Materials Science and Engineering C 78 (2017) 452–456

Contents lists available at ScienceDirect

Materials Science and Engineering C

journal homepage: www.elsevier.com/locate/msec

A novel chondroitin sulfate decorated nano platinum for the treatment of osteoarthritis

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ARTICLE INFO

Article history:
Received 19 March 2017
Received in revised form 3 April 2017
Accepted 5 April 2017
Available online 6 April 2017

Keywords:
PtNPs
Chondroitin sulfate
Osteoarthritis

ABSTRACT

The present work showed the biofabrication of platinum nanoparticles (PtNPs) using chondroitin sulfate via a facile, eco-friendly route by just heating leaf extract and $H_2PtCl_6 \cdot 6H_2O$ (Chloroplatinic acid) solution which gave a brown-colored PtNPs dispersion. The assynthesized PtNPs were analyzed by using Transmission electron microscopy (TEM), Energy dispersive spectroscopy (EDX), X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy and Selected area electron diffraction (SAED). TEM analysis showed PtNPs of irregular shape with a size existed in the range from 3 to 5 nm. From zeta potential studies it is found the surface charge of the synthesized PtNPs is negative (−25.6 mV). FTIR analysis and zeta potential measurements of PtNPs confirm the capping of chondroitin sulfate onto the surface of nanoparticles. XRD and SAED pattern revealed the crystalline nature of synthesized nanoparticles. Further, the in-vitro cytotoxicity of PtNPs against the osteoarthritis chondrocytes showed their biocompatibility, hence the obtained nanoparticles may have future scope in the treatment of osteoarthritis. Also, the present approach is green alternative to the traditionally available chemical methods that are currently been used now a days using chemical reagents such that are hazardous to human and environment.

1. Introduction

In the developing field of nanoscience and nanotechnology, green synthesis of metal nanoparticles such as gold, palladium, silver, platinum, copper oxide and zinc oxide is found to be the best environment friendly substitutes to the chemical and physical methods, which are harmful to environment and human beings. Application of green synthesis principles to develop new synthetic approaches using proteins/enzymes [1–3], microbes [4] and extracts of plants [5,6] play an important role in the synthesis of nanomaterials. On the other hand, increasing the environmental related problems raised by chemical mediated preparation of nanoparticles has led to attempts to advance the preparation of nanoparticles using extracts of plants. A green synthetic approach for nanoparticle preparation which does not use any hazardous reagents during synthesis procedure will play an important role to obtain nanoparticles in bulk scale.

Green approaches for the preparation of PtNPs using plant extracts have not been broadly explored. Literature reports showed that the biofabrication of PtNPs employing extracts of plants have been reported using *Cacumen platycladi* [7], *Anacardium occidentale* [8], *Bidens Tripartitus* [9], *Ocimum sanctum* leaf extract [10], *Punica granatum* [11], *Diospyros kaki* [12], *Cochlospermum gossypium* [13] and honey [14].

Present report investigated the green synthesis of PtNPs using chondroitin sulfate. The synthesized NPs have been studied using various microscopic and spectroscopic characterization techniques. The obtained PtNPs are studied for their in-vitro cytotoxicity against human osteoarthritis chondrocytes

2. Materials and methods

2.1. Materials

$H_2PtCl_6 \cdot 6H_2O$ (Chloroplatinic acid), chondroitin sulfate and Hoechst (2-[4-ethoxyphenyl]-5-[4-methyl-1-piperazinyl]-2,50-bi-1H-benzimidazole trihydrochloride trihydrate) were purchased from Sigma-Aldrich Chemicals. Double distilled water was used throughout the experiments.

2.2. Green synthesis of nanoparticles

For preparation of PtNPs initially 10 mL of 2 mM of $H_2PtCl_6 \cdot 6H_2O$ was added to 10 mL of chondroitin sulfate (20 mM) and heated at 90 °C for about 4 h under magnetic stirring. The change of color of reaction solution to brown indicated the formation of PtNPs.

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http://dx.doi.org/10.1016/j.msec.2017.04.028
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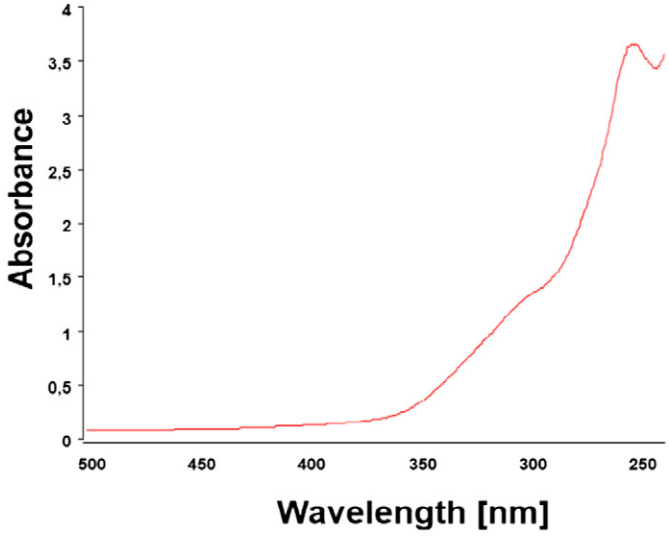

Fig. 1. UV–Visible spectroscopy of PtNPs.

