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A versatile platform for surface modification of microfluidic droplets

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Abstract

To advance emulsion droplet technology, we synthesize functional derivatives of Pluronic F127 that can simultaneously act as surfactants and as reactive sites for droplet surface decoration. The amine-, carboxyl-, N-hydroxysuccinimide ester-, maleimide- and biotin-terminated Pluronic F127 allows ligand immobilization on single-emulsion or double-emulsion droplets *via* electrostatic adsorption, covalent conjugation or site-specific avidin-biotin interaction.

TOC image

Droplet surface decoration: Method for surface modification of microfluidic emulsions, with precise control over droplet surface properties.

Conventional bulk-generated emulsion droplets are often physicochemically heterogeneous and lack batch-to-batch reproducibility. Microfluidics reliably produces highly monodisperse single and multiple emulsions with fine-tunable sizes that can be used in a variety of applications such as material synthesis,^{1–4} high-sensitivity detection,⁵ single-cell analysis,^{6, 7} protein crystallization,⁸ enzymatic activity assay,^{9–11} and bacterial or mammalian cell culture.^{12, 13} The surfactant, an amphiphilic molecule with different affinities for two immiscible phases, plays a crucial role in any droplet-based application.¹⁴ It reduces the surface tension between the two phases, stabilizes the droplet interface and prevents coalescence.¹⁴ In general, oil-in-water (O/W) single emulsion (SE) droplets only

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require one type of water-soluble surfactant to stabilize the oil droplets. However, water-in-oil-in-water (W/O/W) double emulsion (DE) droplets need two types of surfactants: an oil-soluble surfactant for stabilizing the inner water droplets and a water-soluble surfactant in the outer aqueous phase for stabilizing the oil globules.¹⁵

Considering the swelling or shrinkage of poly(dimethylsiloxane) (PDMS) device channels caused by traditional silicon and hydrocarbon oils, fluorinated oil is a promising alternative for emulsion generation due to its low viscosity and low swelling of PDMS microchannels.^{10, 16} So far, a series of fluorosurfactants based on modified perfluoropolyethers (PFPE) or perfluorinated alkyl compounds have been synthesized to serve as fluorocarbon oil-soluble surfactants for the preparation of water-in-fluorinated oil droplets.^{10, 14, 16–21} On the other hand, hydroxyl-terminated Pluronic F127, a neutral and non-ionic tri-block copolymer containing two hydrophilic poly(ethylene glycol) chains and a hydrophobic poly(propylene glycol) segment, can efficiently stabilize aqueous emulsions.^{12, 22}

Among many applications, microfluidic SE and DE droplets can serve as carriers for delivering nutrients, drugs, and cosmetics due to their versatility for encapsulation and release of both polar and non-polar materials.^{23, 24} However, the lack of chemical reactivity of commercial outer-aqueous-phase surfactants, which are on the interface of droplets and involved in their stabilization, limits the option to optimize the microfluidic DE droplets for specific delivery requirements.

In the present study, we report a technique for surface modification of microfluidic emulsions based on terminally-modified derivatives of the surfactant Pluronic F127. The basic principle involves introducing functional chemical groups to the end of the hydrophilic terminal of the amphiphilic surfactant, which will serve as a stabilizer on the droplet surface and facilitate the subsequent *in situ* surface modification based on electrostatic adsorption, covalent conjugation or site-specific avidin-biotin interaction, respectively (Fig. 1). This is the first reported surface modification of microfluidic O/W and W/O/W emulsions, with precise control over droplet surface properties. Here, biocompatible fluorocarbon oil (HFE-7500) was used as the oil layer because of its hydrophobicity, low viscosity and negligible swelling of PDMS.¹⁷ The FDA-approved Pluronic F127 was used as the backbone of the surfactant due to its excellent biocompatibility and extensive applications in biomedical fields.

