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Controlled association and delivery of nanoparticles from jet-sprayed hybrid microfibrillar matrices

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ABSTRACT

To develop bioactive scaffolds of targeted properties for tissue repair or biomedical applications, hybrid microfiber-nanoparticle (MF-NP) matrices capable of controlled nanoparticle (NP) delivery were prepared through two novel approaches. In a first strategy, the suppleness of the jet-spraying method to produce polymer microfibers (MF) was used to deposit poly(D,L-lactide) (PLA) NP on poly(lactic-co-glycolic acid) (PLGA) MF by direct co-projection. The second approach relied on the post-incubation of PLA NP aqueous dispersion with MF preliminarily prepared by jet-spraying. NP coverage density onto MF and NP release was assessed by scanning electron microscopy and fluorescence measurements using coumarin-6 loaded NP. The first process was shown to allow high coverage density of NP onto MF (300 μg/mg MF) and strong association, with no NP release observed over time. In the second approach, direct incubation of PLA NP with PLA MF led to lower NP coverage density (40 μg/mg MF) with very fast release of NP from MF. The pre-coating of MF with poly-L-lysine (PLL) or the one of NP with lysozyme as a model protein drug afforded a higher coverage density and stronger association, coupled with a more sustained release of NP from MF over time. These results show the possibility to control the immobilization density and release of NP through appropriate preparation process and surface modification, and are of prime interest for the development of complex scaffolds with orchestrated bioactivity.

1. Introduction

In the last decade, the need for alternatives to autografts and allografts has triggered tremendous interest for biomaterial scaffold based approaches, able to support tissue regeneration [1]. Aliphatic polyesters such as polylactide/poly(lactide-co-glycolide) (PLA/PLGA) currently remain one of the most potent polymer family for use as scaffolds, due to their biocompatibility and biodegradability [2]. They can be processed in scaffolds of well-defined shape, size and porosity, tuned to the kind of tissue to repair. Particularly, recent research has particularly focused on nanofibrous polyester scaffolds, produced by electrospinning [3] or jet-spraying [4,5] techniques, as they can mimic the size scales of fibers composing the extracellular matrix (ECM) of native tissues, and thus support processes of cell attachment, proliferation and differentiation. Besides the "classical" coupling of scaffold surface with adhesion peptide sequences (such as the tripeptide motif Arg-Gly-Asp, RGD) to improve these processes [6], it is now well-established that tissue regeneration will also largely be impacted by the capacity of the scaffold to provide optimal environment by means of appropriate bioactive agents and drugs (growth factors, anti-inflammatory drugs,. . .) along reconstruction time [7–9]. Direct incorporation of such biomolecules to biodegradable scaffold during its preparation has been reported but this strategy suffers some limitations, such as possible drug degradation following exposure to organic solvents or heating treatments required in the scaffold processing [10], or inappropriate drug release kinetics. In this regard, the use of PLA/PLGA based biodegradable nanoparticles (NP) to mediate localized and temporally controlled delivery of encapsulated or adsorbed biomolecules represents a valuable alternative, considering their reported extensive use in drug delivery applications [11,12], and as they can be tuned to reach suitable properties by means of size, surface nature and charge, drug protection and release [13,14].

Consequently, approaches aiming at combining in a single "hybrid" biomaterial a scaffold edifice as cell support and NP for local and sustainable drug delivery have been the focus of increasing attention over the last years. However, while numerous works

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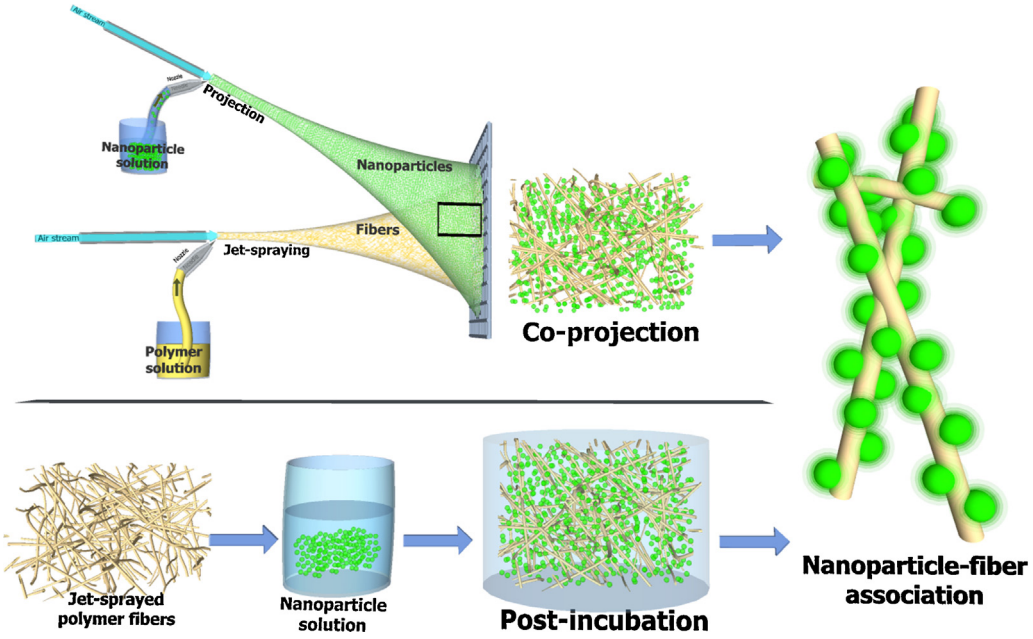

