Acta Biomaterialia 25 (2015) 268–283

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Exploring naturally occurring ivy nanoparticles as an alternative biomaterial

Yujian Huang [a,b,c], Yi-Jun Wang [d], Yongzhong Wang [a,b,c], Sijia Yi [e], Zhen Fan [a,b,c], Leming Sun [a,b,c], Derrick Lin [a], Nagaraju Anreddy [d], Hua Zhu [b], Michael Schmidt [a], Zhe-Sheng Chen [d], Mingjun Zhang [a,b,c,*]

[a] Department of Biomedical Engineering, College of Engineering, The Ohio State University, Columbus, OH 43210, USA
[b] Dorothy M. Davis Heart & Lung Research Institute, The Ohio State University Wexner Medical Center, Columbus, OH 43210, USA
[c] Interdisciplinary Biophysics Graduate Program, The Ohio State University, Columbus, OH 43210, USA
[d] Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. John's University, Queens, NY 11439, USA
[e] Chemistry of Life Processes Institute, Northwestern University, Evanston, IL 60208, USA

ARTICLE INFO

Article history:
Received 3 April 2015
Received in revised form 1 July 2015
Accepted 24 July 2015
Available online 26 July 2015

Keywords:
Ivy nanoparticle
Drug delivery
Tissue engineering
Bio-inspiration
Biocompatibility

ABSTRACT

Arabinoglactan protein (AGP)-rich nanoparticles obtained from the sticky exudates of *Hedera helix* (English ivy), have shown promising potential to be used in nanomedicine owing to their excellent aqueous solubility, low intrinsic viscosity, biocompatibility, and biodegradability. In this study, the feasibilities of utilizing ivy nanoparticles (INPs) as nano-carriers for delivering chemotherapeutic drugs in cancer therapy and as nano-fillers to develop novel scaffolds for tissue engineering in regenerative medicine are evaluated. Via electrostatic and hydrophobic interactions, pH-responsive nanoconjugates are formed between the INPs and the doxorubicin (DOX) with an entrapment ratio of 77.9 ± 3.9%. While the INPs show minimal cytotoxicity, the formed INP-DOX conjugates exhibit substantially stronger cytotoxic activity than free DOX against multiple cancer cell lines, suggesting a synergistic effect is established upon conjugation. The anti-cancer effects of the INP-DOX conjugates are further evaluated via *in vivo* xenograft assays by subcutaneously implanting DOX resistant cell line, SW620/Ad-300, into nude mice. The tumor volumes in mice treated with the INP-DOX conjugates are significantly less than those of the mice treated with free DOX. In addition, the INPs are further exploited as nano-fillers to develop fibrous scaffolds with collagen, via mimicking the porous matrix where the INPs are embedded under natural condition. Enhanced adhesion of smooth muscle cells (SMCs) and accelerated proliferation of mouse aortic SMCs are observed in this newly constructed scaffold. Overall, the results obtained from the present study suggest great potential of the INPs to be used as biocompatible nanomaterials in nanomedicine. The AGP-rich INP renders a glycoprotein architecture that is amenable for modification according to the functional designs, capable of being developed as versatile nanomaterials for extensive biomedical applications.

Statement of Significance

Naturally occurring organic nanomaterials have drawn increasing interest for their potential biomedical applications in recent years. In this study, a new type of naturally occurring nanoparticles obtained from the sticky exudates on the adventitious roots of English ivy (*H. helix*), was explored for its potential biomedical application. In particular, the feasibilities of utilizing ivy nanoparticles (INPs) as nano-carriers for delivering chemotherapeutic drugs in cancer therapy and as nano-fillers to develop novel scaffolds for tissue engineering in regenerative medicine were evaluated both *in vitro* and *in vivo*. Overall, the results obtained from the present study suggest the great potential of the INPs to be used as biocompatible nanomaterials in nanomedicine. This study may open a totally new frontier for exploring the biomedical application of naturally occurring nanomaterials.

* Corresponding author at: The Ohio State University, 340C/D Biomedical Research Tower, 460W 12th Ave., Columbus, OH 43210, USA.
E-mail address: zhang.4882@osu.edu (M. Zhang).

http://dx.doi.org/10.1016/j.actbio.2015.07.035
1742-7061/

1. Introduction

Naturally occurring organic nanomaterials, a group of macromolecules and microorganisms that exist innately in nanoscale within biosphere, are regarded as emerging biomaterials that have drawn increasing interest for their potential biomedical applications in recent years [1–4]. Nature creates a bulky pool of organic nanomaterials with diverse morphological and physicochemical characteristics. Nanofibers observed in the mucilage secreted by sundew plants (*Drosera* spp.) have been applied as scaffolds for tissue engineering, due to their unique capability of enhancing cell adhesion and differentiation [5,6]. Small heat shock protein cage (12 nm) obtained from *Methanococcus jannaschii* provides a nanoscale platform to react with fluorescein, which can be further applied for *in vivo* detection or sensing [7]. Other naturally occurring nanoparticles, including lipoproteins [8–11], viruses [12–14], and ferritin [15,16], have also been exploited as nano-vehicles for drug delivery and reporters for *in vivo* imaging. In regenerative medicine, naturally occurring organic nanoparticles have also shown their great potential and capacity. In particular, attempts to conjugate epidermal growth factors and ferritin nanoparticles have been made for tracing the binding, uptake, and transportation of the growth factors [17–19]. Furthermore, it has been demonstrated that the transforming growth factor-β is capable of being sequestered into the lipoprotein nanoparticles rich in triglyceride, making it feasible to modulate the interactions between growth factors and signaling receptors [20,21]. In the previous studies, our group has discovered that naturally occurring nanoparticles obtained from fungus (*Arthrobotrys oligospora*) [22,23] and green tea [24] can be used as bioactive agents for cancer immunochemotherapy, owing to their effective delivery of chemotherapeutic drugs and the combined immunostimulatory effects. In contrast to traditional synthetic counterparts, naturally occurring organic nanoparticles are usually more biocompatible and biodegradable [22,24], precisely defined in dimensions [2], easier to evade the swift opsonization and clearance of the immune system [2], and amenable to either chemical or genetic modification according to the expected functional design [7]. All these characteristics are exceedingly desirable for drug delivery vehicles and tissue engineering scaffolds.

