# A Novel Fission Process in Mini-emulsion RAFT Polymerization: Shell Crosslinked Nanoparticles as a Model

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Shell crosslinked nanoparticles, prepared from copolymerization of styrene and disulfide crosslinker, using poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA) as stabilizer and macroinitiator, exhibited a special fission behavior during the mini-emulsion RAFT polymerization process.

Keywords: Bioengineering, copolymerization, morphology

#### 1 Introduction

The morphology transformation of living cells is an important stage for evolution of biosomes (1), which has stimulated enormous interest among researchers in recent years (2–4). Due to the similar composition between lipid vesicles (liposomes) and cell membrane, liposomes are always considered to be an ideal model for living cells (5–7). However, the relatively low stability, compared to polymersomes, has become a main problem to expand their applications (8). Therefore, chemists have shed light on robust nanostructures such as micelles or polymersomes in either submicroscopic or microscopic level. Inspired by the pioneer work of Menger and his co-workers (9), numerous cell-like activities including budding and fission have been reported focused on cytomimetic chemistry, which is termed to describe the visible cell-like morphologies of giant vesicles (3, 4, 9–11).

Presently, vesicles can be prepared from compounds such as surfactants, phospholipids and amphiphilic block copolymers. Their common feature is the presence of hydrophilic heads and hydrophobic tails in the molecules. Nolte and his partners found evidence of synthetic phospholipid vesicles fission *via* directly adding calcium ions into the dispersion solution and proposed a possible mechanism (12). Yan and his co-worker then presented a cooperative fission process by using giant polymer vesicles as model membranes and demonstrated that the composition of the solution had an influence on the vesicles fission (3). Recently, Oana *et al.* found that giant unilamellar vesicles could spontaneously and reversibly form from polyion complex (PIC) microdroplets by thermal perturbation. They elucidated that the microphase separation within the PIC droplet and the formation of the hydrophobic ordered PIC layer were the key mechanisms in vesicle formation (13). Although these fission processes induced by post treatments can be well manipulated, the multi-step method is time-consuming, and the precise control over the stimulus becomes a fundamental issue for the membrane fission.

Polymeric vesicles with excellent stability have attracted considerable attention in recent years for both theoretical and experimental studies (14, 15). However, compared with the large amount of articles on the membrane fission of lipid vesicles, papers on polymeric vesicle fission have seldom been reported. The fission of polymeric vesicles has been first investigated by Eisenberg and his colleagues (15), and recent experiments have shown real-time membrane fusion and fission processes of giant polymeric vesicles (3,16). To the best of our knowledge, there is no study describing nanoparticles fission during the polymerization process. Herein, we establish a novel work concerning this special fission process and display a fission sequence by using a number of nanoparticles transformation images obtained from TEM.

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### 2 Experimental

#### 2.1 Materials

Styrene (St) was first washed by 5 wt% NaOH solution and deionized water to remove the inhibitors and then vacuum distilled prior to use. Azobisisobutyronitrile (AIBN, 98%) was recrystallized twice from ethanol and dried in vacuum prior to use. S-1-Dodecyl-S'-( $\alpha, \alpha$ '-dimethyl- $\alpha$ "-acetic acid) trithiocarbonate (DMP) (17), Bis(acryloyloxyethyl) disulfide (BAEDS) (18, 19) and PDMAEMA macroinitiator (20) were synthesized according to the previously published procedures. MilliQ Water (18.2 M $\Omega$ cm<sup>-1</sup>) generated from a Millipore MilliQ Academic Water Purification System was used in all experiments. All other reagents and solvents were of analytical grade and used as received without further purification.

#### 2.2 Synthesis of Shell Crosslinked Nanoparticles via Mini-Emulsion RAFT Polymerization

Shell crosslinked nanoparticles were fabricated in a onepot method via mini-emulsion RAFT polymerization according to the previously published procedure (Sch. 1) (20). In briefly, a dry flask with a magnetic stirrer was charged with cetyltrimethylammonium bromide (CTAB, 0.0033 g, 0.009 mmol) and 5 mL water. After complete dissolution, an equal volume of PDMAEMA (0.1041 g, 0.01 mmol) solution was added into the flask and stirred for 5 min. Meanwhile, an aliquot of organic mixture containing AIBN (0.0003 g, 0.0018 mmol), St (0.25 mL, 2.2 mmol), BAEDS (0.0262 g, 0.1 mmol or 0.0524 g, 0.2 mmol) and cyclohexane (0.055 mL, 0.5 mmol) was then transferred to the flask. After stirring for another 10 min, the reaction mixture turned white, and the flask was immersed in liquid nitrogen followed by three cycles of freeze-pump-thaw procedure. Finally, the flask was flame-sealed under vacuum and placed in a pre-heated oil-bath at 70°C. Reaction mixtures were quenched in liquid nitrogen at intervals for observation of the morphology transformations of the shell crosslinked nanoparticles by TEM.