Fig. 1. Schematic illustration of the different strategies followed to associate nanoparticles and microfibers.

have described the association of PLA/PLGA based NP with natural [15–17] or ECM-derived [18,19] polymer scaffolds for local drug delivery, much fewer have been devoted to their incorporation to scaffolds of the same aliphatic polyester nature, which potentially offer the opportunity to design fully degradable hybrid assemblies. From these few, Ma's group has reported the elaboration of degradable PLGA based scaffold-NP constructs through a post-seeding technique [20–22], consisting in dispersing PLGA NP in organic solvent such as ethanol or hexane, and dripping them into PLGA scaffolds. The latter are then subjected to an appropriate mixture of non-solvent/solvent (hexane/THF, 90/10) to immobilize NP onto the scaffold. However, such technique results in rather poorly conserved NP integrity and morphology, *i.e.*, tendency of filmification/collapsing of NP on the scaffold, probably due to partial dissolution in presence of THF solvent. More recently, DeVolder et al. described an appealing approach through electrostatic attractions between positively charged electrosprayed (VEGF-encapsulating) PLGA microparticles and electrospun PLA microfibers of negative surface charges [23]; but collapsing of microparticle on microfibers was also reported to possibly occur, and be prejudicial for stability of the loaded drug.

In addition to the drawbacks of currently proposed approaches, the crucial issue of NP release from scaffold has been very poorly addressed until now. Therefore, there is a need for developing methodologies of constructs preparation that allow a better control over NP integrity, density of immobilization, and release; which are key issues in the design of scaffolds specifically tuned to repair a precise tissue.

In the present paper, we took profit of the versatile jet-spraying technique, based on projection of a polymer solution to create microfibers (MF), to immobilize NP through two different approaches: (i) a "co-projection" strategy in which an aqueous NP dispersion were projected along with a polymer organic solution during the process of microfiber preparation (ii) a "post-incubation" strategy in which aqueous NP dispersion was incubated with the preliminarily prepared MF. We investigated the properties of hybrid assemblies in regard of NP morphology, covering and release from MF, and particularly showed that the latter could be tuned by choice of NP-MF strategy of association and by control of NP/MF interface through protein surface modification.

2. Experimental

2.1. Materials

Poly(D,L-lactide) (PLA) of Mn = 10,000 g mol^{-1} (polydispersity index, PDI = 1,6) was purchased from Purac Biochem (Netherlands) and PLA of 100,000 g mol^{-1} (PDI = 1.7) was obtained from Anabior (Grenoble, France). Both polymers contained carboxylic end-groups. Poly (D,L-lactic-co-glycolic) acid (PLGA) 50/50 of MW = 110,000 g mol^{-1} (PURASORB® PDLG 5010) was purchased from Purac (Gorinchem, The Netherlands). Lysozyme (14,300 g mol^{-1}, isoelectric point pI = 11.4), poly-L-lysine (PLL, 30,000–70,000 g mol-1), chloroform, dimethylsulfoxyde (DMSO) and coumarin-6 were obtained from Sigma–Aldrich (Saint-Louis, MO, USA). Tris–HCl, sodium dodecyl sulfate (SDS), glycerol, dithiothreitol and bromophenol-blue were purchased from BioRad (Marnes-la-Coquette, France). All reagents and solvents were of analytical grade and received from commercial sources.

2.2. Nanoparticle (NP) preparation

Nanoparticles of PLA (Mn = 10,000 g mol-1) were prepared by nanoprecipitation as previously described [24]. Briefly, the polymer (2 weight percent, wt%) was dissolved in acetone with coumarin-6 as a fluorophore (0.2 wt% relative to PLA) and this solution was added dropwise to an aqueous solution (water:ethanol mixture 2:1) under slow stirring. Organic solvents and part of water were then removed under reduced pressure at 30 °C. The final PLA concentration was typically 50–60 mg/mL, depending on the batch and was accurately measured by weighing the wet and dried materials.

2.3. Protein adsorption and retention on the NP

To prepare NP adsorbed with lysozyme (NP-Lyzo), 200 μL of NP dispersion at 10 mg mL^{-1} were added to 200 μL of lysozyme solution in water (0.13 mg mL^{-1}), and the adsorption medium (NP: 5 mg mL^{-1}; lysozyme: 0.0625 mg mL^{-1}) stirred for 1 h at room temperature. Then, the dispersion was centrifuged at 16,800 × *g* for 10 min and the protein in the supernatant quantified by bicin-