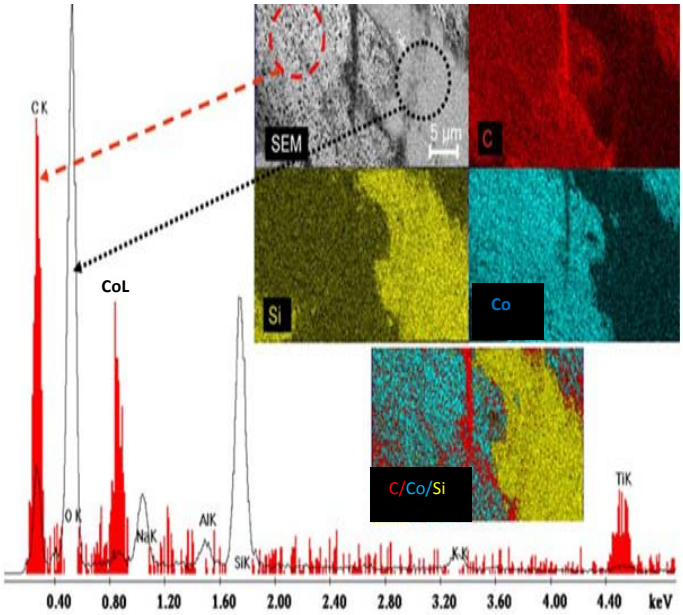

Fig.2. EDX mapping of an FE-ESEM image of adMSCs incubated on a glass plate coated with CoTi nanoparticles. Element mapping of the red (stripe) and black (dots) circled area. Colored SEM images of carbon (red), silicon yellow), and Co (blue), as well as the overlay picture of the three elements.

CONCLUSION

We found several indications for the transformation capability of the fabricated CoTi particles, including the proof that particles around 30-nm size have CoTi alloy composition, and a clear calorimetric behavior typical for SMAs with only slightly shifted transformation temperature.

Particles that are formed in place, e.g., by deposition techniques are difficult to transfer on a desired surface or to disperse into a polymer. Compared to these substrate-bond nano-islands, the laser-fabricated SMA particles are available in stable colloidal state. Since the liquid matrix can be an aqueous or organic solution, potential integration into nanomechanical devices is facilitated, as demonstrated here via embedding of the CoTi nanoparticles into a polymer or coating of a glass substrate.

However, a prerequisite to studies on the nanomechanical cell stimulation is the biocompatibility of the cobalt-containing surface nanostructures. At a surface coverage of 1 lμg /cm^2, no adverse effect on adMSCs was observed, indicating biocompatibility of CoTi nanoparticles. It may be beneficial that the physical laser ablation process allows the fabrication of colloids without using chemical precursors or preservatives, which may affect the purity of a colloid and biocompatibility of the resulting surface coatings.

A comparison of CoTi nanoparticle surface coating with the effect of nanoparticles dispersed in cell culture is projected.

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