### 2.3 Characterization

The <sup>1</sup>H-NMR spectra were recorded on an AVANCE AV 400MHz Digital FT-NMR Spectrometer operating at 400 MHz using deuterated chloroform (CDCl<sub>3</sub>) as a solvent. The chemical shifts were calibrated against residual solvent signals of CDCl<sub>3</sub>. Transmission electron microscopy (TEM) was performed using a JEM-2100 TEM operated at an accelerating voltage of 200 kV, whereby a small drop of micellar solution was deposited onto a copper EM grid, and dried at the same temperature at atmospheric pressure. The sizes and morphologies of the resultant samples were characterized by ULTRA-55 field-emission scanning electron microscopy (FE-SEM). Fourier transform infrared (FT-

# 3 Results and Discussion

The SCL nanoparticles during the polymerization process are chosen to investigate the fission behavior under TEM. Detailed structures of the SCL nanoparticles are described in the Supporting Information. The nanoparticles fission of a daughter vesicle inside a mother vesicle can be observed at the initial polymerization stage (Fig. 1). The daughter shell crosslinked nanoparticles underwent these following fission steps: deformation of spherical nanoparticles, protrusion from the parent nanoparticles to form a budded structure, followed by formation of an internal waist, narrowing the external waist and complete separation. It is interesting to find that fission studied here lasted for hours, which is much longer than that of the previously reported results (3). In addition, the fission phenomena depicted in Figure 1 is very common.

Evidence to support this claim comes from four sources. First, as illustrated in Figure 2a, the mean diameters of SCL nanoparticles decrease with a concomitant increase of reaction time. If the budded structures are formed by coagulation, the mean diameters would increase. Second, it's clearly to find that the intermediate shape displayed in Supporting Information, which looks like two horns tailed on a parent nanoparticle, is a common phenomenon. If the budded structures come from two oil droplets, the intermediate would have an "8" shape (4, 16, 21) because of the coagulation effect. Third, though it's well known that coagulation occurs due to the DLVO theory in emulsion polymerization (21), it's impossible for two independent cores to conglutinate and further become a bigger one because of the hindering effect generated by the crosslinked shells. Fourth, that increasing the amount of crosslinking agent has an impact on the fission. Though the SCL nanoparticles can also undergo a fission process, no separated nanoparticles can be observed by FE-SEM (Fig. 2c) or TEM (Fig. 2d). The reason may be attributed to the crosslinked shells which have become rigid enough before narrowing the external waist. In addition, increasing the molar ratio of crosslinked agent, the nanoparticles with a bigger diameter can be obtained as shown in DLS results (Fig. 2b).

In order to elucidate the fission mechanism, the process of one parent nanoparticle transforming to two daughter ones is selected. We speculate that the fission process of shell crosslinked nanoparticles is in close relationship to the two following reasons. i) After the reaction mixture is mini-emulsified, oil drops with St and BAEDS located in the core are formed, which are stabilized by PDMAEMA macroinitiator and CTAB. Once the polymerization is initiated by free radicals, the monomers would gradually insert between the trithiocarbonate groups and PDMAEMA blocks as the chain extension. This reaction mechanism has



Sch. 1. Synthetic pathway of the fabrication of the shell crosslinked (SCL) nanoparticles via mini-emulsion RAFT polymerization.