Amine- (NH₂), carboxyl- (COOH), N-hydroxysuccinimide ester- (NHS), maleimide- (MAL) and biotin-terminated Pluronic F127s were prepared by chemically modifying the hydroxyl groups of F127 (Scheme 1). Specifically, amine-terminated F127 (F127-NH₂) was obtained by mesylation of F127 in dichloromethane, with subsequent ammonolysis in ammonium hydroxide; carboxyl-terminated F127 (F127-COOH) was synthesized by reaction of F127 with succinic anhydride in pyridine; NHS-terminated F127 (F127-NHS) was conveniently converted from carboxylate by 1-ethyl-(3-dimethylaminopropyl)carbodiimide/*N*-hydroxysuccinimide (EDC/NHS) activation; maleimide-terminated F127 (F127-MAL) was prepared in two steps by acylating chlorination of 6-maleimidoheptanoic acid into 6-maleimidoheptanoic acyl chloride and then directly reacting with F127, thereby forming the

covalent conjugation; biotinylated F127 (F127-Biotin) was synthesized *via* conjugation of biotin *N*-succinimidyl ester (Biotin-NHS) to F127-NH₂ in DMF. The quantitative ¹H NMR spectra of the five surfactants recorded in CDCl₃ or DMSO-*d*₆ are displayed in Fig. S1–S4 (ESI[†]), with the relevant signals labeled. The degree of modification, as determined from the relative integration of proton peaks, was ~100, 100, 100, 100 and 71% for F127-NH₂, F127-COOH, F127-NHS, F127-MAL and F127-Biotin, respectively. The FT-IR spectra (Fig. S5, ESI[†]) also clearly revealed the presence of characteristic absorbance peaks as labeled. The cytotoxicity of these functional surfactants was evaluated against human endothelial progenitor cell (EPC), normal human dermal fibroblast (NHDF), human bone marrow-derived mesenchymal stem cell (MSC), human promyelocytic leukemia cell (HL-60), human non-small lung cancer cell (A549) and human colon cancer cell (Caco-2) by using a tetrazolium salt assay. As shown in Fig. S6 (ESI[†]), both F127 and its functional derivatives were nontoxic against endothelial (EPC), mesenchymal (NHDF, MSC), epithelial (A549 and Caco-2) and white blood cells (HL-60) up to the highest testing concentration of 1 g L⁻¹, indicating the biocompatible nature and potential biomedical application of these functional surfactants.

In the following, PDMS microfluidic devices were fabricated using photo- and soft-lithography techniques,^{12, 13, 25} and monodisperse O/W or W/O/W microdroplets were generated at the flow-focusing junction of the chips (Fig. 1A and Fig. S7, ESI[†]). The PDMS chip, without any special treatment, could generate O/W SE droplets by using the aforementioned F127 derivatives as functional surfactants. In contrast, for DE preparation, a hydrophilic coating was applied to the inner surfaces of the microchannels by following the UV-mediated polymerization procedure developed by Schneider *et al.*²⁶ However, to avoid non-specific interactions between the coating polymer and the surfactants, the neutral monomer poly(ethylene glycol) methyl ether acrylate was chosen instead of the anionic acrylic acid used in the reported method. The resulting SE and DE droplets, as well as HFE-7500 alone, were proven to be biocompatible in subsequent cytotoxicity studies (Fig. S8, ESI[†]), suggesting their potential for bio-applications.

The above five derivatives of F127 can act as surfactants to stabilize the droplet interface and prevent coalescence of both SE and DE droplets, indicating that terminal modification of F127 will not affect its inherent properties as a surfactant. After removing free surfactants from the solution by washing with water 6 times, we implemented three different representative strategies for surface modification of microfluidic droplets (Fig. 1B): 1) electrostatic adsorption; 2) covalent conjugation; and 3) non-covalent, site-specific avidin-biotin interaction.

