Journal of the Taiwan Institute of Chemical Engineers 61 (2016) 46–53

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

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

Artificial oil body as a potential oral administration system in zebrafish

Chung-Jen Chiang[a,1,*], Li-Jen Lin[b], Tsung Ying Yang[a], Yun-Peng Chao[c,d,e,*]

[a] Department of Medical Laboratory Science and Biotechnology, China Medical University, No. 91, Hsueh-Shih Road, Taichung 40402, Taiwan
[b] School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung 40402, Taiwan
[c] Department of Chemical Engineering, Feng Chia University, 100 Wenhwa Road, Taichung 40724, Taiwan
[d] Department of Health and Nutrition Biotechnology, Asia University, Taichung 41354, Taiwan
[e] Department of Medical Research, China Medical University Hospital, Taichung 40447, Taiwan

ARTICLE INFO

Article history:
Received 19 October 2015
Revised 8 December 2015
Accepted 14 December 2015
Available online 14 January 2016

Keywords:
Artificial oil body
Zebrafish
Oral delivery carrier

ABSTRACT

Zebrafish are well recognized as an excellent vertebrate model which, in particular, facilitates the rapid investigation of drug effects on cancers. A prerequisite for this anti-cancer study appears to have a non-invasive and effecicve method for admininstration of drugs. In this study, we explored the potential of artificial oil bodies (AOBs) for the oral administration of a hydrophobic agent in adult zebrafish. A hydrophobic dye was employed for illustration, and AOBs were assembled with phospholipids, a storage protein, and an equal volume of olive oil and brine shrimp oil. Consequently, more than 90% of the dye was encapsulated in AOBs which exhibited a prolonged release profile for the dye cargo. The dye-loaded AOBs collected as an oil cake remained integral within 30 min in the aquatic solution while it was completely consumed by one zebrafish in a few seconds. As revealed by the fluorescence microscopy and frozen section, the fluorescence signal was detected in the zebrafish intestinal tract 2 h after the oral administration. The dye cargo was completely absorbed in 12 h and almost no trace was left in zebrafish feces. Overall, the AOBs system is biocompatible and shows promise in the oral administration of chemical entities for zebrafish.

1. Introduction

Animals are vital to medical breakthroughs over the past century. Among animals most commonly employed in research, zebrafish (*Danio rerio*) are widely recognized as an excellent and valuable vertebrate model [1]. They are easily housed with a large population in a small space because of their small size and high fecundity. Zebrafish embryos are transparent while the embryogenesis completes in a short time, making them favorable for the developmental biology study [2]. With over 75% similarity between zebrafish and human genomes, the feasibility in transferring a gene to zebrafish eggs greatly facilitates gene studies [3,4]. In particular, the responses of zebrafish to drugs and xenobiotic chemicals are equivalent to those of mammals [5]. This renders zebrafish appealing for toxicological and pharmacological investigation and drug discovery [6,7].

Zebrafish offers a promising model in cancer research on the basis of the similarity of their genetic and histopathological features to human [8]. Accordingly, they have been applied to investigate the effect of tested drugs on tumor growth, metastasis, and neovascularization [9,10]. Thanks to the advancement in cancer nanotechnology, delivery carriers that are functionalized and encapsulated with anti-cancer drugs have been explored for the pharmacology study [11]. Among carriers, polymeric nanoparticles and liposomes are two well-known examples exploited for clinical trials [12,13]. In spite of their clinical usefulness, these carriers are mainly employed for cancer investigation in animal models other than zebrafish. Moreover, the formulation of hydrophobic drugs by these carriers remains technically challenging due to the problem of low bioavailability and the aggregate deposition resulting from oral or intravenous administration [14,15].

Intraperitoneal injection and the chemical exposure are two methods commonly employed for administration of chemicals in zebrafish [16,17]. The latter method requires that zebrafish be immersed in the solution containing the tested chemical. Likewise, these methods are still afflicted by the administration of

* Corresponding author at: Feng Chia University, Department of Chemical Engineering, 100 Wenhwa Road, Taichung 40724, Taiwan. Tel.: +886 4 24517250; fax: +886 4 24510890.
E-mail addresses: cjchiang@mail.cmu.edu.tw (C.-J. Chiang), ypchao@fcu.edu.tw (Y.-P. Chao).
[1] Tel.: +886 4 22053366x7227; fax: +886 4 22057414.

http://dx.doi.org/10.1016/j.jtice.2015.12.013
1876-1070/

hydrophobic chemicals. This issue was later addressed by the administration of chemicals via the oral route, including the casein-based formulation and the oral perfusion system [18,19]. However, they are laborious and complicate to perform. In this context, a protocol for the oral administration of chemicals has been recently proposed [20]. This approach is based on the use of gluten as a carrier for oral delivery of tested chemicals to zebrafish.

