Biomaterials 91 (2016) 182–199

Contents lists available at ScienceDirect

Biomaterials

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

Melanin nanoparticles derived from a homology of medicine and food for sentinel lymph node mapping and photothermal *in vivo* cancer therapy

Maoquan Chu [a, *], Wangxi Hai [b], Zheyu Zhang [a], Fangjie Wo [a], Qiang Wu [a], Zefei Zhang [a], Yuxiang Shao [a], Ding Zhang [a], Lu Jin [a], Donglu Shi [c, d]

[a] Research Center for Translational Medicine at Shanghai East Hospital, School of Life Sciences and Technology, Tongji University, Shanghai, PR China
[b] School of Pharmacy, Shanghai Jiao Tong University, Shanghai, 200240, PR China
[c] School of Medicine, Tongji University, Shanghai, PR China
[d] The Materials Science and Engineering Program, Department of Mechanical and Materials Engineering, College of Engineering & Applied Science, University of Cincinnati, Cincinnati, OH, 45221-0012, USA

ARTICLE INFO

Article history:
Received 20 October 2015
Received in revised form
9 March 2016
Accepted 13 March 2016
Available online 15 March 2016

Keywords:
Natural black sesame melanin
A homology of medicine and food
Sheet-like nanoparticles
Sentinel lymph node mapping
Cancer therapy

ABSTRACT

The use of non-toxic or low toxicity materials exhibiting dual functionality for use in sentinel lymph node (SLN) mapping and cancer therapy has attracted considerable attention during the past two decades. Herein, we report that the natural black sesame melanin (BSM) extracted from black sesame seeds (*Sesamum indicum* L.) shows exciting potential for SLN mapping and cancer photothermal therapy. Aqueous solutions of BSM under neutral and alkaline conditions can assemble into sheet-like nanoparticles ranging from 20 to 200 nm in size. The BSM nanoparticles were encapsulated by liposomes to improve their water solubility and the encapsulated and bare BSM nanoparticles were both non-toxic to cells. Furthermore, the liposome-encapsulated BSM nanoparticles (liposome-BSM) did not exhibit any long-term toxicity in mice. The liposome-BSM nanoparticles were subsequently used to passively target healthy and tumor-bearing mice SLNs, which were identified by the black color of the nanoparticles. BSM also strongly absorbed light in the near-infrared (NIR) range, which was rapidly converted to heat energy. Human esophagus carcinoma cells (Eca-109) were killed efficiently by liposome-BSM nanocomposites upon NIR laser irradiation. Furthermore, mouse tumor tissues grown from Eca-109 cells were seriously damaged by the photothermal effects of the liposome-BSM nanocomposites, with significant tumor growth suppression compared with controls. Given that BSM is a safe and nutritious biomaterial that can be easily obtained from black sesame seed, the results presented herein represent an important development in the use of natural biomaterials for clinical SLN mapping and cancer therapy.

1. Introduction

Treating medical malignant neoplasms, also commonly known as cancer, is an ever-growing challenge particularly for the increasing aging population. Nanomedicine is a novel strategy with great potential for the effective treatment of cancer. Multifunctional biomaterials play a key role in medical theranostics, as they can simultaneously fulfil numerous requirements such as imaging (or staining) and therapy *via* various means. For instance, gold nanostructures can be used for both cancer sentinel lymph node (SLN) mapping [1,2] and *in vivo* cancer photothermal therapy [3]. Semiconductor quantum dots have been successfully applied for SLN imaging [4–8] and *in vivo* cancer photothermal/photodynamic double therapies [9]. Several other biomaterials, such as activated carbon [10–12], antimony [13], FeS [14] and indocyanine green (ICG) [15–18] have also been reported to possess both imaging/staining and therapeutic functions.

Compared with the synthetic biomaterials described above, the use of natural biomaterials extracted from edible plants for medical applications has attracted considerable interest from scientists working in numerous fields [19–47]. Furthermore, reports pertaining to the applications of these materials have been published in famous journals such as Nature and its sister journals

* Corresponding author.
E-mail address: mqchu98@tongji.edu.cn (M. Chu).

http://dx.doi.org/10.1016/j.biomaterials.2016.03.018
0142-9612/

[19–28,32–35], with one is concerning the 2015 Nobel Prize [48]. The high level of interest in these food materials can be attributed to the fact that they have no or low toxicity and can be readily derived from a wide variety of natural food resources. For example, (−)-epigallocatechin-3-Ogallate (EGCG), which is a major component of green tea, can be used as anticancer agent [22–26]. Allicin and curcumin, which can be found in garlic (a species belonging to the Alliaceae family) and ginger, respectively, have both been used in clinical applications because of their anti-inflammatory, antioxidant and antimicrobial activities [27–34]. *Artemisia annua* L. represents a good reservoir of nutrients and antioxidants, and has consequently been used as a herbal tonic by humans, as well as food supplement for livestock [47]. Importantly, the artemisinin derived from the *Artemisia annua* L. is an excellent anti-malarial agent, as exemplified by the award of the 2015 Nobel Prize in Physiology or Medicine to Tu for her work towards the discovery of novel therapeutic agents against malaria using artemisinin [48].

