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Multicompartmental Microstructured Materials via Electrohydrodynamic Co-Jetting : A Diagnostic and Biosensing Platform

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Abstract

Control over nano- and microscale architecture of polymeric materials is highly desirable for improved versatility, utility, and performance of biomedical devices, which include smart drug release systems, biomedical coatings and multiplexed bioassays. Apart from size and shape of polymeric micro-objects, phase distribution, or selective material compartmentalization has been shown to be increasingly important for maximizing device performance. In this work, we summarize the recent advances we have made in the synthesis and applications of biodegradable multicompartmental biomaterials from polylactide-co-glycolic acid (PLGA) polymers via electrohydrodynamic co-jetting. In its simplest form, two polymer solutions are flown through a modified side-by-side capillary system. Application of an electric field results in the formation of an electrospray, and solvent evaporation results in particle formation. The interface between two polymer solutions is sustained during jet fragmentation and size reduction. Because of its intrinsic simplicity and generality, the electrohydrodynamic co-jetting process can be applied to a wide range of specialty and non-specialty materials. Furthermore, simple variations of different solution and process parameters, such as concentration, flow rate, applied voltage, etc. provides access to a vast repertoire of shapes and sizes of particles. We herein demonstrate fabrication of a variety of non-equilibrium biphasic shapes, such as discs, rods and others, in addition to spheres. Such novel particle geometries enable independent control of key parameters, such as chemical composition, surface functionalization, biological loading, shape, and size for each compartment. We then demonstrate spatio selective control over surface chemistry of these biphasic particles via Huisgen 1, 3-dipolar cycloaddition. The selective surface functionalization is combined with biotin-streptavidin interactions to show orientation and assembly of bicompartmental particles in suspension. The optical effects achieved through this specific orientation can be employed as diagnostic markers and have applications in developing analyte specific biological assays.

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Introduction

It is increasingly evident that the design of intelligent and stimulus-responsive biomaterials for applications such as tissue engineering, targeted drug delivery, and in vivo imaging depends not only on the chemical composition of the material, but also on physical characteristics, such as the size or shape of a particle used for targeted drug delivery[1]. Hence, materials scientists have increasingly sought to control particle attributes such as size, surface microstructure, or mechanical behavior. In addition to these traditionally investigated parameters, it has been demonstrated that other variables might be crucial in influencing biological function; these include anisotropic distribution of matter and surface functionalization, particle shape, and surface roughness [1]. Although colloidal structures showing individual control over shape [2], size, anisotropy [3] and surface chemistry [3] have been previously fabricated, simultaneous control over multiple particle attributes remains a key challenge. Specific control of more than one of the above mentioned particle characteristics would enable the design of truly multifunctional particles, which would serve as the next generation of smart materials. We herein demonstrate the fabrication of bicompartmental microcolloids with narrow size distributions and controlled shapes. Electrohydrohydrodynamic co-jetting [3] is an attractive technology for formulation of microstructures for aforementioned applications due to low cost, ease of scale up, relatively low loss of material during encapsulation, and above all, the ability to form a vast repertoire of sizes, shapes and surface morphologies by simply controlling a few process and solution parameters. We have previously produced Janus type biphasic nanocolloids via this process from aqueous polymers solutions of hydrogels such as poly (acrylic acid-co-acryl amide) and poly (ethylene oxide) [3-7]. We now demonstrate the fabrication of multicompartmental biodegradable microparticles [8-11] and fibers of different shapes, sizes, and surface features via electrohydrodynamic co-jetting, and illustrate some applications of these particles as potential diagnostic markers via their self assembly. Control over biodegradability, size, shape, anisotropy, and surface structure would provide us with more parameters to create high throughput systems for a variety of therapeutic and diagnostic applications.

Materials

PLGA copolymer with lactide: glycolide ratio 85:15 (Mw= 40-75,000), Chloroform, N, N–dimethylformamide (DMF), ethanol, copper Sulfate Pentahydrate (CuSO4.5H2O), sodium ascorbate, Fluorescein Isothiocyanate (FITC), Serum Bovine Albumin (BSA), Phosphate buffered Saline (PBS) and Tween-20 were purchased from Sigma-Aldrich, USA and used as received. A polythiophene polymer, sold under the commercial name of ADS 306PT (Mw= 20-70,000, American Dye Source, Canada) and a green fluorescent conjugated polymer, poly[tris(2,5-bis(hexyloxy)-1,4-phenylenevinylene)-alt-(1,3-phenylenevinylene), (or PTDPV, Sigma Aldrich, USA) were used for fluorescence labeling. Alexa 633 Streptavidin and Tetrafluorophenylazide-(PEO)3-biotin (or azido-biotin) were purchased from Invitrogen Inc, USA and Pierce Pharmaceutical, USA, respectively. All solvents were ACS reagent grade and used as received from commercial suppliers. Crymolds and Optimal Cutting Temperature gel (OCT) were purchased from Sakura Finetech, USA.

Experimental

Electrohydrodynamic co-jetting

Bicompartmental PLGA particles were synthesized using a previously described protocol [8]. Briefly, for biphasic particles or fibers, two solutions were drawn into syringes (capacity: 1 ml, Becton-Dickinson, USA). The two syringes were held together using a syringe holder and connected to a dual cannula applicator tip (Micromedics Inc, USA) consisting of two capillaries (diameter: 26 gauge, length: 3.25 in) held together in a side by fashion. The needles were connected to the cathode of a DC voltage source (range: 0-30 kV, Gamma High Voltage Research, USA). The flow rate was controlled by a syringe pump (Kd Scientific, USA). A square piece of Aluminum foil was used as collecting substrate (anode). Particles and fibers with three, four or even seven compartments were fabricated by simply increasing the number of capillaries arranged side by side in the desired configuration. In case of fibers, a grounded wheel with a velocity of 16 rpm was employed as the collection substrate.

