## Chemistry enabling "magic bullet"

Since Paul Ehrlich, the Nobel laureate in physiology or medicine, advocates the concept of "magic bullet" in the early 20th century, the selective delivery of drugs to target sites in the body has long been recognized as one of the most difficult challenges in scientific community. Indeed, many drug formulations composed from a combination of various materials have been developed to solve this issue of drug targeting; however, they were encountered many serious difficulties, such as a lack of longevity in blood circulation, limitations in versatility of loadable drugs, uncontrolled drug release at target sites, and concerns of accumulation toxicity. In the early 1980s, when this situation still continued, I started the challenge to solve these difficulties accompanying with drug targeting with a new approach that leveraged my background as synthetic chemist. As a result, based on the ordered formation of core-shell structured assemblies, named polymeric micelles, from molecularly-engineered amphiphilic block copolymers, novel drug nanocarrier with uniform size (~tens of nm) comparable to viruses was successfully developed (*figure 1*) [1-3]. Nowadays, this polymeric micellar nanocarrier (PMN) has come into clinical use as delivery systems for various anticancer agents [4].

As shown in *figure 1*, the polymeric micellar nanocarrier (PMN) we developed has dense outer shell structure composed of tens to hundreds of tethered polymer chains with hydrophilic and flexible nature, thereby revealing to effectively suppress non-specific interactions with blood components when administered intravenously (stealth function). Meanwhile, the inner core composed of polymer chains with high cohesive forces contributes to the stabilization of micellar structures, and functions as nano-reservoir to stably encapsulate delivering drugs. Furthermore, stimuli-responsive characteristics, including concentration changes in physiologically relevant substances (e.g., pH, glutathione, glucose, and ATP), can be introduced into the core-forming polymer chains. In this way, one can expect the smart functionalities to release or activate encapsulated drugs with desired timing of action in response to subtle microenvironmental changes at the target site [5].
The PMN is also characterized by its superior safety aspects, such as prevention of chronic accumulation toxicity, because after the release of encapsulated drugs the PMN loses the stability and dissociates into constituent block copolymers, which are smoothly excreted outside the body. Further appeal is that the PMN is feasible for directing particular cells and tissues with selectively delivering various agents (active targeting), by attaching target-directed molecules (peptides, antibodies, sugars, etc.) onto the periphery of the outer shell. Worth noting is that I focused from the beginning of my research on the PMN availability for the future clinical application. Thus, I chose hydrophilic and highly biocompatible poly(ethylene glycol) (PEG) as the shell forming component and biodegradable poly(amino acid), prepared by NCA polymerization, as the core forming component [1]. Actually, my career in synthetic polymer chemistry (anionic ring-opening polymerization) contributes greatly to pursue the molecular design of various PEG-poly(amino acid) block copolymers suitable for the PMNs with different biomedical applications.

A series of our developed PMNs loaded with hydrophobic anticancer drugs (paclitaxel, epirubicin) and platinum-complex-based anticancer drugs (cisplatin, dahaplatin) has already been derived to companies, and phase I-III clinical trials for the treatment of various cancers are ongoing in Japan, Asia, Europe, and USA [6]. In parallel with these clinical