As a simple and efficient strategy, electrostatic adsorption between oppositely charged entities is an effective method for surface modification.²⁷ Droplets with positively or negatively charged surfaces were produced when amine- or carboxyl-terminated F127 was used as the surfactant during the on-chip generation process, respectively. Subsequently, two model molecules, FITC-labeled anionic bovine serum albumin (BSA-FITC) and Cy5-labeled cationic PAMAM G3 dendrimer (PAMAM-Cy5), could be coated onto the droplets

[†]Electronic Supplementary Information (ESI) available: Experimental section and additional figures. See DOI: 10.1039/x0xx00000x

by electrostatic interaction, as evidenced by fluorescence signals on the surface of both SE and DE droplets (Fig. 2A-a, 2A-b, 2B-a and 2B-b). On the other hand, no fluorescence was detected when neutral F127 was used as the surfactant in both cases (Fig. S9a, S9b, S10a and S10b, ESI[†]).

Compared with the non-covalent electrostatic interaction, covalent conjugation is a more specific and stable method for surface modification. The primary amine group is one of the most commonly targeted functional groups for chemical conjugation. Among the wide variety of amine-based reactions, nucleophilic addition of amine to NHS-ester (a reactive group formed by carbodiimide-activation of carboxylate) results in stable covalent amide linkage with high efficiency.²⁸ This reaction can be executed using a broad range of compounds and proceeds under very mild conditions (*e.g.* room temperature, physiological to slightly alkaline conditions). Here, doxorubicin (DOX) with a primary amine and F127-NHS were used as a pair of model molecule and functional surfactant to test the reactivity of NHS-ester on the droplet surface. After incubation at room temperature for 3 h followed by thorough washing to remove the unreacted fluorescent dye, DOX emitted fluorescence on the surface of both SE and DE droplets (Fig. 2A-c and 2B-c), indicating successful chemical conjugation.

Although amine is a popular target for bioconjugation, there is a risk of low site-specificity due to the abundance of amine groups in many target conjugates such as proteins. An alternative approach to increase site specificity is to target thiol groups *via* maleimide/thiol-mediated Michael addition for bioconjugation. There are two distinct advantages for this reaction: fast kinetics and extremely high selectivity.²⁹ Here, deprotected [5-((2-(or-3)-S-(Acetylmercapto)succinoyl)amino)fluorescein] fluorescein with a thiol group (SAMSA-SH, Scheme S1, ESI[†]) was used as a model molecule to test the reactivity of maleimide on the droplet surface. As shown in Fig. 2A-d and 2B-d, after 2 h incubation of SAMSA-SH and maleimide-modified droplets at room temperature, green fluorescence was specifically detected around the droplet surface, indicating successful chemical conjugation of maleimide and thiol-containing SAMSA-SH. In contrast, the control group did not demonstrate any reaction between the hydroxyl groups of F127 and the two model molecules (Fig. S9c, S9d, S10c and S10d, ESI[†]).

Besides covalent conjugation, affinity-based site-specific avidin-biotin interaction is another highly efficient method of bioconjugation. The classical example is the avidin-biotin system. Specifically, biotin (also known as vitamin B7, vitamin H, or coenzyme R) is a cofactor in the metabolism of fatty acids and leucine.^{30, 31} It has a high affinity for avidin, a glycoprotein produced in the egg white and tissues of birds, reptiles, and amphibians.³⁰ This strong interaction has been used extensively in bioconjugation, purification and detection applications. The dissociation constant K_d , which signifies the affinity for biotin, is on the order of 10^{-15} M. Moreover, the bond formation between biotin and avidin is not only fast but also resistant to harsh chemical conditions (*e.g.* organic solvent and extreme pH) and elevated temperature.³² There are three main types of biotin-binding glycoproteins: avidin, streptavidin and NeutrAvidin. Compared with avidin and streptavidin, NeutrAvidin is a much more ideal biotin-binding reagent because it has the highest specificity. In the present study, Texas Red-labeled NeutrAvidin (NA-TR) was used as a model molecule for surface

modification of F127-Biotin-stabilized droplets. As displayed in Fig. 2A-e and 2B-e, after a 30-min incubation period, NeutrAvidin (red) localized to the surface of both SE and DE droplets. However, using the unmodified F127-stabilized droplets and identical binding conditions with NeutrAvidin, no protein immobilization could be detected (Fig. S9e and S10e, ESI[†]).

