Development of an SS-Cleavable pH-Activated Lipid-Like Material (ssPalm) as a Nucleic Acid Delivery Device

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Gene and nucleic acid-based medication is an ultimate strategy in the field of personalized medicine. A gene or short interference RNA (siRNA) molecule needs to be delivered to the appropriate organelle (i.e., nucleus and cytoplasm, respectively). We recently focused on improving the intrinsic activity of my original material (ssPalm) in terms of endosomal/lysosomal membrane destabilization activity by chemically modifying the tertiary amine structure. In parallel, I have been expanding the range of applications of ssPalsms. The first application is a DNA or RNA vaccine. My crucial finding is that the vitamin E-scaffold ssPalm (ssPalmE) is highly immune-stimulative when combined with DNA. Thereafter, I redesigned the hydrophobic scaffold structure, and found that an oleic acid-scaffold ssPalm (ssPalmO) can confer anti-inflammatory characteristics. Based on this result, I further upgraded the ssPalmO, by inserting a newly designed linker with self-degradable properties.

Key words SS-cleavable pH-activated lipid-like material (ssPalm); endosomal escape; DNA; short interference RNA (siRNA); vaccine

1. INTRODUCTION

The field of medicine is now expanding from low-molecular drugs to recombinant proteins, antibodies, and nucleic acids. This is occurring in parallel with a progress in “-omics” technologies, Genes (i.e., DNA and RNA) for protein complementation and nucleic acids (i.e., short interference RNA; siRNA and micro RNA; miRNA) for protein knockdown are now recognized as an attractive molecule for delivering personalized medication.

Since Glybera® (UniQure) was first approved in Europe in 2012 (discontinued in 2017), the rate of approval of gene therapy drugs has been increasing in recent years. Very recently, for the cure of “CD19-positive relapsed or refractory B-cell acute lymphoblastic leukemia under 25 years of age, including children, and with relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy including diffuse large B-cell lymphoma (DLBCL) not otherwise specified, high grade B-cell lymphoma and DLBCL arising from follicular lymphoma,” a chimeric antigen receptor T cell medicine, KYMRIAH® was approved (April 23, 2019 in Japan).

In these current gene therapies, viral vectors were dominantly used since they mount excellent machinery for the delivery of DNAs to the nucleus in host cells. While gene therapy will be undergoing continuously innovation based on such viral vectors in future, an excessive immune reaction to viral proteins can also be a risk for the development of side effects. Thus, the development of the non-viral vectors using an artificial material is desired.

For many years, plasmid DNA (pDNA) prepared in large quantities from Escherichia (E.) coli has been used for gene therapy. However, especially in cells with slow cell growth or non-dividing cells, the nuclear membrane serves as a large barrier that inhibits the nuclear translocation process of DNA, resulting in a low expression efficiency. Moreover, the risk of carcinogenicity associated with the insertion of the DNA transferred to the nuclear genome of the host cell may be a factor that hampers clinical applications.

Meanwhile, attention has been focused on mRNA as a new modality for gene therapy. Such molecules will be translated to produce a protein once they are introduced into the cytoplasm, thus resulting in the high expression of a protein. Moreover, since it is not inserted into the genome, high safety can be expected. The fundamental technology behind the application of mRNA as a drug modality was the development of the anti-reverse cap analogue (ARCA) method, which adds a Cap structure to the 5’ end of RNA that is transcribed using DNA as a template. In addition, in parallel with progress related to artificial gene synthesis technology, the in silico methods for optimizing codons have also been established. Furthermore, by introducing chemically modified nucleic acids (methylated nucleic acid etc.) into mRNA, immune response and cellular stress would be suppressed. Control of the secondary structure of mRNA can also prolong for the functional stability of mRNA.