Herein, we report the natural melanin extracted from black sesame seeds (*Sesamum indicum* L.) for SLN mapping and laser-driven cancer hyperthermia. Black sesame seeds have been used as a source of food and oil for thousands of years in China and several other East Asian countries. These black seeds contain high levels of important nutrients that can be found in the daily dietary intake of many Chinese families. Furthermore, sesame seeds represent an abundant and safe source of interesting materials for medicinal applications. Black sesame seeds have been reported to possess numerous health benefits, including potent antioxidant, antimutagenic, antihypertensive and anti-inflammatory properties, which have led to them being used as alternative medicines [35–44]. Black sesame melanin (BSM) is extracted from the skin of black sesame seeds and used as a traditional source of nutritional food. BSM has also been reported to exhibit potent antioxidant and antinitrosating activities [42]. The natural black pigment BSM therefore represents an effective homology of medicine and food.

In this study, BSM has been used for SLN mapping based on the staining effects produced by its natural black color. The SLN is defined as the first lymph node to receive lymphatic drainage from a primary tumor. Cancer cells usually spread from a primary tumor site through the SLN into deep or distant tissues [49,50]. Furthermore, the discovery of cancer cells in the SLN is usually an indication that the disease has reached an advanced stage. In the same way, the absence of cancer cells from the SLN indicates that the spread of cancer cells from the primary tumor may have not occurred. SLN mapping and biopsy therefore represent important diagnostic tools for determining the stage of cancer spread and developing a suitable treatment scheme.

Although many researchers [1,2,4–8,10,11,51–55] have developed various nanoparticles (e.g., quantum dots [4–8]) for SLN mapping, most of these technologies are not yet ready for clinical use. In the clinical setting, blue dye staining, radiocolloid tracers or a combination of both of these techniques has been used extensively for SLN mapping. Several synthetic blue dyes have been developed for clinical SLN mapping, including methylene blue (MB). There are several advantages to using BSM for SLN mapping over the conventional MB-dye staining method. First, MB is a small (1.6×0.7 nm) water-soluble molecule [1] that can rapidly migrate into the blood and SLN, with significant quantities of material accumulating in the second LN [7,56,57]. This situation is similar to that observed with ICG, which is rapidly washed out from the tissues after being injected, resulting in a short imaging time [58]. These properties of MB would therefore lead to a decrease in the detection sensitivity of the SLN mapping process. In contrast, the BSM molecules evaluated in the current study were much larger than the individual molecules of MB. The BSM would therefore accumulate in the SLN for a longer period of time than MB, making it much more convenient for to the medical practitioner to identify the SLN site. Second, the use of MB can lead to anaphylactic reaction and life-threatening side effects during SLN mapping [59–67]. For example, the intradermal injection of MB (0.2 mL) into the area surrounding the lesion of a 6-year-old white girl with spitzoid melanoma on her right forearm resulted in wide-complex bradycardia after 5 min, which progressed to asystole in less than 1 min [59]. In contrast, BSM is a much safer material and there have, to the best of our knowledge, been no reports in the literature pertaining to adverse cardiac effects resulting from BSM. Last, MB is a photosensitizer, which means that it can exhibit phototoxicity towards tissues during SLN mapping when the tissues containing MB are exposed to light. However, BSM under light irradiation does not efficiently generate toxic reactive oxygen species (ROS).

In this study, we found that the natural BSM molecules dissolved in water underwent a self-assembly process to give graphene sheet-like nanoparticles. BSM was also found to be particularly suitable for SLN mapping based on its long-term entrapment in the SLN. Importantly, these black nanoparticles allowed for the efficient conversion of light energy into heat energy following their irradiation with near-infrared (NIR) laser light, making them excellent candidates for use in cancer photothermal therapy (PTT). To improve the dispersion properties of these BSM nanoparticles in aqueous solution, they were stabilized with liposomes. The resulting liposome-BSM nanocomposites were used for SLN mapping and cancer therapy (Fig. 1). In recent years, PTT using biomaterials has attracted great attention from scientists and several reviews on this field have been published [68–70]. These biomaterials include organic, metal nanomaterials, metal oxide nanomaterials, semiconductor nanocrystals and carbon-based nanomaterials. However, most of the materials described above are far away from clinical use. In contrast, the liposome-BSM developed by this work may be close to application in the clinic.

We selected esophageal cancer model for studying as this cancer is common worldwide, but with high incidence in China [71]. In clinical practice, local treatments such as laser-induced photodynamic therapy [72,73], hyperthermia [73] and radiotherapy [74], are commonly used to prolong the esophageal cancer patients' survival time. In this paper, liposome-BSM nanocomposites were intratumorally injected into mice for inhibiting esophageal tumor growth *via* photothermal effect, which was consistent with the method of clinical application.

Given that BSM and liposomes have both been used in clinical applications as natural and biocompatible materials, it is envisaged that the liposome-BSM nanocomposites developed in this study may also be used in clinical applications. To the best of our knowledge, this work represents the first reported account of the use of liposome-coated BSM nanomaterials for both SLN mapping and cancer PTT.

2. Materials and methods

2.1. Materials

2.1.1. Reagents and materials

Black sesame seeds were purchased from a local Carrefour supermarket (Shanghai, China). Soybean lecithin was purchased from Aladdin Industrial Corporation (Shanghai, China). Other chemical reagents, such as methylene blue (MB) powders, OsO4, epoxide resin, ethanol as well as HCl, NaOH, chloroform, etc., were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

The RPMI-1640 culture medium and fetal bovine serum were obtained from Gibco (Carlsbad, CA, USA). 2,7-Dichlorodihydrofluorescein diacetate (DCFH-DA) was purchased