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FRET-enabled monitoring of the thermosensitive nanoscale assembly of polymeric micelles into macroscale hydrogel and sequential cognate micelles release

Pingsheng Huang [a, 1], Huijuan Song [a, 1], Yumin Zhang [b], Jinjian Liu [b], Zhen Cheng [c], Xing-Jie Liang [d], Weiwei Wang [a, *], Deling Kong [a], Jianfeng Liu [b, **]

[a] Tianjin Key Laboratory of Biomaterial Research, Institute of Biomedical Engineering, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300192, China
[b] Tianjin Key Laboratory of Radiation Medicine and Molecular Nuclear Medicine, Institute of Radiation Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin 300192, China
[c] Molecular Imaging Program at Stanford and Bio-X Program, Canary Center at Stanford for Cancer Early Detection, Stanford University, Stanford, CA 94305-5484, USA
[d] CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, Beijing 100190, China

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ABSTRACT

Thermosensitive "micellar hydrogel" is prepared based on poly(ε-caprolactone-*co*- 1,4,8-trioxa[4.6]spiro-9-undecanone)-*b*-poly(ethylene glycol)- *b*-poly(ε-caprolactone- *co*-1,4,8-trioxa[4.6]spiro-9-undecanone) (PECT) triblock copolymer. Fluorescence resonance energy transfer (FRET) is adopted to explore its assembly (formation) and disassembly (degradation) mechanism within the range of 10 nm. Results prove that the thermosensitive non-covalent aggregation of micelles facilitates the hydrogel formation and the sustained shedding of cognate micelles induces the hydrogel degradation, during which polymers are steadily incorporated in micelles without any micelle disassembly or reassembly. It is confirmed that using multiple-tags based imaging technology, such as FRET imaging, the fate of macro biodegradable materials *in vitro* and *in vivo* can be followed at a precise nano even molecular level. Such an unique hydrogel composed of nothing more than PECT micelles can act as not only an injectable nanomedicine reservoir by subcutaneous or peri-tissue administration, but also an advanced "combo" macroscale platform for co-delivery of multi-modal therapeutic agents. Our findings also indicate that biological stimuli (e.g., temperature, enzymes)-induced non-covalent micelle self-assembly may provide us an effective strategy to prepare a macroscale device from nanoscale subunits.

1. Introduction

Functional structures assembled from simple building blocks are of widespread interest for engineering materials with enhanced and synergistic properties, which have shown great potential in biomedical applications [1–5]. Specifically, nano/macro drug delivery systems (DDSs) assembled by amphiphilic block copolymers have significantly advanced the development of formulations for treatment of diseases including cancer and inflammations [6–10]. However, limitations including low delivery efficiency (~0.7%), poor tissue accumulation and insufficient retention at the target tissue and undesired detention by reticuloendothelial systems seriously restrict the clinical translation of nanomedicines [11,12]. Alternatively, macro scale DDSs (eg. injectable hydrogels, implantable scaffolds) provide precise spatiotemporal control over the presentation of encapsulated therapeutic payloads, which are capable of sustaining the drug release over a prolonged time period and improving the local drug concentrations [13–19]. Unfortunately, relying on the free drug diffusion pattern, such systems suffer from poor drug penetration through cell membranes or into tissues, as well as rapid drug clearance. Recently, it has been suggested that organized assembly of nanoparticles into macrostructures through

* Corresponding author.
** Corresponding author.
E-mail addresses: wwwangtj@163.com (W. Wang), lewis78@163.com (J. Liu).
[1] Pingsheng Huang and Huijuan Song contributed equally to this work.

