SELF ASSEMBLED NANOSTRUCTURE OF PLGA-GLUCOSAMINE COMPOSITE NANO PARTICLES

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1 General Introduction

For decades, the self assembly of complex nanostructures out of simple colloidal nanoparticles (NPs) is of practical interest for building materials with unique properties to be used as drug delivery carrier. Self-assembly is the process of inter- and intra-molecular bonding by means of Van der Waals forces or hydrophobic interactions which normally resulted in close-packed structures. This close-packed structures may be either colloidal crystals or particle clusters depends on the place where the process of assembly takes place such as in the bulk fluid [1] or in the liquid-liquid interface [2], respectively. Polymer based self-assembled nanostructures are one of the potential nano vehicles for delivering wide range of pharmaceutical agents. Due to their good biocompatibility and natural degradation/resorption pathways, some polymers like poly(lactic acid), poly(glycolic acid), and poly(lactide-co-glycolide) (PLGA) were studied extensively as drug delivery carrier in the form of NPs[3]. Several studies on NPs based oral and parenteral formulations of PLGA were studied and due to high stability, they are found to be advantageous than liposomes [4-6]. There are reports showing the efficiency of PLGA based nanoformulation for transdermal delivery of drugs such as flufenamic acid and bioactive agents like plasmid DNA [7,8].

For tissue engineering applications, the foreign body giant cell response after scaffold implantation is one of the major problems in the field of biomedical nanotechnology. Glucosamine hydrochloride (GlcN Hcl), an aminosugar found abundantly in articular cartilage matrix, is studied extensively for the treatment of osteoarthritis. Evidence suggested that glucosamine (GlcN), has natural COX-2 inhibitory activity [9]. The crosslinking of GlcN, a natural anti-inflammatory drug, with biodegradable PLGA polymer can render its form of biomedical composite nanostructure, a dual characteristic of anti-inflammatory effect and protein carrier.

The presented work have utilized sonication to increase the scale and throughput of this method to produce self assembled nanostructure out of colloidal nanoparticles of biologically inspired polymer composite, PLGA-GlcN.

2 Experimental

2.1 Preparation of PLGA-GlcN composite

Chemical grafting of the PLGA-g-GlcN was prepared with EDC system with various modifications from the previous literature [10]. Briefly, PLGA (0.5 g) and DMAP (0.047 g) were dissolved in 18 ml of DMSO by ultrasonication. The solutions were then mixed with 1 ml of GlcN solution (1g, in deionized water (DI H2O). The GlcN solution was mixed dropwise to the PLGA/DMAP solution and an EDC solution (0.55 g, in 2 ml of DMSO) was then added at room temperature. After 3 h, the mixed solution was then poured into excess amount of acetone to produce the precipitates. The precipitates were then dissolved in phosphate-buffered saline (PBS) and dialyzed with a membrane for two days to remove the ungrafted GlcN. After the sample had been lyophilized until dryness and stored. The schematic of the chemical reaction happens when PLGA conjugated with GlcN was given in Fig. 1.

2.2 FT-IR analysis and NMR analysis

The Fourier transform infrared (FT-IR) spectra for free glucosamine, free PLGA and conjugated
PLGA-Glu were obtained from Fourier transform infrared (FT-IR) spectrophotometer using a TENSOR 27 instrument (Bruker) for analyzing the chemical modification after conjugation of PLGA with Glu. Detection of spectra obtained in the range of 4000-500 cm⁻¹.

Nuclear magnetic resonance (NMR) spectra were recorded on a 400MHz ¹H NMR spectrometer (AVANCE 400 WB) using commercially available deuterated solvent (D₂O). Proton chemical shifts are reported in parts per million (ppm, δ).

2.3 Preparation of self assembled nanostructure

PLGA-Glu (7.5%) with polyvinyl alcohol (PVA, 5%) was mixed in 1ml DI H₂O. The mixture was probe sonicated for 5 min. Add the above mixture dropwise to 3 ml of diethyl ether (DEE) under probe sonication for 5 min to form w/o emulsion. In the mean time mix 1% tween 20 with 9 ml of ethanol and sonicate. Followed by, the above formed w/o emulsion was added dropwise to 9 ml of ethanol and continue sonication for 2 min. Finally, the mixture was subjected to magnetic stirrer at 1200rpm for 2 h. This w/o/w emulsion of milky white solution contains the self assembled raspberry like nanostructure of PLGA-Glu. The DEE was removed under reduced pressure using a rotary evaporator. The final concentration of Glu in the emulsion is 5%.

