Expert Opinion

Self-assembled filamentous nanostructures for drug/gene delivery applications

Jun-hyung Lee, Young Joo Choi & Yong-beom Lim†
†*Yonsei University, Translational Research Center for Protein Function Control, Department of Materials Science and Engineering, Seoul 120-749, Korea*

Importance of the field: Among many nanostructural shapes, filamentous shapes can have unique advantages over the others, including longer persistence in the bloodstream. With the advent of nanotechnology in recent years, a variety of shape-controlled nanostructures has been fabricated. As a wide variety of building blocks can self-assemble into filamentous nanostructures, there are many options available for biomaterial developments with filamentous nanostructures. Similarly to conventional spherical micelles, most filamentous nanostructures have hydrophobic cores where hydrophobic guest molecules can be encapsulated, which enable them to be used in drug delivery applications. Moreover, on suitable molecular design and self-assembly process control, filamentous nanostructures can also be made to deliver nucleic acids or even both drugs and nucleic acids simultaneously.

Areas covered in this review: This review describes the self-assembly process, current developments, and prospects of filamentous nanostructures in drug and gene delivery applications.

What the reader will gain: This review should be helpful in gaining insight into the self-assembly process and nanostructural shape control, the advantages of constructing filamentous nanostructures, and the design of more advanced nanobiomaterials.

Take home message: At present, the development of multifunctional nanostructures is one of the main focuses in nanobiomaterial developments. In this regard, filamentous nanostructures can be good initial targets for tailor-made nanostructure developments because they have more structural variables, as compared with spherical micelles.

Keywords: biomaterials, drug delivery, filamentous nanostructures, gene delivery, nanotechnology, self-assembly

Expert Opin. Drug Deliv. (2010) 7(3):341-351

1. Introduction

Nanometer-sized structures/materials have recently gained intensive research interest owing to their unique and valuable properties, as compared with conventional bulk materials. Nanomaterials can be used in various applications, including biomaterial development. Numerous advantages accompany biomaterials on the nano scale, which typically ranges from tens to hundreds of nanometers. For example, with respect to *in vivo* delivery, some nanometer-sized materials show enhanced permeability and retention (EPR) effect, which may be useful for the selective delivery of nanomaterials to a tumor site [1,2]. Coating nanomaterials with multiple bioactive ligands can be used to take advantage of the multivalent effect, whereby the binding affinity and specificity of biomolecular interactions can be increased dramatically [3-5]. Nanomaterials are in fact similar in size to subcellular organelles (endosomes, lysosomes, mitochondria), biomacromolecules (proteins, ribosomes,

informa
healthcare

10.1517/17425240903559841 © 2010 Informa UK Ltd ISSN 1742-5247

Article highlights.

- Nanostructures, depending on their physical parameters such as shape and size, can display remarkably different biological activities and cytotoxicity profiles.
- Self-assembly is a force balance process in which the most important driving force underlying self-assembly is non-covalent interactions or weak covalent bonds.
- Potential advantages of filamentous structures in biotechnology including their applications in drug and gene delivery.
- Filamentous nanostructures have been shown to be more persistent than spherical ones under *in vivo* flow conditions.
- With recent advances in nanostructural control, trial and error with many different types of nanostructure will provide unprecedented opportunity to develop an ideal drug/gene delivery vehicle.

This box summarises key points contained in the article.

DNAs) and microorganisms (viruses). Although these biological nanostructures can be referred to as 'natural bionanostructures', elaborately designed 'synthetic bionanostructures' can substitute the function of the natural bionanostructures, and can even have enhanced properties or have functions that are unprecedented in nature. One of the most fascinating aspects of the synthetic bionanostructure development is that their numerous advantageous properties can be combined and incorporated into a single nanomaterial to create a multifunctional bionanomaterial.

