Heparin/Poly(l-lysine) Nanoparticle-Coated Polymeric Microspheres for Stem-Cell Therapy

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Heparin/Poly(l-lysine) Nanoparticle-Coated Polymeric Microspheres for Stem-Cell Therapy

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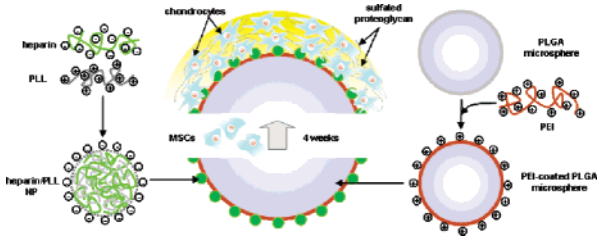
The design of nanostructures and nanometer-scale fabrication is driven not only by the novel, yet unexplored properties associated with nanoscale materials, but also by the continuously increasing demand for further miniaturization of electronic components, optical detectors, chemical and biochemical sensors, and other devices. Recent conceptual advances, which have taken advantage of new and practical techniques for size distribution and stabilization control, have created novel routes for the synthesis of nanoparticle-based materials, in which nanoparticle (NP) building blocks can be spatially ordered in a controlled manner.[1−4] The unique properties of NPs have compelled the development of novel methods for their preparation on (or in) organic or inorganic templates.[5−7] Of particular interest is the formation of NP clusters, which retain their surface plasmon absorption bands when immobilized onto functionalized silica nanoparticles.[8]

Layer-by-layer (LbL) assembly has also been suggested as a method by which to direct the formation of thin films from oppositely charged NPs and polymer layers which have been sequentially adsorbed onto a substrate. The incorporation of NPs on the polymeric surface via the LbL method may allow for the construction of polymer substrates with high surface-to-volume ratios, and may also cause the concentration of the immobilized entity to be considerably higher than that afforded by experimental protocols predicated on the immobilization on matrix. The immobilization of nanosized materials was detected in different assemblies on a variety of substrates, including planar two-dimensional or spherical three-dimensional configurations. Among the substrates, polymer microspheres have been successfully incorporated using inorganic nanoparticles, including gold, silver, and silicone.

Herein, we present a simple and highly efficient method for the complete stabilization of three-dimensional nanostructures via physical immobilization to polymeric microspheres. To the best of our knowledge, no one has, thus far, prepared or evaluated the qualities of organic microspheres constructed from organic NP clusters.

In this work, we attempted to devise a simple and highly efficient method for the complete stabilization of three-dimensional nanostructures via their embedment into a microspherical structure. As a result, a new type of flexible nanoparticular structure was generated on the microspheres using this method. To fabricate the nanoscale structure on a 3D matrix for cell delivery vehicles, heparin/poly(l-lysine) nanoparticles were prepared as an initial step (Scheme 1). Heparin is a highly sulfated, anionic polysaccharide that is composed of repeating glucosamine and uronic acid residues. In addition, heparin can interact with a variety of proteins that have heparin-binding domains, including various growth factors that enable the growth factors to cross-link their receptors. On the other hand, poly(l-lysine) has been used as a commonly utilized model cationic polymer to fabricate the polyionic complexes with anionic polymer. In cell delivery vehicles, heparin-functionalized hydrogel was used for human mesenchymal stem cells (hMSCs) for osteogenic differentiation.[9] In this system, heparin-functionalized hydrogel supported hMSC viability and induced osteogenic differentiation. Similar to the heparin-functionalized hydrogel, our polymeric matrix system has good viability and induces chondrogenic differentiation.