Furthermore, short nucleic acids (i.e., siRNA and miRNA) can be used to knock-down or down-regulate disease-related genes. The siRNA against transthyretin familial amyloid (ATTR), and antisense oligonucleotides for muscular dystrophy (ONPATTRO®) have currently been approved worldwide. However, the RNA molecule per se is a quite hydrophobic molecule, and readily degradable, especially in the body. Thus, to realize RNA-based therapeutics, an innovative Drug Delivery System (DDS) will be needed. In this review article, our recent approaches for achieving DNA/RNA de-
livery using an intracellular environment-responsive lipid like material (ssPalm) are summarized.

2. THE CONCEPT OF ssPalm

For successful DNA or RNA therapeutics, a rational design that can deliver a gene or a nucleic acid to the target organelle (nucleus and cytoplasm, respectively) is needed. Biomembranes such as endosomal membrane, as well as nuclear membrane in the case of gene delivery are one of the major barriers to be overcome. In the past 30 years, a large variety of “cationic” liposomes and polymers have been reported as DNA carriers based on the following two advantages. First, they can readily form a positively charged nanoparticle by electrostatic interactions with negatively charged DNA/RNA that permits a strong association with negatively cellular-surface constituents (i.e., heparan sulfate proteoglycans) for achieving an enhanced cellular uptake. Second, a cationic charge is the essential driving force for the subsequent destabilization of the endosomal/lysosomal membrane for cytoplasmic delivery. However, when a delivery system that can be applied via intravenous administration is designed, we will also need to consider the ultimate dilemma: the nanoparticle must be robust in the extracellular domain (i.e., the blood circulation), as well as in storage conditions, while the particle must be highly unstable at the target organelle of action to allow the gene and nucleic acids to be released.

Unfortunately, a conventional cationic material is not an ideal design for overcoming this dilemma. While an excess amount of cationic materials are needed to protect the DNA/RNA molecules from enzymatic degradation in the serum and extracellular space, these highly cationic particles form large aggregates with erythrocytes or platelets after their intravenous administration, and subsequently become stacked in the lung microvessels. This event may cause tissue ischemia, and possible myocardial damage because of microinfarction, and undesirable transgene expression in the lung. In addition, the injection of these cationic materials trigger cytokine production. From the point of view of intracellular events, our previous imaging-based quantitative analyses clarified that the use of the cationic materials is also attended with a demerit, in that the robust and continuous electrostatic interaction with DNA/RNA inhibits the cytoplasmic release of these cargos, thus preventing the transcription and the translation processes, respectively. Our quantitative analysis revealed that this inhibition is the major mechanism responsible for the 3 orders of magnitude poorer DNA transfection efficiency of cationic liposome in comparison with adenovirus.

Based on our previous findings, the development of a neutrally charged particle prepared by excluding cationic materials is the next-generation design. we developed an original material: an SS-cleavable pH-Activated Lipid-like Material (ssPalm). This material contains two hydrophobic moieties. Thus, similar to a lipid, it can form a lipid nanoparticle. The material can also be functionalized so as to contain dual sensing motifs that can respond to the intracellular environment (Fig. 1). The first one is a tertiary amine, a unit that acquires a positive charge in response to an acidic compartment (endosome/lysosome), for membrane destabilization. Another one is disulfide bonding, a unit that can be cleaved in response to a reducing environment (cytosol) for spontaneous collapse.

The design of the former units (tertiary amines) were inspired by the pioneering technology (DLin-MC3-DMA: MC3), which is a key material used in an ONPATTRO®, a siRNA-based first approved drug for the treatment of hereditary transthyretin-mediated amyloidosis (hATTR). The lipid

**Fig. 1. Fundamental Concept and Design of an ssPalm**

The ssPalm mounts dual sensing motifs that can respond to the intracellular environment; a proton-sponge unit (tertiary amines) that functions in response to an acidic environment (endosome/lysosome), and disulfide bonding that can be cleaved in a reducing environment (the cytosol). (Color figure can be accessed in the online version.)