Nanomaterials can be prepared in two different ways, namely, a top-down approach or a bottom-up approach. In the top-down approach, the final nanostructure is carved from a larger block of matter into the desired shape and order, consequently without atomic-level control. By contrast, in the bottom-up approach, the desired nanostructures can be built from molecular building blocks that self-assemble or self-organize by means of principles of molecular recognition. Indeed, by manipulating the structure of the molecular building blocks, atomic and/or molecular-level control of self-assembled nanostructures is possible. As such, the formation of most natural bionanostructures is driven by a bottom-up self-assembly process, examples of which are found in protein folding, self-assembly of phospholipids into cellular membranes, and the formation of DNA double helices and viral coat proteins.

Many types of synthetic molecule are now being used as building blocks for self-assembly. Block copolymers are one of the most representative examples [6-10]. Self-assembled nanostructures of block copolymers have been used in a variety of applications, including biomaterials developments, nanolithography and photonics. Other examples of building blocks include peptides [4,11-17], DNA [18-22], rod-coils [23-26], foldamers [27-29], dendrimers [30,31], and other types of amphiphilic molecule. Furthermore, hybrid nanostructures composed of the organic soft materials described above and inorganic hard materials have been developed and show new and/or superior properties, as compared with their pure counterparts [32,33].

Nanostructures, depending on their physical parameters such as shape and size, can display remarkably different biological activities. For example, it has been shown that gold nanoparticles of different sizes can elicit size-dependent cellular responses [34]. Likewise, functionalized nanoparticles of different shapes (spherical or cylindrical) have been shown to have distinct cell internalization efficiencies [35,36]. Carbon nanotubes (CNTs) have size-dependent cellular uptake efficiency, in which shorter CNTs are more readily internalized through an energy-independent pathway [37]. It has also been reported that ligand-coated nanostructures have size and shape-dependent binding modes when they interact with their binding partner in a multivalent manner. Such behavior has been demonstrated clearly in carbohydrate–bacterial cell and carbohydrate–virus multivalent interaction systems [38-41].

Apart from their biological activity, nanostructures can display size and shape-dependent cytotoxicity profiles. Recent toxicological studies have shown that the degree of accumulation of rigid multi-walled carbon nanotubes (MWCNT) longer than 20 μm is significantly higher, compared with low-aspect ratio (ratio of length to width) CNTs and tangled nanotube aggregates [42,43]. It has also been suggested that, whereas macrophages can engulf MWCNTs with a low aspect ratio before their clearance by draining lymph vessels, MWCNTs with a high aspect ratio cannot be cleared and accumulate in tissues, where they may promote carcinogenesis. Although gold nanoparticles are known to be fairly non-toxic, it has been reported that gold nanoparticles of very small size show relatively high toxicity [44-47]. In addition, cellular responses to gold nanoparticles appear to be size-dependent.

Together, the evidence described above suggests that the control of physical properties of nanostructures is highly important for their successful utilization in biological systems. In other words, nanostructures designed to have specific shapes and sizes are likely to have advantageous properties, as compared with those that are not, in specific bioapplications. In this review, the benefits of using filamentous nanostructures, such as cylindrical micelles, nanotubes, nanoribbons, nanohelices and nanobelts, in drug and gene delivery applications are introduced. First the basics of self-assembly and nanostructure control are described, then the current status of the field and future prospects of filamentous bionanostructures (**Figure 1**).

2. Control of physical properties in self-assembled nanostructures

2.1 Driving forces for self-assembly: non-covalent interactions

Self-assembly is an autonomous formation of well-defined structures or patterns from building blocks without human intervention. Self-assembly is a force balance process between three classes of force: a driving force that brings self-assembly