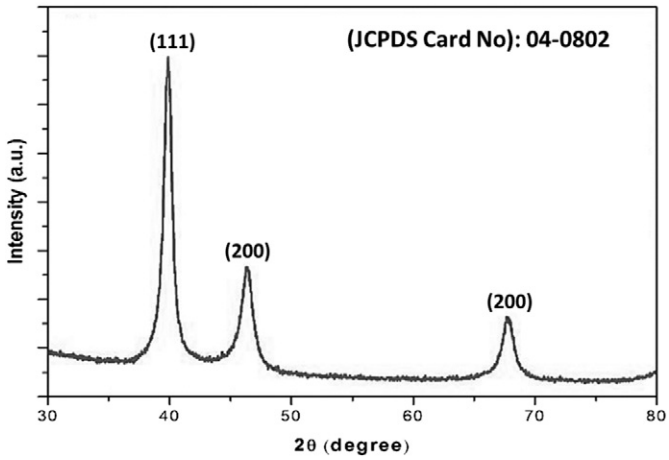

Fig. 2. XRD pattern of PtNPs.

2.3. Characterization of nanoparticles

JEOL JEM 2100 high resolution transmission electron microscope was used to know about the morphology (size, shape) of PtNPs, Energy Dispersive Spectroscopy (EDS) and selected area electron diffraction (SAED) pattern analysis. Instrument was operated at an accelerating voltage of 200 KV. A BRUKER D8 Advanced X-ray diffractometer was used to record X-ray diffractometer (XRD) pattern for the dried, purified

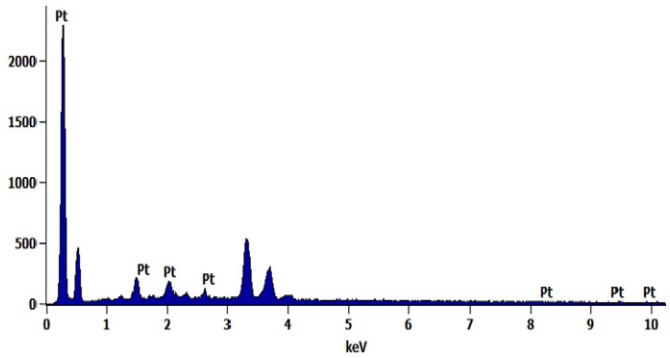

Fig. 4. EDS spectrum of PtNPs.

sample of PtNPs with Cu Kα source (λ = 1.5406 A°), scanning rate of 4°/min and step size of 0.02°. Malvern Zetasizer Nano ZS90 counter was used for Zeta potential and dynamic light scattering (DLS) measurements for the synthesized nanoparticles to know the details of size and stability. Samples for size distribution were prepared by diluting 1 mL of obtained PtNPs colloid with 4 mL of double distilled water. Fourier transform infrared (FTIR) spectrum for the PtNPs was recorded to know about the surface capping using SHIMADZU, IRAffinity 1 spectrometer.

2.4. In-vitro cytotoxicity assessment

2.4.1. Cell culture

Osteoarthritic chondrocytes were isolated from the fragments of articular cartilage by following the reported procedure [15]. In brief, a sequential enzymatic digestion of articular cartilage fragments was conducted for half an hour using 0.1% of hyaluronidase and 1 h with 0.5% of pronase, which then followed by digestion with 0.2% of collagenase for about 1 h at 37 °C in a solution mixture consisting of penicillin/streptomycin, Dulbecco's modified Eagle medium (DMEM) and amphotericin. The consequently obtained cell suspension was then filtered two times with 70 μm nylon meshes and centrifuged at 700 rpm for about 10 min. The resulting suspension consists of 90–95% viable cells, which were later tested by Trypan blue viability test. The resulting primary chondrocyte cultures were then incubated in 5% CO_2 at 37 °C for about two weeks.

2.4.2. Cytotoxicity evaluation

The Osteoarthritic chondrocytes were initially seeded at 4×10^4 cells/well in 24-well microplates and then about 1 mL of DMEM medium consisting of 200 mg mL^{-1} streptomycin, 2 mM glutamine, phenol red with 10% fetal calf serum, 200 U mL^{-1} penicillin was added and the cells were allowed to become pre-confluent. Then after, the cells are treated with various concentrations of PtNPs (10, 20, 40, 80 and

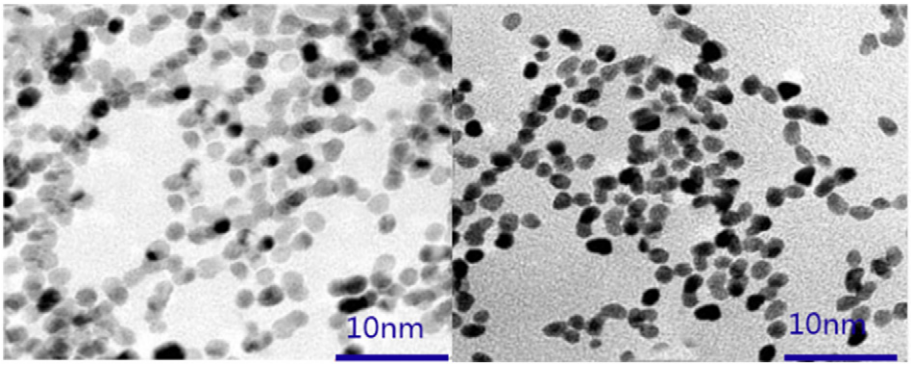

Fig. 3. TEM image of PtNPs synthesized using chondroitin sulfate.