**Biography**

After graduating from the University of Tokyo, School of Pharmacy (B.S. Pharmacy) in 1997, he entered graduate school (M.S. Pharmaceutical Science in 1999). Ph.D. degrees (Pharmaceutical Sciences) at the University of Tokyo in 2002. After a Research Fellowship for young scientists from the Japan Society for the Promotion of Sciences (JSPS), he was appointed to the Faculty of Pharmaceutical Sciences, Hokkaido University. He was promoted to the rank of Associate Professor in 2010. In 2016, he became a Professor in the Graduate School of Pharmaceutical Sciences, Chiba University.
nanoparticle (LNP) formed with an ionizable lipid has a neutral charge. However, after cellular uptake, the LNPs are taken up into the endosome. In this compartment, the particle is exposed to an acid environment, resulting in the tertiary amines being protonated. This protonation results in the LNP developing a positive charge, thus triggering an interaction with the endosomal membrane that is generally charged negative.25) Thereby, it adopts a nonbilayer hexagonal HII structure.25) As I designed, the LNP accommodates LNP ssPalmE with various hydrophobic scaffolds.25) As an example, the LNP ssPalmM with double hydrophobic chains is converted into two sets of products with single hydrophobic chains. This event triggers the collapse of the particle, and decapsulation (release) of encapsulating cargo (nucleic acids) to the cytoplasm. In fact, more extensive release of the rhodamine-labeled DNA from the NBD-labeled LNP formed with the ssPalm (LNP ssPalm) was observed in comparison with that formed with the ccPalm (LNP ccPalm) in that the disulfide bonding was replaced with a non-cleavable carbon bonding.22) The cleavage of the disulfide bonding occurred within 10 min in a buffer containing 10 mM glutathione (GSH), that mimics the reductive environment in cytoplasm. By this cleavage, the parent ssPalm with double hydrophobic chains is converted into two sets of products with single hydrophobic chains. This event triggers the collapse of the particle, and decapsulation (release) of encapsulating cargos (nucleic acids) to the cytoplasm. In fact, more extensive release of the rhodamine-labeled DNA from the NBD-labeled LNP formed with the ssPalm (LNP ssPalm) was observed in comparison with that formed with the ccPalm (LNP ccPalm) in that the disulfide bonding was replaced with a non-cleavable carbon bonding.22)

Fig. 2. Structure of ssPals with Various Hydrophobic Scaffolds

The employment of physiological substances (i.e., vitamin A or vitamin E) allows the trafficking and bio-reaction of the nanoparticle to be controlled. (Color figure can be accessed in the online version.)

The ternary amine structure of the ssPalmE was originally developed for improving endosomal escape.33,34) Since nanoparticles are mainly taken up by the liver after being intravenously administered, this organ is the most promising in vivo target for siRNA delivery. Our initial screening indicated that the gene knockdown activity of siRNA against a hepatocyte-specific marker (factor VII) was the highest for the LNP ssPalmE in comparison with the LNP ssPalmM or the LNP ssPalmA after intravenous administration. Consistent with this result, fluorescence-labeled siRNA encapsulated in the LNP ssPalmE accumulated more extensively in the liver than the others.33) Thus, I tuned-up the tertiary amine structure to allow the particle to detect a slight change of pH in endosome, which then adopted a positive charge for rapid endosomal escape at an earlier stage. The apparent pKₐ value of the preparation is a key parameter that reflects the activity of pH-triggered positive charging. Current studies indicate that the maximum gene knockdown activity occurred when the apparent pKₐ values were adjusted to approximately 6.4.

