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Polyaniline sheathed electrospun nanofiber bar for in vivo extraction of trace acidic phytohormones in plant tissue

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ABSTRACT

For in vivo extraction of trace and polar components in tissues, extraction materials should be biocompatible, highly permeable to matrix and have high extraction capacity for polar and ionic compounds. In this study, nanofibers (~200 nm) with rough polyaniline (PAn) sheath were prepared successfully for the first time by coaxial electrospinning and coelectrospun polymer sacrificing method. The nanofibers (0.5 mg) were then compressed into tiny bar (Φ1 mm × 2 mm) and inserted directly into the aloe leaf for in vivo extraction of trace phytohormones. Due to the large surface area (13.97 m^2/g) of nanofibers and large through pores formed between fibers, high extraction capacity (up to 10 μg/bar) and permeability to tissue matrix of the tiny bar can be obtained. Moreover, PAn sheath has high adsorption equilibrium constants (10^7–10^8 mL/mol) for acidic phytohormones in aloe leaf. Four phytohormones were detected by in vivo extraction with a spatial resolution of 3–8 mm^3, temporal resolution of 20 min, and limit of detection down to 60 pg/g. The quantification results were well met with that by traditional in vitro organic solvent extraction.

1. Introduction

In traditional in vitro semisolid sample analysis [1], the natural spatial distribution and dynamic content change of chemicals in living tissues cannot be obtained, as the tissue damage during sampling would make the living system different from the natural state. Moreover, the true target content at the time of sampling also could not be accurately predicted due to compounds decomposition during sample transport, solvent extraction process and complex sample preparation procedures. For real-time and in situ tracking of their distribution in the living tissues, a lot of in vivo analysis or sampling methods were developed such as microdialysis (MD) sampling [2,3], in vivo desorption electrospray ionization (DESI) [4] and in vivo solid-phase microextraction (SPME) [5–7]. Among them, in vivo SPME coupled with chromatography separation developed by Janusz Pawliszyn group [5–7] is more suitable for analysis of organic compounds with wide polarity range in complex matrix, and the needlelike device would be favorable for spatial resolution which is important for in vivo tissue extraction [5].

For in vivo SPME, various biocompatible coatings of SPME fibers have been developed for application to both biofluid and tissue samples [8–11]. However, to the best of our knowledge, most of polar biocompatible coatings such as porous polypyrrole (PPY) [9] and mixed-mode sorbent covered by biocompatible polymer [11] were just applied to extraction from biofluids (such as blood). Different from biofluids, tissue matrix always has higher viscosity and mass transfer resistance, so its permeation into extraction material will become difficult and then the analyte mass transfer flux in it is decreased. PPY [9] coating exhibited limited extraction capacity due to its low specific surface area and coating thickness [6,9], which would further decrease the mass transfer flux of analytes to extractive phase leading to low sensitivity of analysis. On the other hand, although coatings of commercial sorbents (mixed-mode) covered by biocompatible polymers [11] have larger extraction capacity, the biocompatible polymers layer would largely impede the permeation of tissue matrix to active sites on the sorbents and increase the mass transfer resistance between phases. Moreover, the half open nanopores on both PPY and commercial sorbents are not favorable for permeability and mass transfer.

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Until now, for in vivo semi-solid tissue extractiononly nonpolar poly(dimethylsiloxane) (PDMS) fiber was widely used [7,12–14], and most studies on in vivo tissue SPME were focused on less polar exogenous drugs and pollutants from fruits or fish tissues [7,12–14]. Actually, most of bioactive endogenous chemicals in tissues which would be of greater interest to biologists are usually trace, polar or even ionic. Thus, it is urgent to develop the extraction material, which is biocompatible to living tissues, has large extraction capacity to polar and ionic compounds, and can provide high mass transfer rate.

Electrospun nanofibers [15] possess unique properties to fulfill above mentioned requirements. Nanofibers could form nonwoven, self-supported membrane with high surface area [15–17]. Instead of deep nanopores in the extraction phase, there are only much larger through pores formed between nanofibers, so the surface active sites are fully exposed, yielding high permeability and mass transfer flux [18]. In our previous work, polystyrene (PS)/crosslinked collagen core-sheath electrospun nanofibers have been developed for in vivo fast equilibrium-extraction of less polar basic drugs from living blood [19]. However, the PS core has poorer extraction ability to very polar and ionic compounds, and the biocompatible collagen sheath, which was used to resist protein in blood, has no extraction ability and would impede the permeation of tissue matrix into PS core. Thus, for polar or ionic compound extraction from static semi-solid tissues, new electrospun nanofibers should be developed. Unfortunately, the widely used biocompatible polymers [20] with extraction ability to polar compounds like PPY [9] or polyaniline (PAn) [21–23] are difficult to be electrospun directly due to their low molecular weight and low solubility in volatile solvent [24,25]. For PAn, only PAn's solution in concentrated sulfuric acid [26,27] and its solution mixed with other polymers [28] have been electrospun successfully. However, the former strategy leads to thick fibers (micrometer-level) [27], and the latter will weaken the PAn properties due to dilution of the PAn content by other polymers.