been well documented by the previous reports (22). When the earlier transferred monomers are exhausted, the new monomers would spontaneously transfer to fill the blank until all the monomers consumption. So a "channel" would form during the monomer transfer process, which is called an internal waist. In the meanwhile, the chain extension also occurs *in situ* within the parent nanoparticles, so the rigidity of the shell is enhanced with a concomitant increase of reaction time. After a certain polymerization period, the fluidity of the shell decreases rapidly, which causes the shrinkage rate of the shell not keeping pace with that of the monomer transfer, and results in forming a cavity in parent nanoparticles (Fig. S4). It further causes the morphology of parent nanoparticles changing from spheres to shriveled ones because of the pressure generated by the aqueous dispersion which has been confirmed by the previously reported results (Fig. S3) (20). When the transfer rate of monomers is much faster than that of the polymerization *in situ*, the deformation of the spherical nanoparticles and the formation of a budded structure are formed. As the polymerization continues, the budded structure transforms to a more symmetrical shape, giving rise to the twin structure. Due to the limitation of mass transport through aqueous dispersed media in mini-emulsion polymerization (23), the space of the core in the whole nanoparticles shrinks gradually. Therefore, external waist is narrowed. In addition, when the nanopaticles are in a budded structure or a more symmetrical shape, the lateral tension is greater than that of the rest (4), which also has an influence on the external waist narrowing. At last, the complete separation



Fig. 1. The sequential fission process of SCL nanoparticles during mini-emulsion RAFT polymerization. The amount of crosslinker is 0.1 mmol.



**Fig. 2.** The mean diameter *vs.* time of SCL nanoparticles (a), DLS curves of SCL nanoparticles prepared by different amount of crosslinker (0.1 mmol and 0.2 mmol) (b), FE-SEM (c) and TEM (d) images of SCL nanoparticles prepared by 0.2 mmol of crosslinker. (Color figure available online.)



Fig. 3. The fission mechanisms for the shell crosslinked nanoparticles during the mini-emulsion RAFT polymerization process. (Color figure available online.)

of the nanoparticles would occur when the monomers are exhausted. ii) The differences of thermal expansivity between reaction components also play an important role in nanoparticles fission. In general, the thermal expansivity of the monomers is relatively larger than that of PDMAEMA macroinitiators. When PDMAEMA macroinitiators and the monomers are heated at the same time, the expansion extent of the monomers would be greater than that of PDMAEMA macroinitiator which results in the shape change of the nanoparticles and formation of an internal waist. In other words, the fluidity of monomers is better compared to that of the PDMAEMA macroinitiators. It is in accord with the previously reported results (4). The rest fission process may be the same as aforementioned. The detailed fission mechanisms are displayed in Fig. 3.

Then, FT-IR spectroscopy was employed to trace the structure changes of the shell crosslinked nanoparticles (Fig. 4). At the initial stage, C–N stretching vibration at 1148 cm<sup>-1</sup> and C=O stretching vibration at 1726 cm<sup>-1</sup> can be detected, which are the characteristic absorption bands of PDMAEMA (20). After reacting for 3 hours, the characteristic absorption bands for polystyrene can be seen from Fig. 4B, though the intensity of these bands is not strong enough. Besides, all the characteristic absorption bands of PDMAEMA can also be detected, indicating that a successful chain extension of PDMAEMA. It is

noteworthy that the intensity of characteristic absorption bands for polystyrene becomes stronger with a concomitant increase of reaction time. When the fission process finished, the phenyl ring stretching vibration at 1492 and 1600.8 cm<sup>-1</sup>, the ring in phase C–H stretching vibration



**Fig. 4.** FT-IR spectra of SCL nanoparticles during mini-emulsion RAFT polymerization. The amount of crosslinker is 0.1 mmol. (Color figure available online.)

at 1028 cm<sup>-1</sup> and the ring out-of-plane bend at 697 cm<sup>-1</sup> can be clearly seen in Fig. 4F. Furthermore, the relatively intensity of two absorption bands at 2924.6 cm<sup>-1</sup> and 1728.4 cm<sup>-1</sup> have changed significantly, which is attributed to the relative amount of C=O and St. Though the amount of C=O and St in resultant nanoparticles increases, the proportion of St is larger than that of C=O.

# 4 Conclusion

In summary, we have demonstrated a novel fission process by using shell crosslinked nanoparticles as a model. The fission is induced by the different rate between the monomer transfer and the polymerization *in situ*, along with the differences in thermal expansivity of the monomers and the PDMAEMA macroinitiator. Our finding also extends the fission time to hours and provides a possibility for researchers to easily observe the fission process. Furthermore, it is believed that soft matters such as membranes or nanoparticles can undergo a fission process.

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**Supporting Information Available:** <sup>1</sup>H-NMR spectra of PDMAEMA, PDMAEMA-*b*-PS and the synthesized shell crosslinked nanoparticles in CDCl<sub>3</sub>, TEM images for morphology transformations of shell crosslinked nanoparticles,

FE-SEM image of the synthesized shell crosslinked (SCL) nanoparticles.

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