The ternary amine structure of the ssPalmE was originally flexible. For the efficient and stable protonation of these tertiary amines, they were fixed in a ring structure (piperidine).27) In contrast, once taken up by cells, it is spontaneously degraded, releasing its cargo in response to the intracellular environment.22,26,28)

Historically, we developed a series of ssPalm molecules with various types of hydrophobic chains: myristic acid (ssPalmM).22) vitamin A (ssPalmA)29) and vitamin E (ssPalmE)30–32) (Fig. 2). Some advantages accrued as the result of using these vitamins. First, these vitamins have unique physicochemical properties. For instance, the stability of the particle can be controlled by selecting the appropriate hydrophobic scaffold since self-assembly of such particles are driven by the hydrophobic interactions. Second, vitamins are processed by a unique transport system. One example is that cellular retinoic acid-binding protein (CRABP), a nuclear transport system is available as machinery for nuclear delivery of vitamin A. In fact, the LNP ssPalmA exhibited a 1 order of magnitude higher transgene expression in comparison with the LNP ssPalmM.29) The LNP ssPalmE also allowed a more efficient hepatic delivery of siRNA than other types of ssPals, most probably because the LNP ssPalmE was recognized by serum ApoE, and these ApoE-bound particles were then dominantly taken up by hepatic low density lipoprotein (LDL) receptors.33) Finally, it is possible to mount a unique pharmacological activity in a hydrophobic group. As I attempted to apply this strategy to a DNA or RNA vaccine, the combination of a ssPalmE with DNA/RNA was found to activate the immune-response. An example of this is described in the later section.

3. TUNING OF THE TERTIARY AMINE STRUCTURE FOR IMPROVING ENDOSOMAL ESCAPE33,34)

![Diagram of ssPals with Various Hydrophobic Scaffolds](image-url)
It is thus concluded that the co-delivery of anti-inflammatory glucocorticoid compound, was simultaneously loaded into the particle with DNA, this inflammatory response was relieved, and the efficiency of gene expression was enhanced. This is thus concluded that the co-delivery of anti-inflammatory drug is a promising approach for reducing these risks and improving the function of DNA-encapsulating LNP(ssPalmE) preparations.

4. APPLICATION OF A VITAMIN E-SCAFFOLD ssPalm (ssPalmE) AS A DNA/RNA VACCINE PLATFORM

Personalized medicine is one of the currently growing concepts for the creation of safe medications and for curing intractable disease. The cancer vaccine that targets unique and significant genetic changes in individual cancers is one of the new-generation technologies. In this concept, neo-antigens predicted by the analysis of genetic changes in cancer are used for vaccinations. However, since the physicochemical properties of these antigens are potentially affected by their sequence, and even by single amino acid mutations, the peptides per se may not be an adequate modality for a vaccine. Alternatively, a DNA/RNA vaccine, in which DNA or RNA encode for a series of neo-antigens are used for immunizations is powerful technology, since the insertion or mutation of the DNA/RNA sequence has only a marginal effect on the physicochemical property. Thus, once the formulation is optimized, it can be applied to a large variety of antigens. To realize such a DNA/RNA vaccine, an innovative technology for DNA/RNA transfection to the antigen presenting cells (i.e., dendritic cells and/or macrophages) is needed to educate them as to what is a tumor-specific antigen (neo-antigen) or foreign substances. In parallel, technologies for immunological activation are necessary.

As described in section 3, the intravenous injection of the DNA-encapsulating LNP_{ssPalmE} resulted in extensive cytokine production. These drawbacks per se are not desirable for applications that involve intravenous administration. However, these findings prompted us to use this property in DNA vaccine/adjuvant technology via subcutaneous administration.

First, we simply focused on the immune-stimulative activity of DNA-encapsulating LNP_{ssPalmE}. Incubating this particle with macrophage-derived cells caused a significantly higher production of IFN-β, a key cytokine that induces a Cytotoxic T-Lymphocyte (CTL) in comparison with the LNP_{ssPalmM}. It is noteworthy that the production of IFN-β was under the detection limit in the case of naked DNA, and the DNA-free LNP_{ssPalmE}. This indicates that a combination of DNA and ssPalmE is needed in order to produce an adjuvant function. Moreover, an inhibitor of TBK1/IKKe (inhibitor of kappaB (IκB)-kinase epsilon) significantly suppressed the IFN-β production. These results indicate that the LNP_{ssPalmE} triggers the stimulation of a cytoplasmic DNA sensor, an immunological defense machinery that prevents viral infections, and the following STING/TBK1/IRF3 pathway.