To circumvent those problems, thin core-sheath eletrospun nanofibers (~200 nm in diameter) with pure PAn sheath and PS core (PS-PAn CSEF) were prepared for the first time successfully with coelectrospun polymer sacrificing method in this study. At first, core-sheath fibers were co-axial electrospun with mixture of collagen and PAn as the sheath and PS as the core, and then collagen was selectively removed to leave pure PAn sheath. The fibers had strong extraction ability and large extraction capacity to acidic phytohormones which play a crucial role in the regulation of plants germination, growth, reproduction, and response to various stresses [29]. In addition, the fibers had good matrix resistance in aloe leaf tissues. We compressed the fibers into an integrated monolithic tiny bar (Φ1 mm × 2 mm) and applied it to in vivo extraction of trace acidic phytohormones in aloe leaf. The quantification results were validated by comparing it with that by in vitro organic solvent extraction.

2. Experimental

2.1. Chemicals and materials

Standard gibberellin A3 (GA3, >90%) and gibberellin A7 (GA7, >90%) were purchased from OlChemIm Ltd. (Olomouc, Czech Republic). Jasmonic acid (JA, >99%), abscisic acid (ABA, >99%), *p*-hydroxycinnamic acid (p-HCA, >99%) of HPLC-grade were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). HPLC-grade acetonitrile, 2-propanol and methanol were purchased from Merck (Darmstadt, Germany). Methanol solutions of 1 mg/mL phytohormones were stored at −20 °C. Deionized water used in this experiment was Wahaha® purified water (Hangzhou, China). NaCl, Na_2HPO_4, NaH_2PO_4, H_3PO_4 and NaOH (analytical grade) used to prepare phosphate buffer saline (pH = 2–9) were bought from Shenyang Chemical Reagent Co. (Shenyang, China). Dichloromethane and oxalic acid (analytical grade) were obtained from Kermel Chemical Reagent Co. (Tianjing, China). Lactic acid (analytical grade) was purchased from Xin Xi Chemical Reagent Co. (Shenyang, China). Salicylic acid and succinic acid (>90%) were purchased from J&K Scientific Ltd. (Beijing, China).

PS (M_w = 250,000–270,000, general type I) was purchased from Aladdin industrial Co. (Shanghai, China). PAn (the emeraldine base form, M_w = 50,000–60,000, powders with 7 μm average diameter) was purchased from Ji An Ya Da Scientific Ltd. (Shijiazhuang, China). Collagen (M_w = 80,000–100,000) was purchased from Sichuan Ming-rang Bio-Tech Co. Ltd. (China). 1,1,1,3,3,3-Hexafluoroisopropanol (HFIP) and 10-camphorsulfonic acid (HCSA) were purchased from Aladdin Reagent Co. (Shanghai, China). Chloroform was obtained from Kermel Chemical Reagent Co. (Tianjing, China). The materials were used without any purification.

Plant samples (potted aloe) are purchased from the local market without any special treatment.

2.2. Preparation of electrospun fibers

For eletrospinning core-sheath nanofibers with PAn/collagen sheath and PS core, 5% (w/v) PS solution (A solution) in chloroform and 4% (w/v) collagen and 2% (PAn.HCSA) solution in HFIP (B solution) were prepared at room temperature and stirred for 5 h, respectively. For preparing B solutions, the emeraldine base PAn and HCSA (w/w = 1/2) were firstly dissolved in HFIP (3:7, v/v). Then the resulting solution was filtered, in which the corresponding amount of collagen was dissolved. The spinneret was composed of two co-axial capillaries. Both capillaries are connected to the same electrical potential applied by a high voltage power supply (ADW300-0.5, Dongwen high voltage power supply factory, Tianjing, China), and the applied voltage was 20 kV. The tip-to-collector distance was set to 15 cm, and a grounded aluminum foil (5 cm × 4 cm) was used for the fiber collection. Via syringe pumps (LSP01-1, Longerpump, Baoding, China), B solution and A solution were introduced from the outer and inner capillary with the flow rate at 0.4 mL/h and 0.2 mL/h, respectively. The spinneret and collector were housed in a transparent close polymethylmethacrylate box. The humidity and temperature during electrospinning was kept within 45–60% and 25 °C. For removing of collagen, the as spun core-sheath fibers were immersed into distilled water for 12 h and then was immersed in 0.2% formic acid-water for 5 h. Then, fibers were immersed in 3% ammonia solution and 1 M HCl solution (pH = 2) sequentially for 3 h to dedope the PAn-HCSA emeraldine salt and redope it with HCl.

For fibers of PS and PAn blends, the detailed eletrospinning method is in Method S1.

The detailed characterization processes of the nanofibers are described in Method S2.

2.3. Extraction procedure

Firstly, 0.5 mg PS-PAn core-sheath electrospun fibers were weighed precisely and then put into a 10 μL pipette tip and compressed in the tip by a 1 mm o.d. stainless steel rod for 1 min (Fig. S1). After compaction, the fibers were pushed out, and the final integrated monolithic tiny bar (Φ1 mm × 2 mm) was shown in Fig. S1b.

2.3.1. In vitro extraction

Stock solutions of 1 mg/mL investigated target analytes were prepared in methanol and kept at −20 °C until analysis. For analysis,