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Biodegradable polycation and plasmid DNA multilayer film for prolonged gene delivery to mouse osteoblasts

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Abstract

Sustained release of functional plasmid DNA from the surfaces of materials which support cell adhesion for tissue formation could have a significant impact on gene therapy and tissue engineering. We report here layer-by-layer assembled multilayer film from a degradable cationic poly(2-aminoethyl propylene phosphate) and plasmid DNA encoding for enhanced green fluorescent protein (EGFP) for mouse osteoblast cell adhesion and prolonged gene delivery. Multilayer film growth was monitored by UV spectrophotometry and intensity of absorbance at 260 nm related to incorporated DNA increased in an exponential manner with increase the number of deposited polymer and plasmid layers. It degraded upon incubation in phosphate-buffered saline (PBS) at 37 °C and sustained the release of bioactive plasmid DNA up to 2 months. The multilayer film facilitated initial mouse osteoblast cell adhesion onto the surface and enhanced cellular alkaline phosphatase activity and calcium accumulation. It sustained delivering transcriptional active DNA to mouse osteoblast cells cultured on the film, and directly prolonged gene expression in the presence of serum without any exogenous transfection agent. This biodegradable multilayer assembly is promising for the local and sustained delivery of plasmid DNA and such a layer-by-layer system suggests an alternative method for plasmid DNA incorporation which may be useful for surface modification of implanted materials or scaffold for gene therapy and tissue regeneration.

Keywords: Gene delivery; Layer-by-layer assembly; Biodegradable polyphosphoester; Osteoblast cells; Tissue engineering

1. Introduction

The combination of gene therapy and tissue engineering represents a promising approach, and the advantages of this have been demonstrated in many contexts including bone, cartilage, wound, urothelial, and nerve tissue regenerations [1–5]. Scaffolds such as polymeric materials, which can be derived from natural sources (e.g. chitosan, collagen [6]) or synthetic materials (e.g. poly[lactide-*co*-glycolide] [7]), provide the physical support for cell adhesion as well as the template for tissue formation. On the other hand, gene therapy utilizes localized and sustained gene delivery to transfect cells cultured with scaffolds, and therefore generates the cellular machinery for therapeutic protein production that facilitates specific cellular progress.

To achieve localized gene delivery over an extended period in tissue engineering applications, naked plasmid DNA can be distributed in polymeric scaffolds. Sustained plasmid DNA delivery from polymer matrices via different fabrication methods led to long-term transfection of large numbers of cells both in vitro and in vivo [8–11]. Plasmid DNA can also be incorporated into scaffolds in the condensed states, where DNA formed complexes with cationic lipids such as DOTAP/DOPE [12] or polymeric carriers such as polyethyleneimine (PEI) [13,14]. The complexes can be physically distributed in scaffold, or tethered in substrate or on surface via either non-specific

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adsorption or specific interaction [15–17]. In addition to non-viral delivery, viral vectors have also been encapsulated in biomaterials, typically in collagen scaffold for effective protein expression and tissue regeneration [18].

Layer-by-layer (LBL) assembly methods introduced by Decher [19] have been extensively used for biomedical applications due to the ease of preparation and versatility [20–22]. For tissue engineering applications, this technique has been frequently applied for scaffold surface modification to effectively control cellular function such as cell adhesion and growth behavior [23–27]. Besides exploration on cell behaviors of charged polyelectrolytes (e.g. poly (allylamine hydrochloride), poly-L-lysine, chitosan, hyaluronic acid) that may cover the hydrophobilicity or alter the cell-resistant property to polymer materials [21,22], extracellular matrix proteins such as fibronectin [28–30], fibroblast growth factor [31,32], or collagen [33] have also been assembled onto the surface of polymer scaffolds by the LBL technique and the multilayer films can be therefore rendered bioactive to promote cell adhesion and cellular function.

Self-degradable multilayer thin films were fabricated at least with one polyelectrolyte that degrades under either hydrolysis (it can be enzymatically catalyzed) or reductive degradation [23,34,35]. Such degradation properties have been utilized for controlled DNA release, where the multilayer films were fabricated with positively charged but degradable polyelectrolytes and negatively charged DNA molecules [35–37]. With the degradation of polycations, DNA was released in a sustained manner. Moreover, Lynn and co-workers reported that by alternate deposition of plasmid DNA and hydrolytically degradable poly (β-aminoester) they fabricated multilayer films, which promote the sustained release of the incorporated DNA in aqueous medium at neutral pH [36–38]. They have demonstrated released plasmid DNA from the multilayer film could be taken up by cells cultured beneath the film, resulting in successful gene expression [38]. It has also been reported that the rate of DNA release can be adjusted by altering the chemical structure of the poly(β-aminoester) [39]. This implies that localized delivery of DNA is achievable from surfaces of tissue engineering scaffolds through plasmid DNA deposition with a degradable polycation using LBL assembly approach.