In vivo CTL activities were highly induced after the co-immunization of DNA-encapsulating LNP_{ssPalmE} and antigen protein (Ovalbumin: OVA as a model antigen). Furthermore, this CTL activity was higher than that of the LNP_{ssPalmM}. Consistent with this result, this immunization resulted in a prophylactic anti-tumor effect (complete rejection of tumor engraftment) in an antigen-dependent manner. Furthermore, a combination of an anti-PD-1 antibody, an immune checkpoint inhibitor synergistically improved the therapeutic anti-tumor effect.
We then applied this immune-stimulative activity of LNP_{ssPalmE} to DNA vaccine technology, in that antigen information was encoded in the encapsulated DNA.\textsuperscript{32} Immunization with an OVA-encoding DNA by means of an LNP prepared with a series of ssPalmss (ssPalmM, ssPalmA and ssPalmE) revealed that the LNP_{ssPalmE} exhibited the highest CTL activity, as well as the highest DNA transfection activity. As a demonstration of its pharmacological activity, a prophylactic anti-tumor effect was evaluated. As a result, immunization with the LNP_{ssPalmE} with antigen-encoding DNA nearly completely suppressed tumor engraftment in 4 out of 5 mice.

Second, we assessed the protective immunity for a protozoan (Toxoplasma gondii), an obligate intracellular parasite that causes congenital disorders or abortion in pregnant humans and domestic animals. A DNA vaccine is one such therapeutic candidate, since it is known that CD8\textsuperscript{+} T cell-mediated cellular immunity plays a key role in protecting and controlling intracellular parasite infections. After 3 sequential immunizations, the immunization of the mice with its antigen (Toxoplasma gondii profiling: TgPF)-encoding DNA by means of LNP_{ssPalmE} dramatically increased their survival rate (87.5%, p < 0.05), while all of the mice were dead in the non-coding DNA-immunized or water-injected group. Collectively, the LNP_{ssPalmE} represents a highly potent DNA vaccine platform that can be used for the prevention of cancer and protozoan infections.

In general, lymph nodes consist of various types of immune-responsive cells such as B cells, T cells and Antigen Presenting Cells (APCs); Dendritic Cells (DCs) and macrophages. Since it is well known that the CD169-positive macrophage functions as antigen presenting cells against cancer-derived antigens, I hypothesized that these CD169-positive macrophages might be a key player for immune-stimulation and antigen presentation. However, unexpectedly, the CTL activity induced by the pDNA-encapsulating LNP_{ssPalmE} was not reduced, but rather increased in CD169-DTR transgenic mice, in that the CD169-positive cell population is conditionally ablated following the administration of Diphtheria toxin. Thus, at least regarding DNA vaccines, other immune-responsive cells might play a key role in immune-activation.\textsuperscript{32}