Scheme 1. Schematic Diagram of a Heparinized Nanoparticle Coated on PLGA Microspheres for Cell Delivery



The morphology of the heparin/poly(l-lysine) nanoparticles was imaged using an atomic force microscope (AFM) (AutoProbe CP, ThermoMicroscope) with tapping mode ultrasharp tips and a ca. 3 N/m force constant. The AFM images show a typical densely packed heparin/chitosan nanoparticle structure (see Supporting Information, Figure SI1). A polyelectrolyte layer can be deposited onto an oppositely charged substrate via a simple adsorption step. A monolayer is adsorbed when the substrate is dipped into the polyelectrolyte solution. This procedure is generally referred to as self-assembled monolayer deposition.

The second step involves the immobilization of the heparin/poly(l-lysine) NPs on PLGA microspheres, used as microcarriers of MSCs. Figure 1 shows representative SEM images of the drop-cast films of PEI-coated PLGA microspheres and the heparinized NPs coated onto PLGA microspheres pretreated with PEI. Figure 1B, C, D, E, and F show the middle, upper, and bottom sites of the PLGA microspheres that immobilized the heparinized NPs. The heparin/poly(l-lysine) polyelectrolyte complex is highly distributed on the PLGA microspheres. As can be clearly observed, the specific binding activity of heparin in the bioconjugate is not reduced in the results of the immobilization process, which may be due to the presence of heparin within the outer shell of NPs on the surfaces of the PLGA microspheres. However, the heparin/poly(l-lysine) NPs did not entirely coat the three-dimensional structure, in contrast to the two-dimensional substrates in the geometrical structures of the PLGA microspheres. Although the heparin/poly(l-lysine) NPs did not thoroughly coat the PLGA microspheres, the heparin/poly(l-

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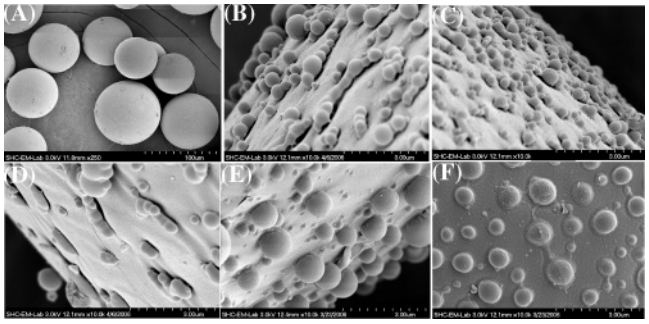

Figure 1. The SEM images of PLGA microspheres fabricated with heparin/poly(l-lysine) nanoparticles: (A) entire morphology of PLGA microspheres; (B) upper site (left part) of microspheres; (C) upper site (right part) of microspheres; (D) bottom site (right part) of microspheres; (E) bottom site (left part) of microspheres; and (F) middle site of microspheres. The bar means 3 μm.

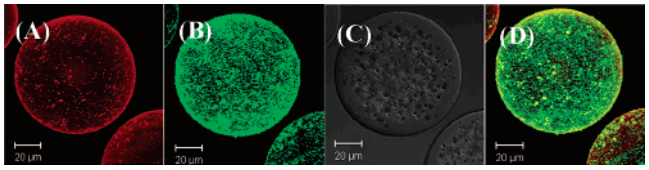

Figure 2. Confocal laser scanning micrographs of heparin/poly(l-lysine) nanoparticles attached to PLGA microspheres: (A) red-wavelength detected RITC-conjugated PEI; (B) green-wavelength detected FITC-conjugated heparin/poly(l-lysine) nanoparticles;, (C) DIC image; and (D) merged image of panels A and B.

lysine) NPs occupied more than 70% of the surface, which is satisfactory for other materials, most notably inorganic materials.