Zebrafish are particularly useful for rapid screening and investigation of the drug effects on cancers [21]. Given that many potent drugs are hydrophobic, the pressing need calls for a simple, reliable, and effective method for the oral administration of insoluble chemicals in zebrafish. Resembling plant seed oil bodies, artificial oil bodies (AOBs) have been illustrated as a potential drug delivery carrier [22–24]. Self-assembly of AOBs *in vitro* undergoes in one step upon mixing plant oils, phospholipids (PLs), and the storage protein such as oleosin (Ole) [25]. As a result, the structure of AOBs is mainly composed of a triacylglycerol (TAG) matrix surrounded by a monolayer of PLs. The structure integrity is maintained by Ole which is closely associated with PLs. AOBs assume a hydrophobic core consisting of natural lipids, which endows them with the ability for encapsulation of hydrophobic agents. As illustrated with the mouse model, AOBs exhibited high encapsulation capability and enabled effective delivery of camptothecin (CPT) after injection [26]. CPT is a cytotoxic alkaloid of low solubility and shows variable pharmacokinetics due to its poor absorption [27]. The success of the study prompted us to explore the potential of AOBs as an oral administration system in zebrafish. As proof of principle, a hydrophobic fluorescence dye serving as the cargo entity was exploited to facilitate the qualitative and quantitative assessment. AOBs were formulated with the mixture of shrimp oil and olive oil. The result shows that the AOBs formulation is promising for the oral administration of water-repellent agents in zebrafish.

2. Materials and methods

2.1. Self-assembly of AOBs

The method for preparation of AOBs essentially followed our previous report [24]. In brief, recombinant Ole was overexpressed in *Escherichia coli* strain BL21/pJO1-Ole grown in a shake flask [28]. Bacteria after receiving the induction were harvested by centrifugation and resuspended in 10 mM sodium phosphate buffer (PBS) at pH 7.5. Followed by disruption with sonication, bacterial proteins were analyzed by sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The production of Ole was quantified using the Image Analyzer GA90000 (UVItech, England).

The assembly solution consisted of PBS (1 ml) with the following composition: PL of 150 μg, Ole of 100 μg, yellow GGK dye (YGGK) of 1 μg (Widetex Co., Taiwan), and the oil mixture (100 μl). The oil mixture was made of the various volume ratios of shrimp oil to olive oil (the S/O ratio). AOBs were then assembled by subjecting the solution to sonication on ice. Followed by centrifugation, YGGK-loaded AOBs were recovered from the top and washed with PBS for use.

2.2. Characterization of AOBs

The morphology and the mean particle size of YGGK-loaded AOBs were analyzed by a transmission electron microscope (Jeol JEM-1400, Japan) and an N4-submicron particle size analyzer (Beckman Coulter, USA), respectively. Moreover, the zeta potential of AOBs was determined using Zetasizer HSA 3000 (Malvern Instrument Ltd, UK) while the stability of AOBs was determined by the turbidity test. The analysis methods were essentially based on the reported protocols [23].

2.3. Stability of oil cakes

The assembly solution of AOBs was centrifuged after sonication. The oil layer floating on the top of the solution was recovered as the oil cake. The oil cake was placed in a cuvette containing the aquatic solution (1 ml) at 28 °C. The fluorescence (A) of the disintegrated part of the oil cake in the lower portion of the cuvette was measured by spectrofluorometer FP-6200 (Jasco, Japan) at time intervals. The disintegration percentage of the oil cake was then calculated by dividing A with the total fluorescence (A_0) of the loaded YGGK.

2.4. Dye cargo loading and release study in vitro

The loading and released level of YGGK in AOBs were determined using a dialysis bag with the 12,000–14,000 molecular weight cutoff (Spectrum Laboratories, USA) as described previously [23]. In brief, the dialysis bag containing YGGK-loaded AOBs (1 ml) was immersed in 0.01 M PBS (20 ml) at 37 °C with the constant stirring. Aliquots of the solution were withdrawn along the time course and YGGK was measured by spectrofluorometer FP-6200. All experiments were performed in triplicate. The encapsulation efficiency was calculated as follows: (YGGK in AOBs/ total YGGK) x 100%

2.5. Regular maintenance of zebrafish

The animal work was approved by Committee of China Medical University with the Permit Number 99-18-N. All procedures for experiments are complied with guidelines for use of zebrafish in the NIH Intramural Research Program (http://oacu.od.nih.gov/ARAC/documents/Zebrafish.pdf). Indigenous wild type male adult zebrafish (5–6 months old) were supplied by Vikrant Aquaculture (Taichung, Taiwan) and maintained in the facility according to the established protocol [29]. Fish were maintained in tanks equipped with the water recirculation system containing 0.2% sea salt at 28 °C under a 14:10 h light and dark cycle. Fish were regularly maintained with commercial granular fish food (Hikari Tropical Fancy Guppy, Japan) fed twice daily. During the experimental period, fish were fed with oil cakes (10 μl). All tested fish were fasted overnight and weighed prior to the study.

2.6. Oral administration of AOBs

Adult male zebrafish at the age of 6 months (3.5–4 cm body length; average weight: 0.40 g) were used and fasted overnight before the experiments. On the day before the oral administration, fish were divided randomly (one fish per 0.2 l water tank). Oil cakes made of AOBs (0.1 μg YGGK per fish, $n = 6$) were added to each tank. For further analyses, fish were anesthetized and observed under a fluorescence microscope (IX71 Olympus, Japan). Parts of the zebrafish abdominal skin were removed to observe the morphogenesis of visceral organs. Frozen sections were obtained from parts of the zebrafish intestine samples and examined using a fluorescence microscope.

3. Results

3.1. Characterization of brine shrimp eggs-loaded AOBs

Brine shrimp (*Artemia sp.*) eggs are widely used for regular maintenance of zebrafish. However, AOBs are conventionally assembled from plant oils. To make AOBs favorable for zebrafish, shrimp oil was extracted from brine shrimp eggs and utilized as the plant oil substitute. Our previous study illustrated that AOBs made of olive oil exhibited high stability [23]. Therefore, AOBs