Concerning an RNA vaccine, the first in-human trial was reported in 2017. In this study, a total of 10 neo-antigens were predicted, and humans were then immunized with 2 sets of mRNA encoding 5 antigens in a tandem manner. However, in this study, a large amount of mRNA (0.5–1.0 mg) were directly injected into the lymph nodes. Thus, DDS that can confer effective immune activation with a smaller dose, and would also be applicable for use via a more conventional route such as subcutaneous administration, would be highly desirable. We are currently attempting to apply the ssPalmE to the development of an RNA vaccine. In a first attempt, I compared the RNA transfection efficiency between the ssPalmE and the ssPalmM.\textsuperscript{38} In this experiment, the ssPalmE and ssPalmM particles were modified with KALA,\textsuperscript{39} a \(\alpha\)-helical cationic peptide that induces cellular uptake by dendritic cells,\textsuperscript{40} and were then complexed with mRNA. These nano-sized complexes were incubated with the mouse bone marrow-derived DCs. As a result, the ex vivo transfection with mRNA using the ssPalmE conferred a significant gene expression accompanied by the induction of cytokine production compared to the ssPalmM (Fig. 4). Therefore, as in the case of DNA vaccine development, the ssPalmE is also a promising material for use as an RNA vaccine. The mechanism underlying this high mRNA transduction (i.e., high mRNA translation activity) and immune-activation (cytokine production) is now being clarified by means of a phosphorylation proteomics analysis. Based

Fig. 4. ssPalmE as a RNA Vaccine Platform

Particles prepared with vitamin E-scaffold ssPalm were modified with dendritic cells (DCs)-targeting peptide (KALA), and then complexed with mRNA. The ssPalmE conferred the effective antigen expression/presentation and cytokine production to dendritic cells in comparison with ssPalmM particle. Thus, the ssPalmE can be a promising RNA vaccine platform. (Color figure can be accessed in the online version.)
on this result, the development of a directly injectable RNA vaccine using a ssPalmE derivative is now underway.

5. OLEIC ACID-SCAFFOLD ssPalm (ssPalmO) FOR AN INTRAVENOUSLY INJECTABLE DDS PLATFORM

As described in section 4, I found that the ssPalmE, when combined with DNA, is highly immune-stimulative. These data per se are quite promising for the development of vaccine technologies. However, such an immune-stimulative molecule is not adequate for the delivery of a nucleic acid via intravenous administration, or topical administration for gene knockdown or protein replacement therapy. Therefore, I attempted to identify a hydrophobic scaffold with less immune-stimulative activity.

As a first process of the development of a ssPalm that is optimized for intravenous administration, the hydrophobic scaffold with an anti-inflammatory property was identified by using a dextran sulfate sodium-induced colitis (DSS colitis) mouse model. The ssPalm can intrinsically form a lipid droplet structure in a large variety of sizes (30–200 nm) depending on the preparation conditions (salt concentration and temperature).41) The intravenous administration of particles in different sizes indicated that an ssPalm particle with a size of approx. 110 nm in average accumulated in colitis tissue to a higher extent compared to either smaller (54 nm, in average) or larger (180 nm, in average) sized particles.42) I thus prepared LNPs with myristic acid (ssPalmM), oleic acid (ssPalmO), and linoleic acid (ssPalmL) with sizes of approximately 110 nm, and then compared the inflammatory reactions of the preparations after their intravenous injection. After the daily administration of a high dose of LNP ssPalm, the reduction in colon length was also significantly shortened by the administration of all three LNP ssPalm compared to the PBS group. Among them, the LNP prepared with the ssPalm with oleic acid (LNP ssPalmO) tended to alleviate the inflammation more efficiently than the LNP ssPalmM and LNP ssPalmL.41) Thus, oleic acid is one of the most potent hydrophobic scaffolds available for ionizable lipids from the point of view of biocompatibility and anti-inflammatory.

The tertiary amine groups of the ssPalmO were then modified to increase the efficiency of endosomal escape of the nucleic acids. As I attempted to improve the ssPalmE (see section 2), the tertiary amine was fixed into a ring structure (a piperazine ring in this study: ssPalmO-Paz4) to stabilize the protonated state of the tertiary amine groups. This structural modification resulted in an enhanced transgene expression of encapsulating mRNA by over 10-fold in the inflammatory colon of DSS-colitis model.41)

Based on this scaffold structure, I additionally inserted a reductive environment-triggered “self-degradable linker” to confer active degradation based on the concept of “Hydrolysis accelerated by the intra-Particle Enrichment of Reactant (HyPER)”26) (Fig. 5). In this concept, the thiol groups produced from the ssPalm attack the phenyl linker in the particle, and then further undergo self-cleavage to accelerate the release of cargos. (Color figure can be accessed in the online version.)

