Increasing the yield of iron-dextran nanoparticles by ultrasonic treatment

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***Abstract* — Iron-dextran nanoparticles are very useful for separating ciliate conjugating pairs. However, due to the low yield, their application is greatly limited. By ultrasonic treatment, their yield is greatly increased, which makes their application without any hesitation. This method will be introduced here.**

***Keywords* — Iron-dextran particles, Nanoparticles, Ultrasonic treatment, Ciliate, Conjugating pairs**

I. INTRODUCTION

Ciliates are a huge group of eukaryotic unicellular organisms with two important characteristics. One is nuclear dimorphism, means two types of nuclei, micronuclei and macronuclei, co-existing in the same cells. The second is conjugation, the ciliate sexual reproduction mode[1]. *Paramecium caudatum* is the representative of ciliates having one micronucleus and one macronucleus[2], whose classical conjugation was reported as early as in 1907[3]. In the past decade, we had some new findings of conjugation[4-6], mostly depending on the adequate conjugating pairs separated by the method of feeding magnetic iron-dextran nanoparticles (MIDPs)[7,8].

Other magnetic particles, such as superparamagnetic iron oxide (SPIO) particles have been used as carriers of drugs or enzymes due to their good biocompatibility and excellent targeting performance including in clinic diagnosis, such as hepatocellular carcinoma[9,10]. Both MIDP and SPIO are conglomerates of Fe_3O_4 and dextran made through chemical reactions. According to Vosskühler and Tiedtke[11], MIDPs have been made and used for the first time for phagosome separation in ciliate study, and then for dividing cell collection[12] and conjugating pair separation[7]. However, MIDP preparation is a time consuming process taking two days obtaining 0.5g, being enough only for once phagosome separation in the experiment of Vosskühler and Tiedtke[11]. How to increase the yield and make magnetic nanoparticles easy to be used in research as well as in clinic?

In fact, these nanoparticles are separated from suspension of magnetic particles with different sizes by centrifugation, through which larger particles are excluded. This operation made us to consider if there is a way to break the larger particles into smaller ones. Ultrasonic treatment is our choice and it does solve the problem of low yield of MIDPs, making great increase of the yield[13]. This improved method will be introduced here.

II. Materials and Methods

Main Equipments and Reagents

Centrifuge (Heal Force Neofuge 1600R, Shanghai, China), stirrer (Qilinbeier GL-3250A, Haimen, China), peristaltic pump (Longerpump BT01-100, Baoding, China), NdFeB Magnets (Jiangdong Mingda, Ningbo, China), dextran-70, $FeCl_3 \cdot 6H_2O$, $FeCl_2 \cdot 4H_2O$, 7.5% $NH_3 \cdot H_2O$.

Methods

Step 1: Preparation of primary conglomerate of magnetic particles

According to Vosskühler and Tiedtke[11], 100 mL of 7.5% $NH_3 \cdot H_2O$ was added into the mixture of dextran-70, $FeCl_3 \cdot 6H_2O$ and $FeCl_2 \cdot 4H_2O$ (dissolved in 60 mLH_2O, 60°C) at the speed of 0.8 mL/min with peristaltic pump (Fig. 1). After the completion of 7.5% $NH_3 \cdot H_2O$ addition, the primary conglomerate was obtained containing different size brown precipitations, the so-called magnetic particles.

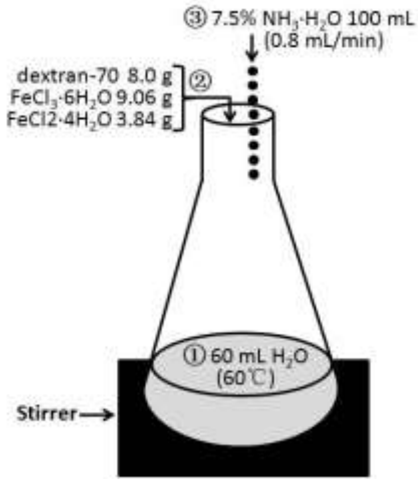

Fig. 1 Preparation of primary conglomerate containing magnetic particles of different sizes

Step 2: Ultrasonic treatment

Usually, the primary conglomerate was centrifuged to eliminate the larger particles according to Vosskühler and Tiedtke[11]. Here we add one step of 5 min ultrasonic treatment before centrifugation, which is the point of our improvement.

Step 3: Collection of nanoparticles with NdFeB Magnets

After ultrasonic treatment, centrifugation was performed, then the remaining steps for collection of MIDPs with NdFeB Magnets were followed the protocol of Vosskühler and Tiedtke[11].

III. Results and Discussions

About 10 g of wet MIDPs could be obtained, which is 20 times of amount obtained by the original method[11]. Due to some unexpected reasons, the centrifugation interval as well as ultrasonic treatment time might be necessary to adjust slightly based on each case. However, as described below, the advantages of this approach are real and objective.

1. Greatly increasing MIDP yield by short interval of ultrasonic treatment

Only 5 min ultrasonic treatment makes the MIDP yield increase to 20 times of the original amount, which greatly improved the MIDP yield.

2. Application of ultrasonic treatment to other magnetic nanoparticle preparation

Ultrasonic treatment could be expanded to the preparation of other magnetic nanoparticles, such as SPIO[9,10].

3. No hesitation for researchers to use magnetic nanoparticles

Higher yield makes the researchers use the magnetic nanoparticles without hesitation. In our cases, the sufficient MIDPs ensure the separation of enough conjugating pairs of *P. caudatum*[4-6], which guarantees the materials for the study of ciliate conjugation.

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