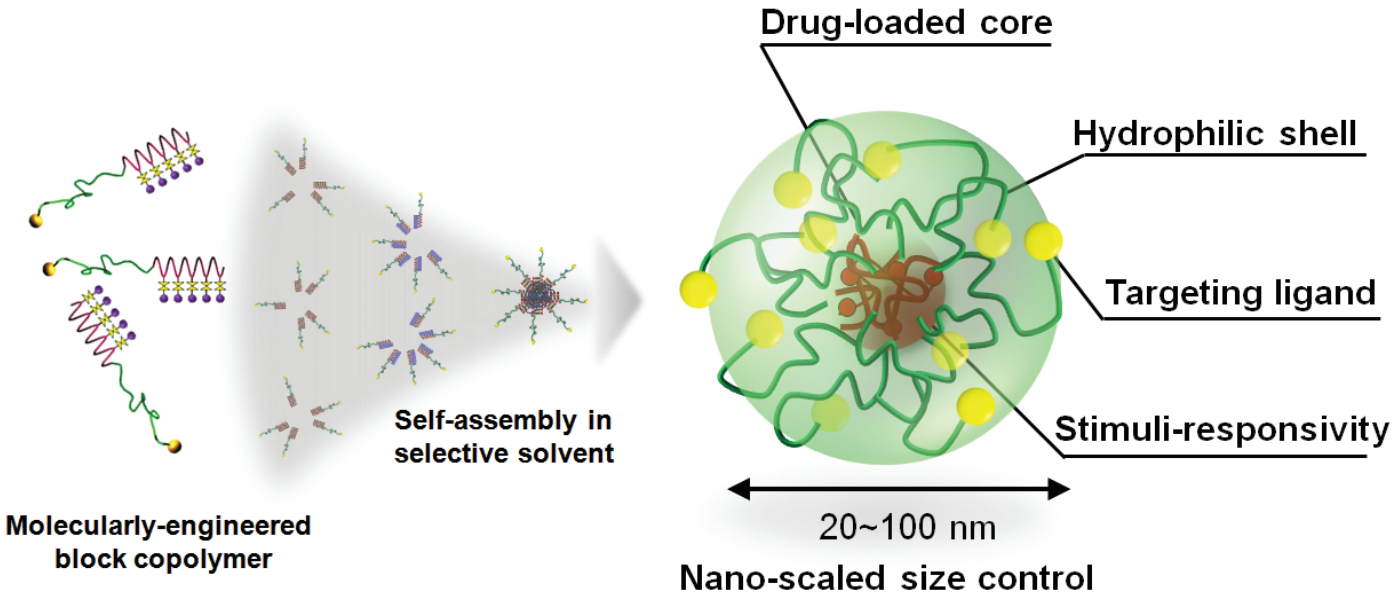


Figure 1 - Polymeric micellar nanocarrier (PMN) self-assembled from molecularly-engineered block copolymers.

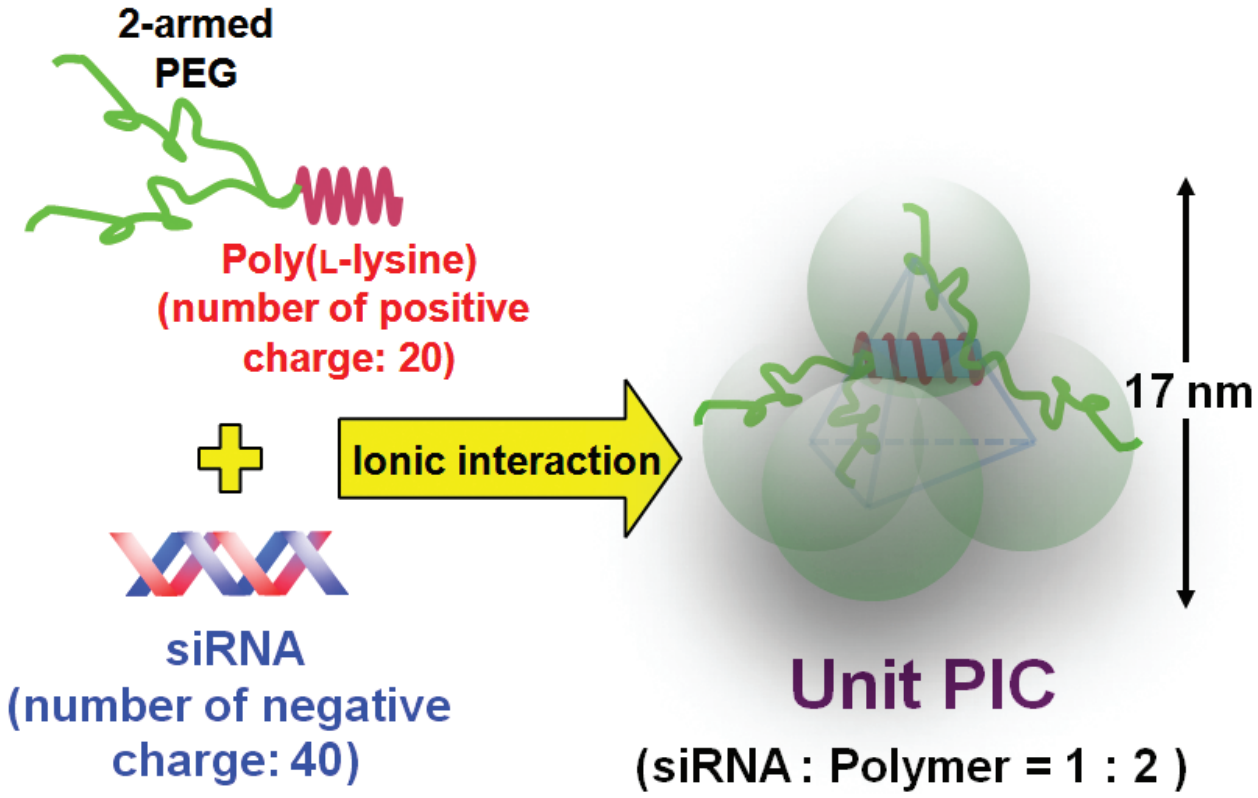


Figure 2 - Formation of unit PIC through charge-matched interaction of siRNA and 2-armed PEG-polycation block copolymer.