The above three types of interaction and reaction are highly efficient and orthogonal, offering potential for making multiple modifications simultaneously. In the following, we demonstrate the feasibility of multifunctional modification by implementing orthogonal modification of microfluidic droplets. For this purpose, droplets masked by two different types of reactive chemical groups were first obtained by using surfactants with two chemically different terminal groups. Then, relevant model molecules were added simultaneously for surface conjugation. More specifically, F127-MAL plus F127-NHS and F127-MAL plus F127-Biotin were used as surfactants to stabilize the droplets, followed by incubation with SAMSA-SH plus DOX and SAMSA-SH plus NA-TR. The co-localized fluorescence was uniformly distributed on the surface of the droplets, indicating successful orthogonal modification (Fig. 3A and 3B).

The stability of the droplets was verified by tracking and recording their morphology and fluorescence signal. As shown in Fig. S11 (ESI[†]), F127-stabilized SE and DE droplets were stable in aqueous medium at room temperature for at least 3 days. When functionalized-F127s were used for the three types of modifications (Fig. S12–S16, ESI[†]), both SE and DE droplets were stable within 72 h. Different degrees of reduction in fluorescence intensity on the droplet surface were observed, which can be attributed to fluorescence quenching over time.^{33, 34}

In summary, we have developed a simple yet versatile platform for functional F127 surfactant-based surface modification of microfluidic droplets *via* covalent or non-covalent interactions. This is the first example reported on the surface modification of microfluidic droplets using end-group-functionalized surfactants. Taking advantage of the unique characteristics of surfactant-assisted surface decoration of droplets, this technique offers a broad spectrum of interaction/reaction, facile surface conjugation and simultaneous orthogonal modification. Notably, the present work suggests that surface modification is a promising technique for engineering microfluidic droplet-based multifunctional drug delivery systems with tunable surface properties. Moreover, our work will also facilitate the application of surface-functionalized microfluidic droplets in biomedical engineering and biotechnology. Stemming from this concept, other surfactant-based surface modifications can also be envisioned.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

(A) Generation of single and double emulsion droplets in flow-focusing devices by using different functional surfactants. (B) Schematic illustration of surface modification *via* electrostatic adsorption, covalent conjugation and site-specific avidin-biotin interaction.

Note: x, y and z represents degree of polymerization of F127 segments. x = 100, y = 65, z = 100.

Fig. 2.

Surface modification of microfluidic (A) single and (B) double emulsions using one type of end-group functionalized surfactant. Microscopy images of (a) F127-NH₂-, (b) F127-COOH-, (c) F127-NHS-, (d) F127-MAL-, and (e) F127-Biotin-stabilized droplets after coating with (a) BSA-FITC and (b) PAMAM-Cy5 *via* electrostatic adsorption, conjugation with (c) DOX and (d) SAMS-A-SH *via* covalent conjugation, and (e) linkage with NA-TR *via* site-specific avidin-biotin interaction. The scale bar represents 200 μm.

Fig. 3. Orthogonal surface modification of microfluidic (A) single and (B) double emulsions using two different end-group functionalized surfactants. Microscopy images of (a) F127-NHS plus F127-MAL-, and (b) F127-MAL plus F127-Biotin-stabilized droplets, after (a) simultaneous covalent conjugation with DOX and SAMSA-SH, and (b) simultaneous functionalization with SAMSA-SH and NA-TR. The scale bar represents 200 μm .

Scheme 1.

Synthetic routes for the preparation of (a) F127-NH₂, (b) F127-COOH and F127-NHS, (c) F127-MAL, and (d) F127-Biotin.