2.4 FE-SEM analysis, Dynamic light scattering and zeta potential measurements of PLGA-GlcN nanostructure

The synthesized PLGA-Glu NPs were characterized for their surface morphology by using field emission scanning electron microscope (FE-SEM; JEOL-ISM-7500F). A single drop of emulsion was placed on a pre-cleaned glass cover slip, air dried, and was sputter-coated with platinum and visualized using FE-SEM.

Size distribution and zeta potential were determined using Malvern Zetasizer Nano ZS series, with a laser at 633 nm and a scattering angle of 90°.

3 Results and discussion

The IR spectra of PLGA, GlcN, and the PLGA-GlcN are shown in Fig. 2. On the IR spectrum of the PLGA-GlcN, several characteristic peaks from GlcN such as 3290, 1538, 1092 and 1030cm⁻¹ for primary amine -NH stretch, -NH bend, -CN stretch, and primary alcohol –C-O stretch respectively, appeared in addition to the peaks due to PLGA. This proved that the PLGA contained GlcN moieties.

In addition, PLGA-GlcN conjugate was further analyzed by 1H-NMR obtained from BRUKER 400 MHz NMR spectrometer using the solvent deuterated water (D₂O) (Fig. 3). The resonance from 8.7 to 6.7 ppm was due to NH of primary amine linkage proton of GlcN [11]. 5.4 ppm chemical shift was brought by SP² hybridized CH proton [11]. 3.8 to 3.2 ppm chemical shift were brought by proton from GlcN OH [12]. The shift at 2.6 ppm corresponds to proton CH of GlcN and to proton COOR of PLGA. The shift at 2.73 ppm is due to terminal OH of PLGA. Also resonance at 1.07 and 1.2 ppm were due to SP³ hybridized CH proton (CH₃-CH-) of PLGA. It is evinced from these data that the successful conjugation of PLGA was taken place with GlcN.

A novel new EDC based reaction method is used to graft PLGA with a natural amino monosaccharide, GlcN. Probe sonication offers a means to accelerate reaction kinetics via the formation of high-energy cavitation bubbles and microdroplets created by low frequency (20—100 kHz) ultrasound with an output of 50W. This successfully induces the self assembly of 15-25nm sized PLGA-GlcN nanoparticles resulting in a final size of 200-300nm sized nanostructure as shown in field emission scanning electron microscope (FE-SEM) image (Fig. 4). Dynamic light scattering technique confirms the size distribution of self assembled nanoparticles determined by FE-SEM analysis. PLGA-GlcN nanoparticles assemble at the water/oil interface due to the presence of the hydrophilic GlcN and hydrophobic PLGA group. The accelerated formation of nucleation centers on the cavitation bubbles through high-intensity sonication facilitates the transport and self assembly of polymer particles into the water/oil interface which leads to the monodispersed self assembly of nanoparticles with
increased surface area [13]. It is believed that GlcN usage will minimize the in vivo immune rejection and so the PLGA-GlcN nanostructure formed is found to be biologically inspired.

4 Conclusion
The present research synthesized a novel biologically inspired polymer composite, PLGA-GlcN, and utilized probe sonication technique for the monodispersed fabrication of self assembled nanostructure of PLGA-GlcN nanoparticles with high surface area. These self assembled PLGA-GlcN nanostructures are intended to be used as potential drug delivery carrier, powerful molecular imaging system and implantation material.

References

Fig.1. Chemical reaction of PLGA-GlcN conjugation using EDC coupling reaction.
Fig. 2. FT-IR spectrum of GlcN, PLGA and PLGA-GlcN.

Fig. 3. $^1$H NMR spectrum of PLGA-GlcN conjugated composite material.

Fig. 4. FE-SEM image of self assembled PLGA-GlcN nanoparticles (top) and size distribution analysis results (bottom).