developments, I noted that in real clinical cases, the stroma is abundant in many cancers, which constitutes a barrier to the intratumoral penetration of drug-loaded nanocarriers. This fact led me to the idea that the drug could be efficiently penetrated into stromal rich tumors, such as pancreatic cancer, by the strict size control of PMNs. Then, we established the chemical procedure to control the size of PMNs loaded with anticancer drugs in the range of 30-50 nm to circumvent penetration barrier of stroma-rich intractable cancers [7-8]. As this finding is leveraged in the clinical trials of our developed PMNs, as well as being the study to clearly quantify the penetrability of drug-loaded nanocarriers (nanomedicines) in the intratumoral microenvironment, it has received a substantial acknowledgment in the field of cancer nanomedicine. In addition, I have recognized the importance of polymeric micellar-type MRI-contrast agents that can detect local fine-pH changes to predict the tumor malignancy [9]. Worth noting is that these PMN-based MRI-contrast agents are useful to estimate the efficacy of nanomedicines in individual patients who may have variations in their tumor microenvironment, including vascular and stromal permeability. Combination system of drug delivery and imaging functionality is termed "theranostics", and has been recognized as an emerging field with high attention [10].

I also inspired that polymeric micelles could also be formed based on ionic interactions between a pair of oppositely charged polyelectrolytes, given that at least one of the pairs is a block copolymer composed of charged and non-charged hydrophilic segments. Because, in this way, polyion complexed core of the micelles is sealed from outer environment by non-charged hydrophilic shell, thereby avoiding further progressive aggregation of polyion complex to form precipitates. Based on this idea, in 1995, the first examples of monodispersive polymeric micelles were prepared by our group, and are named as polyion complex micelles (PIC micelles) [11]. Worth noting from the standpoint of molecular recognition in this self-assembly process is that a strict chain-length recognition occurs upon the formation of PIC micelles from block copolymer pairs with opposite charges, and when a mixture of block copolymers of different lengths is solubilized in aqueous solution, PIC micelles are selectively formed from pairs of oppositely charged block copolymers having the same length of charged segments [12]. This is the manifestation of a new molecular recognition mechanism based on the requirement of the homogeneous distribution of the charged segments in the micellar core and the distinct phase separation of the outer shell/inner core interface.

Importance of PIC micelles in nanomedicine to enable "magic bullet" is their application to PMNs delivering charged biopolymers such as proteins and nucleic acid pharmaceuticals. We actually revealed this possibility in a series of studies done in the late 1990s to the early 2000s [13-17], and PIC micelles are now widely appreciated as useful nanocarriers in the research field of nanomedicine. More recently, we have established an approach of rigorous particle size control for PIC micelles, and successfully aligned their sizes to the same levels as antibodies (unit PIC) (*figure 2*) [18]. Thanks to their antibody-comparable size, the unit PICs easily reached the deep part of the tumor while repeatedly binding and dissociating with oligonucleotide pharmaceuticals bioorthogonally in the bloodstream, realizing the molecularly targeted treatment of intractable cancers such as malignant glioblastoma and stroma-rich pancreatic cancers. Furthermore, because of the high safety and easy formulation process of unit PIC, it has already reached the production stage satisfying GMP (good manufacturing practice), and clinical trials are planned to be initiated during this fiscal year in Japan.

The research results described above is positioned as a new interdisciplinary field research of nanomedicine based on the fusion of medicine, chemistry, pharmacy and engineering. Drug development is becoming increasingly diverse, including biopharmaceuticals such as antibodies and gene and nucleic acid pharmaceuticals, in addition to traditional small molecule pharmaceuticals, and many of them are required to optimize biodistribution and improve selectivity for target cells and organs. We expect that our initiated PMNs can greatly contribute to the practical application of these new pharmaceuticals due to their versatility in molecular design.


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