Selective Surface modification and self assembly

For selective surface modification of particles, acetylene groups were introduced by blending in 30% by wt. of poly(lactide-co-propargyl glycolide) along with PLGA in the jetting solution [8, 12]. The resulting acetylene groups on the surface of particles were modified with azido-biotin via click chemistry according to a previously described protocol [8, 11]. Self assembly was carried out via addition of 1 µg/ml of streptavidin to a 1mg/ml particle suspension in PBS containing 0.1% tween-20 and 1% BSA. Samples were analysed using Scanning Electron Microscopy (SEM,

Philips XL30 FEG), Confocal Laser Scanning Microscopy (CLSM, Olympus Fluoview 1000) and Flow Cytometry (BD Biosciences, FACS Calibur).

Results and Discussion

During electrohydrodynamic co-jetting, two or more jetting solutions are pumped through a side-by-side capillary system under laminar flow. Similar to conventional electrospinning, the application of an electric potential results in distortion of the pendant droplet into a Taylor cone [13]. The rapidly stretching jet results in formation of well-defined particles through solvent evaporation [14, 15]. Owing to the low propensity of organic solvents to accumulate surface charges, a fine interplay of several forces, such as electrical, surface tension, combined with rapid solvent evaporation is necessary to drive the co-jetting of non-polar, organic solutions. Thus, by shifting this equilibrium within the laminar flow regime, there exists a distinct possibility of fabricating non-equilibrium shapes from electrohydrodynamic processing of organic solutions.

With these opportunities in mind, we proceeded to systematically investigate the effects of various process parameters on the particles. We hypothesized that the fluctuations from equilibrium would be maximized at low polymer concentrations while earlier high to very high polymer concentrations would yield fibers. Hence, we focused on the polymer concentration as the first variable to be investigated. Electrohydrodynamic co-jetting of a 1.3% w/w solution (in 95:5 v/v chloroform: DMF) of each polymer (PLGA 85:15 and PLGA 50:50), at a flow rate of 0.15 ml/h, produced discoid biphasic particles. Observed disc morphologies ranged from smooth to slightly rough predominately at the surface ridges. Rods, and spheres were observed at concentrations of 3.5% w/w and 4.5% w/w respectively, with small amounts of triethylamine added to the jetting solution that produced rods [9]. A relatively high flow rate of 0.4 ml/h was also found to facilitate rod formation. Increasing the concentration to 18% w/w resulted in well aligned fiber sheets at flow rates of 0.02 ml/h. Furthermore, this process was extremely conducive for the production of fibers with three, four, and even seven compartments [10]. This can be attributed the design of the jetting solution and process parameters, which offers a unique combination of low conductivity, high viscosity and rapid solvent evaporation, resulting in a stable jet at low flow rates, which in turn, produces a single, continuous fiber for long periods of time (~5h). By rotation of the wheel assembly (16rpm), the fiber could be directed to deposit as an ordered bundle. Since this bundle results from continuous deposition of a single fiber, Scanning Electron (SEM) micrographs of the bundles revealed near-perfect monodispersity with respect to diameter [10]. The fiber bundles, ~ 3 cm in length were held together by adhesive forces, most likely due to low distances between the cone and substrate which cause fiber deposition before complete evaporation of the solvent. The bundles were harvested, embedded in optimal cutting temperature gel and cryosectioned. Cryosectioning resulted in bundles of microcylinders, whose length could be controlled by adjusting the slice thickness and speed of the cryotome. After dissolution of the gel in water, the bundles were resuspended in PBS buffer and subjected to ultrasonication in order to remove the cohesive forces. This resulted in uniform individual microcylinders with controllable number of compartments as well as aspect ratios [11]. By combining electrohydrodynamic co-spinning with microsectioning, we have established a versatile, low-cost, and potentially high throughput method, and also made possible the fabrication of monodisperse microstructures directly via electrospinning.

The selective modifition of biphasic nanoparticles with biotin was carried out via incorporation of acetylene-functionalied PLGA [8, 12] in one compartment of particles. The resulting acetylene groups on particle surface were reacted with azido-biotin in presence of copper sulfate pentahydrate and sodium ascorbate. For self assembly, the concentration of streptavidin was optimized to ensure that so that there would be a mixture of bound and unbound biotin moieties on the surface of particles, which causes inter-particle self assembly since a streptavidin molecule can bind four biotin molecules. CLSM analysis showed that the particles assembled into dimers, trimers, and tetramers. The compartments labeled with biotin were seen to be bound together, indicating that self assembly occurred only via specific biotin-streptavidin interactions. In order to account for the non specific interactions, a control experiment was also performed in the absence of streptavidin. Self assembly was significantly more pronounced in presence of streptavidin, and the number of non specific dimers, trimers, and tetramers was more abundant in the control sample [16].

Conclusions

The fabrication of multicompartmental microparticles and fibers with two or more compartments via electrohydrodynamic co-jetting from biodegradable materials was demonstrated. Biphasic particles of different shapes, such as discs and rods were also fabricated. The monodisperse aligned fibers resulting from this process were utilized to fabricate monodisprse microcylinders. The number and

configuration of these compartments was controlled by varying the number and configuration of the capillaries employed during the electrohydrodynamic co-jetting process, whereas the aspect ratio was controlled during cryosectioning by adjusting the length of the sections. Spatioselective surface modification of particles with biotin via click chemistry, followed by addition of streptavidin, resulted in specific self organization into dimmers, trimers, and tetramers. Such particles and their assemblies have applications in theranostics, bioimaging, and sensing.

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