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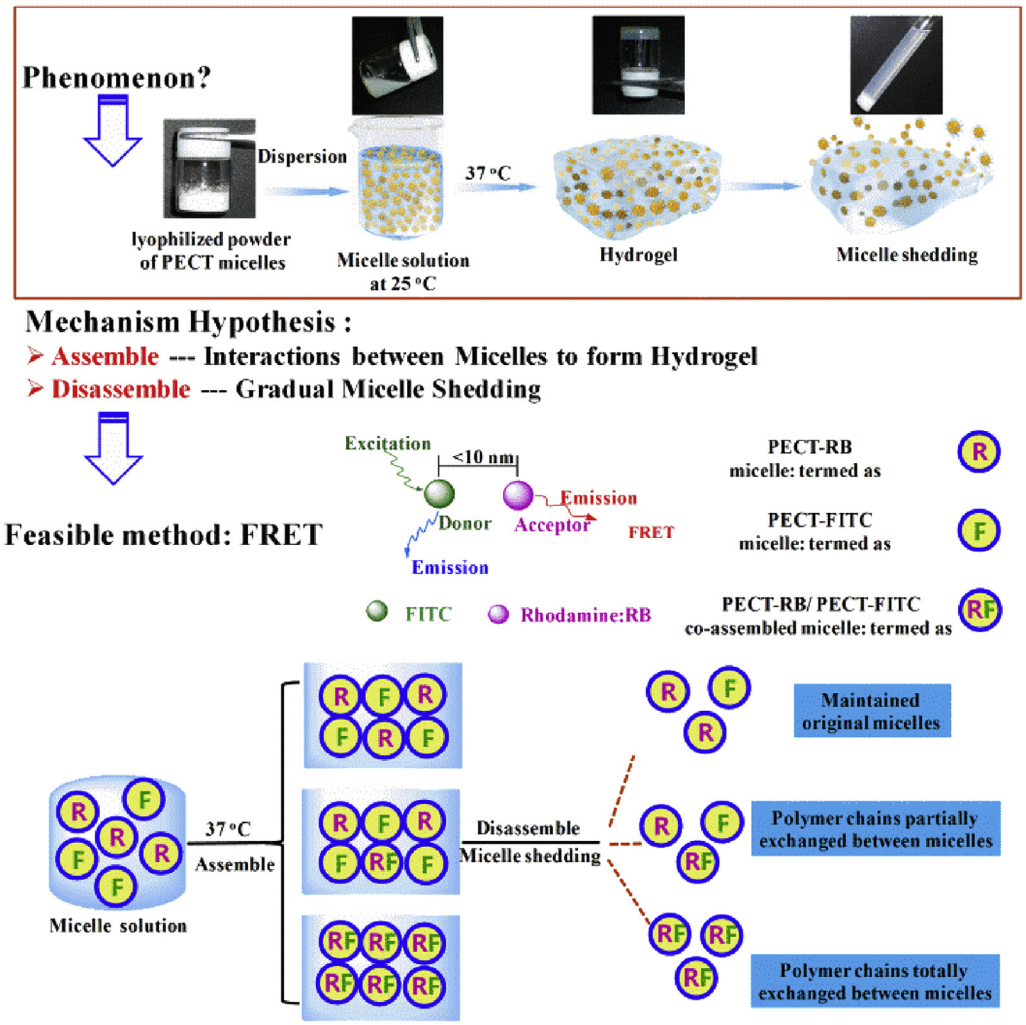
governing the specific molecular interactions or external directing factors associated with the nanoparticle building blocks could be an efficient approach to combine the merits of nano and macro DDSs [20–25], which would be beneficial for further improving the therapeutic effects.

In our study, for the first time, a new type of hydrogel was constructed by the thermosensitive assembly of micelles self-assembled by poly(ε-caprolactone-*co*- 1,4,8-trioxa[4.6]spiro-9-undecanone)-*b*-poly-(ethylene glycol)-*b*- poly(ε-caprolactone -*co*-1,4,8-trioxa[4.6]-spiro-9-undecanone) (PECT) triblock copolymer [26]. As shown in the phenomenon part in Scheme 1, the construction protocol is uniquely characterized by the easy dispersion of lyophilized powders of PECT micelles in water or saline solution at 25 °C with a mass fraction of 25%, which transformed into hydrogel rapidly upon heating to 32 °C. Subsequently, micelles were observed in the medium during hydrogel degradation. Model anticancer drugs, such as paclitaxel or doxorubicin, could be encapsulated and released in a well-controlled single or "cocktail" pattern. The hydrogel-based drug formulations showed significantly improved antitumor activity *in vivo* compared with free drugs or drug-encapsulated nanoparticles [27–31]. Despite these significant progresses in therapeutic applications, insights into the mechanism of hydrogel assembly (formation) and disassembly (degradation) in biological systems remain to be revealed.

Commonly, the amphiphilic balance and hydrophobic interaction of copolymers are considered as pivotal factors for the formation of thermosensitive hydrogels, which are usually verified by the rheology analysis [32–34]. On the other hand, the degradation mechanism is a critical issue associated with the hydrogel duration and drug release profiles *in vivo* [35,36]. The dissolution of polymers or their oligomer fragments generally determines the degradation of macro DDSs. Traditional approaches, including the evaluation of mass loss [37,38], measurement of mechanical properties such as morphology or viscosity [39,40], urine analysis of degradation products [41,42], and the advanced non-invasive fluorescence imaging [43–45], have been adopted to track the rough surface or bulk degradation of macro DDSs. Obviously, as to our macro PECT hydrogel, the above-mentioned formation and degradation mechanisms and evaluation methods cannot fully reveal the unique "micelles-to-hydrogel-to-micelles" transformation process, which occurs on molecular, nano to macroscopic length scales.



Scheme 1. The "from phenomenon to the mechanism" logical route for study on the formation and degradation of the micellar hydrogel. ***Phenomenon:*** a micelle-hydrogel-micelle transformation process has been observed in the formation and degradation process of PECT hydrogel materials; ***Mechanism Hypothesis:*** thermo-induced micelles aggregation facilitates the hydrogel formation and consecutive micelle shedding induces the hydrogel degradation. ***Feasible method:*** FRET has been employed to detect the distance and distance change between biomacromolecules within the range of 10 nm. Micelles composed of PECT-RB or PECT-FITC are termed as R or F within a circle, respectively, and micelles co-assembled by PECT-RB and PECT-FITC polymers are termed as RF within a circle. Hydrogel was prepared by dispersing the lyophilized powder of micelles in water.