When aiming at delivery of genes from tissue engineering scaffolds using the LBL assembly, both long-term gene transfection and cell adhesion on the surface of LBL film are essential to support tissue regeneration. It is believed that cell adhesion and function are affected by the property of applied polyelectrolyte as well as its degradation rate. For example, fast degradation of poly(β-aminoester) may not be suitable for cell adhesion in long-term gene-induced tissue engineering applications. In this study, using the LBL approach, we demonstrated that by deposition of plasmid DNA and a biodegradable polycation poly(2-aminoethyl propylene phosphate) (PPE-EA), we fabricated a biodegradable multilayer film, which released incorporated DNA in a sustained manner. More importantly, it facilitated primary mouse osteoblast cell adhesion on the surface while localized gene delivery from this multilayer film resulted in long-term gene transfection into osteoblast cells cultured on such films.

2. Materials and methods

2.1. Materials

PEI (branched, MW = 25,000) was obtained from Aldrich Chemical Co. and used directly. PPE-EA (MW = 30,300) was synthesized as previously described [40]. Streptomycin, penicillin, fungizone, and ascorbate were purchased from Sigma Chemical Co. (St. Louis, MO). MTT (3-{4,5-dimethylthiazol-2-yl}-2,5-diphenyltetrazolium bromide) was purchased from BDH Laboratory Supplies (Poole, Dorset, England). Dulbecco's modified Eagle's medium (DMEM), L-glutamine, Terrific Broth were purchased from Gibco BRL (Grand Island, NY). Fetal bovine serum (FBS) was the product of HyClone (Logan, UT). Qiagen Giga plasmid purification kit was purchased from Qiagen in China (Hong Kong, China).

2.2. Plasmid DNA

pEGFP-N2 plasmid from BD Biosciences Clontech (4.7 kb, Palo Alto, CA, USA) was amplified in *Escherichia coli* DH5α and purified by Qiagen Giga plasmid purification kit according to the manufacturer's protocol. The purified plasmid was dissolved in Tris–EDTA buffer and kept at $-20\,^{\circ}C$ in aliquots at a concentration of 1–2 mg/mL.

2.3. Layer-by-layer assembly

Quartz substrates ($8 \times 24\,mm^2$, or Ø 12 mm plates) were cleaned for 20 min in the mixture of concentrated sulfuric acid and a 30% solution of hydrogen peroxide (1:1, v/v), washed with Milli-Q water (18.2 MΩ, Millipore Milli-Q Synthesis System), and rendered hydrophilic with a mixture of $H_2O/H_2O_2/NH_3$ (1:1:5, v/v/v) for 20 min at 80 °C, washed with a copious amount of Milli-Q water, and dried under a stream of dry nitrogen prior to use. Silicon substrates for atomic force microscopy analyses were treated in the same method.

Multilayer films of PPE-EA and pEGFP-N2 plasmid DNA were fabricated on quartz substrates pre-coated with PEI using the alternate-dipping method according to the general protocol published in the literature [23,34,41]. Substrates were rinsed in 20 mM PEI solution for 15 min and dried, followed by immersing in a solution of plasmid DNA (0.1 mg/mL, in 0.1 M phosphate-buffered saline, PBS buffer, pH 5.9) for 15 min, and rinsed in Milli-Q water for 1 min twice. Substrates were further immersed in a solution of PPE-EA (20 mM of amino groups, in 0.1 M PBS, pH 5.9) for 15 min and rinsed by Milli-Q water to finish the first cycle and obtain one pEGFP-N2/PPE-EA bilayer. The above alternate procedures were repeated until the desired number of DNA and PEE-EA layers was achieved. The multilayer film was dried under a stream of nitrogen. pEGFP-N2/PEI multilayer film was also fabricated as the control in this study using PEI (20 mM of amino groups, in 0.1 M PBS, pH 5.9) instead of PPE-EA solution.

2.4. Characterization of multilayer film assembly

UV–Vis spectrophotometry (UV-2802 PC, UNICO Instruments) was employed to monitor the buildup of the film by exploiting the characteristic absorbance of plasmid DNA at 260 nm.When each bilayer deposition was completed, the dried quartz substrate was immediately used to measure the UV absorbance in the range of 200–300 nm.