To verify the prepared LbL-type coated polymeric matrix, the heparin/poly(l-lysine) NPs on PLGA microspheres were observed by confocal laser microscopy in order to assess the degree of immobilized heparin/poly(l-lysine) NPs. Figure 2A and B show PEI-coated PLGA microspheres and immobilized heparin/poly(l-lysine) NPs, which were conjugated by fluorescence. TRITC-conjugated PEI was precoated onto the PLGA microspheres (Figure 2A). The heparin-FITC was mixed with poly(l-lysine) for the polyelectrolyte complexes and immobilized onto TRITC-conjugated PEI-coated microspheres (Figure 2B). FITC-conjugated heparin/poly(l-lysine) images of the obtained NPs evidenced green fluorescence, and PEI coated onto PLGA microspheres evidenced red fluorescence. Figure 2D shows the merged images of microspheres, and the completely overlapped image of heparin/poly(l-lysine) NPs by FITC-labeled heparinized NPs, and PEI by TRITC labeling can be observed. These images indicate that the positively charged PEI readily coated the negatively charged PLGA microspheres, as well as the adherence of the heparinized NPs to the PEI-coated surfaces of the PLGA microspheres.

Conventional cell culture methodologies are inadequate for coping with the scale of cell production required for the manufacturing of engineered cartilage tissue products. In vitro cell expansion has become an essential step in the processes of tissue engineering and regenerative medicine, and the optimization of expansion protocols is a fundamental issue. The expansion suitability of stem cells isolated from the superficial zone was then evaluated in both a conventional monolayer and a macroporous microcarrier in spinner flask cultures. Although monolayer systems promoted the rapid in vitro expansion of undifferentiated cells, they evidence limited scalability. Both its satisfactory expansion potential and, more importantly, its economical and operational advantages over traditional monolayer cultures indicate that this system constitutes a feasible alternative method for the extensive expansion of progenitor cells. To determine the properties of MSC adhesion on

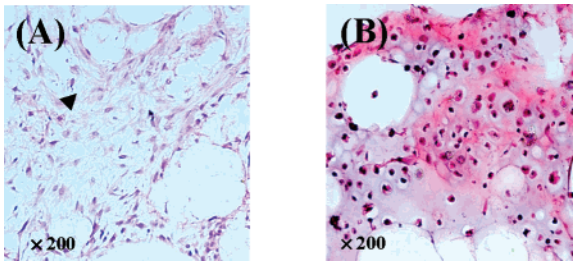

Figure 3. Safranin-O staining of mesenchymal stem cells embedded on PLGA microspheres: (A) image of MSCs adhered to conventional microspheres; (B) image of MSCs adhered to heparin/poly(l-lysine) NP-coated microspheres.

the surfaces of the PLGA microspheres modified by heparin/poly-(l-lysine) NPs, MSCs were cultured in spinner flasks containing microspheres. To confirm the cartilage regeneration, the specific marker of collagen type II was observed (see Supporting Information, Figure SI3 and 4). After 4 weeks of culture, Safranin-O staining indicated that MSCs adhered and differentiated onto the heparin/poly(l-lysine) nanoparticle-embossed PLGA microspheres, which accumulated an abundant extracellular matrix that was rich in glycosaminoglycans and polysaccharides (Figure 3). Safranin-O staining indicated the presence of highly sulfated proteoglycans, which compose the extracellular matrices produced by differentiated MSCs. In contrast, the cells adhering to the PLGA microspheres produced extracellular matrix only in the immediate vicinity of each cell. These results indicate that MSCs mixed with PLGA microspheres and transplanted into bodies should adhere to the heparin/poly(l-lysine)-coated PLGA microsphere surfaces in vivo, which may result in cell proliferation, differentiation, production of the extracellular matrix, and tissue formation in vivo.

In conclusion, two-dimensional assemblies of various NPs can be both simply and efficiently stabilized via embedment onto PLGA microspheres for cell delivery vehicles. In this way, heparin/poly-(l-lysine) NPs coated onto PLGA microsphere surfaces were found to be an excellent structure for cell adhesion and growth, and these methods may constitute new and valuable systems for a host of applications, including bioreactor, cell delivery vehicle, and protein or peptide delivery systems. The unique properties of NPs, including their biocompatibility, uniform distribution on a substrate, and chemical or biological action in cells, may help to solve some of the problems thus far associated with tissue regeneration.

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Supporting Information Available: Detailed experimental procedures and background on the heparinized nanoparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

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