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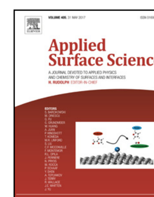

Full Length Article

Poly(lactide-co-glycolide) nanofibrous scaffolds chemically coated with gold-nanoparticles as osteoinductive agents for osteogenesis

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ABSTRACT

Poly(lactide-co-glycolide) (PLGA) is a biocompatible and biodegradable polymer that has been widely used in devices for tissue engineering and drug delivery applications. Gold nanoparticles (GNPs) have also been used as biomaterials and have been found to have a positive effect on bone formation. In this study, we synthesized thiol end-capped PLGA (PLGA-SH) and used it for binding GNPs. This PLGA was processed into a sheet form via electrospinning. GNPs with an approximate size of 30 nm were attached onto the PLGA-SH sheet surfaces (PLGA-GNPs). This membrane was characterized by thermogravimetric analysis, ultraviolet/visible spectrophotometry, field emission scanning electron microscopy, energy dispersive X-ray spectroscopy, X-ray photoelectron spectroscopy, and confocal laser scanning microscopy. Characterization results show that the GNPs are well attached on the PLGA-SH sheet and it is possible to control the GNPs load. Additionally, in-vitro results showed that PLGA-GNPs have good biocompatibility. They were also found to enhance osteogenic differentiation of human adipose derived stem cells. From these results, we have determined that the PLGA-GNP fibers can be useful as materials for bone regeneration and can also potentially serve as drug carriers.

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1. Introduction

The aim of tissue engineering is to replace or repair damaged tissue with an artificial replacement that has the same biological function as the original tissue. Tissue engineering and regenerative medicine is supported by the continuous development of materials, enhanced engineering, and improved medical technology. Recently, micro- and nano- technologies have emerged as valuable technologies in this field. Nanotechnology is defined as a technology which deals with matter at a scale of 1–1000 nm. In the field of biotechnology, nanotechnology has been applied to the development of bio-chips, nano-biosensors, drug delivery systems, and diagnostics. Development of nanotechnology has enabled the production of biological substitutes having more precise biomimetic environments for use in tissue engineering. Various nanomaterials have been used to deliver drugs or peptides. Other nanomaterials have been designed to present a structure similar to that of extracellular matrix [1,2]. Electrospun nanofibers are nanomaterials that have been utilized for many state-of-the-art industries such as medicine, biotechnology, and military applications [3]. This is because of the beneficial attributes that electrospinning offers, including well defined nm-sized pores. In biotechnology, electrospun nanofibers are utilized for biosensors, tissue regeneration scaffolds, and drug delivery systems [4,5]. In particular, poly(lactide-co-glycolide) (PLGA) nanofiber sheets have been used as scaffolds for tissue engineering. This is because of PLGA's biodegradability, biocompatibility, and mechanical properties [6–8]. However, PLGA fiber sheets alone are not enough for effective tissue regeneration. For this reason, additional functionalities are required for an effective material.

Many researchers have utilized growth factors, drugs, or proteins to aid osteogenesis of tissue scaffolds. However, these materials have some drawbacks due to their cost and difficulty in handling. Gold nanoparticles (GNPs), a bone morphogenetic substance, have been widely used in many fields such as drug-delivery,

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bio-sensing, and tissue-engineering. This is because of their unique properties [9–12]. GNPs also have a powerful bonding force with thiol-groups which allows them to be conjugated to a wide variety of materials [13,14]. In recent studies, GNPs not only inhibited the formation of osteoclasts, but also supported osteo-differentiation [15–17]. Heo et al. established that 14 μg GNPs have a higher osteoinduction effect than 10 ng of bone morphogenetic protein-2 in a hydrogel environment [18]. These results indicate that GNPs can be a good replacement for expensive bone morphogenetic protein-2.

Based on these findings, we designed and prepared a thiolated PLGA (PLGA-SH) nanofibrous sheet for binding with GNPs using a simple process. The objective of this study was to design a manufacturing process for PLGA-GNPs nanofibrous sheet. This sheet can find many applications within the field of tissue engineering, more specifically orthopedic materials. The manufactured membrane was characterized by thermogravimetric analysis (TGA), Field emission scanning electron microscopy (FE-SEM), energy dispersive X-ray spectroscopy (EDX), X-ray photoelectron spectroscopy (XPS), Attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR), and ultraviolet/visible spectroscopy (UV-vis). It was also evaluated for cytotoxicity and osteogenic effects using human adipose derived stem cells (hASC).

2. Materials and methods

2.1. Materials

Poly(D,L-lactide-co-glycolide) (PLGA, lactic acid: glycolic acid ratio 75: 25) (Mw 20,000), N,N'-dicyclohexylcarbodiimide (DCC), ethylene diamine, Gold (III) chloride hydrate (99.999% trace metals basis), sodium citrate, formaldehyde, β-glycerol phosphate disodium salt hydrate, ascorbic acid and dexamethasone were purchased from Sigma-Aldrich (St. Louis, MO). N-hydroxysuccinimide (NHS) and 1,1,1,3,3,3-Hexafluoroisopropyl Alcohol (HFIP) were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Dichloromethane (DCM) was purchased from Junsei Chemical Co., Ltd (Tokyo, Japan). Anhydrous diethyl ether was purchase from JT Baker (Phillipsburg, NJ, USA). EZ-Cytox (enhanced cell viability assay kit) was purchased from Dogen (Seoul, Korea). Human ASCs and MesenPRO RS^{TM} medium (MPRO medium) were obtained from Invitrogen (Carlsbad, CA, USA). Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate buffered saline (PBS), fetal bovine serum (FBS), trypsin–EDTA, and penicillin streptomycin were purchased from Gibco (Gran Island, NY).