![Fig. 5. Development of 3rd Generation ssPalm (ssPalmO-Ph-P4C2)](image-url)

The reductive environment-triggered degradation of ssPalm was intensified by the insertion of a phenyl ester linker based on the “Hydrolysis accelerated by the intra-Particle Enrichment of Reactant (HyPER).” In this concept, the thiol groups produced from the ssPalm attack the phenyl linker in the particle, and then further undergo self-cleavage to accelerate the release of cargos. (Color figure can be accessed in the online version.)

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MC3-DMA is a key material employed in ONPATTRO®, the 3rd generation ssPalm: ssPalm-Phe-P4C2 conferred a slightly higher gene knockdown activity: 0.004 mg/kg (ssPalm-O-Ph-P4C2) vs. 0.005 mg/kg (DLin-MC3-DMA). This system is also applicable to the hepatic delivery of mRNA. In this case, the ssPalmO-Ph-P4C2 had a higher activity than DLin-MC3-DMA and a commercially available transfection reagent. Furthermore, by the co-delivery of Cas9-encoding mRNA and chemically modified single guide RNA (sgRNA) targeting a transthyretin (TRT) gene, allowed the editing of this targeting genome with an efficiency of 55%, and resulted in a 95% reduction in serum TTR concentration. Therefore, this system represents a good platform for the in vivo administration of siRNA or mRNA via intravenous administration.

6. OTHER APPLICATIONS

In addition to nucleic acids (DNA, siRNA and mRNA), applications of ssPalm are now expanding. This includes the delivery of colloidal nanoparticle (gold nanorods: AuNRs) towards photothermal therapy. The gold nanorods (AuNRs) produce heat in response to near infrared (NIR) irradiation and are an attractive particle for the cancer photothermal therapy. However, since AuNRs are usually prepared by using cetyltrimethylammonium bromide (CTAB), a highly toxic detergent, the removal of CTAB, as well as the further stabilization of the AuNRs is required. For the stabilization of AuNRs, they were encapsulated in the ssPalm LNPs (LNPssPalm/AuNRs). The in vitro photothermal cytotoxicity of the LNPssPalm/AuNRs was further evaluated in 4T1 breast cancer cells. After NIR radiation, the temperature of the medium was increased to approximately 60°C. In parallel, cell viability was decreased by approximately 90%. However, this cytotoxic effect cannot simply be explained by heating. Rather, the intracellular delivery of AuNRs with an aid of ssPalm is a key event for achieving a high degree of cytotoxicity. Thus, delivering AuNR by means of functionalized ssPalm nanoparticles represents a promising approach for inducing NIR-triggered apoptosis to cancer cells.

7. SUMMARY

Collectively, I have succeeded in improving the intrinsic function of the ssPalm by molecular tuning by fixing the tertiary amine in a ring structure (piperidine or piperazine) for the promotion of endosome/lysosome escape properties, and the insertion of a self-degradable linker (a phenyl linker) to enhance the degradability of the material in the cell. I also succeeded in the clear classification of the hydrophobic structure depending on the desired application: immune-stimulative ssPalmE (vitamin E-scaffold) as a DNA/RNA vaccine, and anti-inflammatory ssPalmO (oleic acid-scaffold) for the intravenous delivery of nucleic acids (Fig. 6). The application is also now being expanded to the delivery of low-molecular drugs and colloidal particles. Thus, ssPalm particles can now be considered to be a multi-nanoDDS platform. Given the strong indications that this ssPalm molecule can contribute to the innovation of drug discovery, a series of ssPalm materials has now been commercialized by the NOF CORPORATION (Tokyo, Japan). I hope these materials will be used in a large variety of applications in the pharmaceutical industry.

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