Herein, a new type of naturally occurring nanoparticles obtained from the sticky exudates on the adventitious roots of English ivy (*Hedera helix*) (Fig. 1A) [25], was explored for its potential biomedical application. Ivy nanoparticles (INPs) are a group of macromolecules composed of arabinoglactan proteins (AGPs) which belong to the family of hydroxyproline-rich glycoproteins [25–30]. The core protein of the INPs consists of 132 amino acids, including 15 prolines (or hydroxyprolines) [25–30]. Type II arabinogalactans (AGs), as well as short oligoarabinosides, are repeatedly anchored on the hydroxyproline residues in the protein backbone via *O*-glycosylation [25–30]. In light of the proposed biosynthetic procedure for classic AGPs, the assembly process of the INPs presumably starts in the endoplasmic reticulum and is accomplished within Golgi [25–30]. The physiological function of the INPs has recently been revealed, aiding in the generation of strong adhesive force that gives rise to the surface climbing of English ivy, via a calcium-mediated cross-linking with pectin in the secreted mucilage [25–30]. A platform, involving a hormone-induced cultivation system and an highly efficient purification approach relying on gel-filtration, has been developed earlier for massive production of the INPs [27,28]. The purified INPs are uniform and spheroidal, with an average diameter of approximate 100 nm and a negatively charged surface in aqueous suspension at pH 7.0, as measured by atomic force microscopy (AFM) and dynamic light scattering (DLS) [26,27]. Compared to similar-sized synthetic metal nanomaterials, such as TiO_2 or ZnO nanoparticles, INPs are exceptional in exhibiting great aqueous solubility, well biocompatibility, and excellent biodegradability [27,31]. All these characteristics indicate that the INPs might be an excellent alternative to traditional synthetic nanoparticles used in nanotechnology, and potential candidates for versatile nanomaterials that are highly desired in nanomedicine. More importantly, in light of physiological function that the INPs exert in the sticky exudates of *H. helix* [25–30], it seems reasonable to hypothesize that the INPs may continuously perform an adherence function upon being applied to a biomedical device, which may aid in the cell adhesion, proliferation, migration, cellular uptake, or other bioactivities. To explore these possibilities, in this study, INPs were evaluated as potential carriers for delivering chemotherapeutic drug in cancer therapy and as possible nano-fillers to construct novel scaffolds for tissue engineering.

2. Materials and methods

2.1. Plants, chemicals, and cell lines

Juvenile shoots of *H. helix* were collected from Knoxville, TN. A549 human non-small-cell lung cancer cells (CCL-185), MC3T3-E1 mouse pre-osteoblastic cells (CRL-2593), NIH3T3 mouse embryo fibroblast cells (CRL-1658), and CCD-18Co normal human colon fibroblast cells (CRL-1459) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Mouse aortic smooth muscle cells (MSMCs) and rat aortic smooth muscle cells (RSMCs) were prepared from explants of excised aortas of mice or rats using a method described earlier [32]. B16BL6 murine melanoma cells were obtained from the National Cancer Institute-Central Repository (Frederick, MD). SW620 human colon cancer cell line and its doxorubicin-selected P-gp-overexpressing

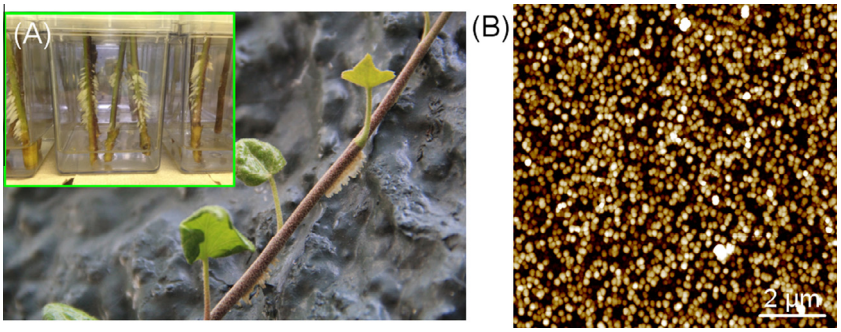

Fig. 1. The INPs isolated from the adventitious roots of *H. helix*. (A) Adventitious roots observed on the ivy shoots, aiding in the surface affixing and the climbing. Inset, *H. helix* was *in vitro* cultivated in the vessels, after induction with potassium salt of indole-3-butyric acid for 3 h, to massively collect adventitious roots of English ivy for further isolation of the INPs [27]. (B) AFM image of the INPs isolated and purified from the mucilage secreted by the adventitious roots of English ivy. The scale bar represents 2 μm.