2.2. Equipment

XPS was performed using a K-Alpha (TFS, USA). Field Emission Scanning Electron Microscopy (FE-SEM) and Energy-dispersive X-ray spectroscopy (EDX) observations were carried out using a Field Emission model S-4700 microscope (Hitachi High Technologies Corp., Tokyo, Japan). Thermogravimetric analysis (TGA) was performed using a SDT Q600 (TA instrument, USA). Attenuated total reflectance fourier transform infrared Spectroscopy (ATR-FTIR) measurements were carried out using an HYPERION model 3000 equipped with an IFS66V/S module (Bruker Optiks, Germany). The ultraviolet/visible (UV/Vis) spectrum was measured using a UV-1650 (Shimadzu Europe, Germany). Dynamic light scattering (DLS) was performed using a 90 Plus Particle Size Analyzer (Brookhaven Instruments Corporation, NY, USA). Light scattering images were obtained using a Kodak Image Station 4000 MM (Digital Imaging Systems, New Haven, CT, USA). Confocal laser scanning microscopy (CLSM) observations were carried out using a C1si (Nikon, Japan).

2.3. Preparation of thiolated PLGA and GNPs

GNPs were produced using the citrate reduction of HAuCl4 [19]. Briefly, a 0.02% solution was stirred at 100 °C, and then 2% sodium citrate was added with rapid stirring. After the solution color changed to dark red, the reaction temperature was held at 100 °C for 15 min. The reaction solution was then cooled slowly to room temperature. The solution was subsequently stored refrigerated.

The process for generating PLGA-GNPs is shown schematically in Fig. 1. PLGA (RG 757 S, 75:25, 5 g) was dissolved in dichloromethane (DCM, 100 ml) along with DCC and NHS at a molar ratio of PLGA:DCC:NHS = 1:10:10. The reaction was allowed to proceed at room temperature. After 3 h, ethylene diamine was added to the solution with rapid stirring. This was allowed to react at room temperature overnight. The mixture was then filtered and precipitated in 4 °C anhydrous ether. The precipitate was then dried using a vacuum oven at room temperature overnight. The amine-terminated PLGA was then dissolved in 100 ml of DCM and 2-iminothilane hydrochloride in methanol was added to the amine-terminated PLGA solution with rapid stirring. After the reaction, the mixture was kept at room temperature for 24 h. Subsequently, it was precipitated in 4 °C anhydrous ether. The precipitate was completely dried in a vacuum oven at room temperature.

2.4. Fabrication of thiolated PLGA fiber via electrospinning (ELSP) and binding of the GNPs

The thiolated PLGA was dissolved in 10 ml HFIP to form an 8% solution. To prepare the nanofiber sheets via ELSP, the polymer solution was put into a luer-lock syringe connected to a 20 gauge stainless-steel blunt-ended needle by a Teflon tube. The distance between the needle tip and the collector was set at 15 cm. The rotating mandrel-type collector was covered with aluminum foil and the polymer solution was electrospun onto the aluminum-covered collector at 18 kV positive voltage using a high-voltage DC power supply (Nano NC, Korea) with a flow ratio of 1 ml/h (KDS-200, KD Scientific Inc.). In order to remove residual solvent, the nanofiber sheets were vacuum dried overnight at room temperature. After vacuum drying, the PLGA sheets were separated from the aluminum foil and the sheets were cut to an appropriate size (5 by 6 cm). The cut PLGA-SH sheets were immersed in 30 ml solutions of GNPs which had been diluted to various concentrations (0 μM, 50 μM, and 200 μM, respectievly). Subsequently, the sheets were shaken at 70 rpm in an incubator set to 37 °C for 24 h. The different loads of GNPs (50 μM and 200 μM loading solutions, respectively) were attached to PLGA-SH sheets (50 μM: PG-L, 200 μM: PG-H and PLGA-GNPs), and the sheets were cleaned three times with distilled water. An un-coated control PLGA sheet (PLGA-SH) also underwent the same washing process.

2.5. Characterization

The manufactured sheets were prepared for FE-SEM and EDX measurements by coating them with platinum. EDX analysis was performed at 3.0 kV. The sheets that had been treated using different concentrations of GNPs-solutions were observed by FE-SEM at 3.0 kV and 10,000 X magnification. XPS was utilized to determine the GNPs-bound on the surface of the PLGA sheet. TGA was performed under a 100 ml/min high purity nitrogen flow. The temperature was increased at a rate of 10 °C/min until it reached a maximum of 800 °C. The PLGA, PLGA-SH and PLGA-GNPs sheets were also analyzed by ATR FT-IR.

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