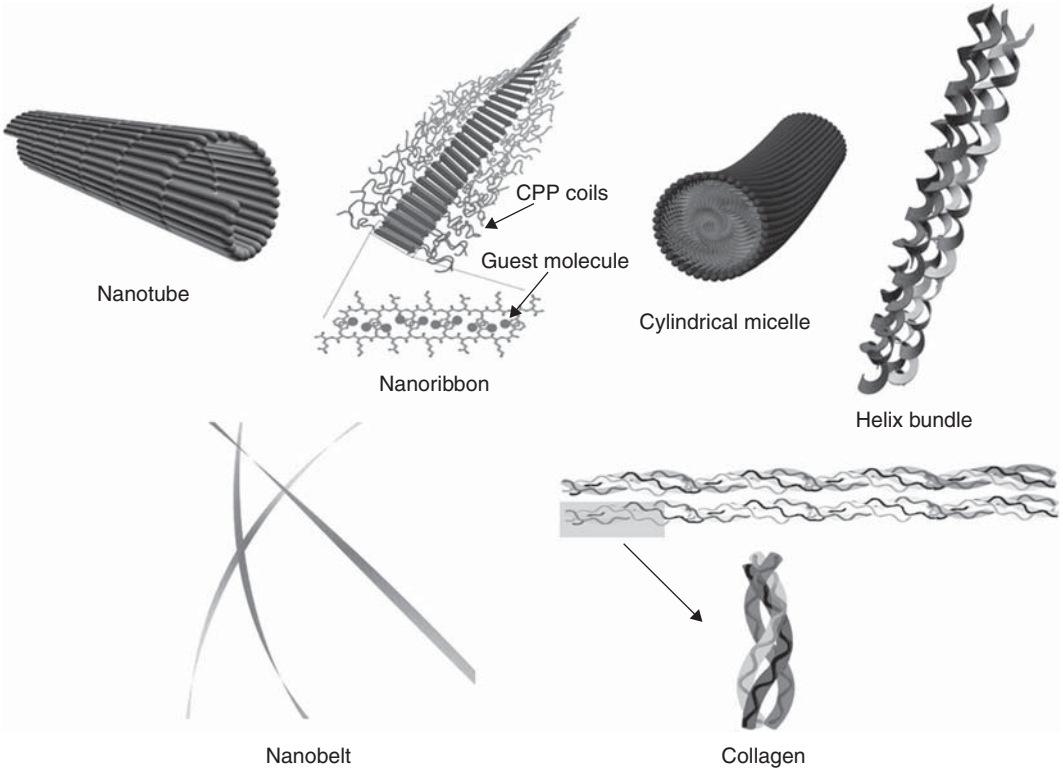

Figure 1. Various types of filamentous nanostructure.
Reproduced with permission from [86].

units together, an opposing force that balances with the driving force, and a functional force that determines the directionality and functionality of self-assembled aggregates [48]. The most important driving force underlying self-assembly is non-covalent interactions or weak covalent bonds, such as van der Waals interactions, the hydrophobic effect, electrostatic interactions, hydrogen bonding and coordination bonding. Non-covalent interactions are weaker and longer than covalent bonds but collectively have a very significant influence on the detailed structures of self-assembled nanostructures. Thus, a full understanding of non-covalent interactions can provide us with the ability to control the physical properties of self-assembled nanostructures such as size and shape.

2.1.1 *van der Waals interaction*

Van der Waals interactions are the attractive or repulsive forces between molecules other than those due to covalent bonds or to the electrostatic interaction of ions. The van der Waals interaction is a dispersion force, of which there are three different types, namely, dipole–dipole, dipole–induced dipole, and induced dipole–induced dipole interactions (London dispersion force). Depending on conditions, van der Waals interactions can be either attractive or repulsive. Therefore, it is important to understand the nature of van der Waals interactions both at the molecular level and in relation to objects ranging from supramolecular to macroscopic in size.

2.1.2 *Hydrophobic effect*

Most of the synthetic building blocks for self-assembly are amphiphiles. For the aggregation of amphiphiles in aqueous solution, hydrophobic interactions are the most important determinants. Many biomolecules such as proteins and lipids are also amphiphilic because they contain both polar and nonpolar regions. The self-assembly of amphiphiles is directed by microphase separation of two dissimilar blocks. When the selective solvent (which is to say a good solvent for one region and a poor solvent for the other region) is water, the nonpolar regions of the amphiphile cluster together to expose the smallest possible hydrophobic area to the aqueous solvent, whereas the polar regions arrange to maximize their interaction with the solvent. The forces that hold the nonpolar regions of the molecules together are called hydrophobic interactions. Hydrophobic interactions are not due to any intrinsic attraction between nonpolar regions, but rather are the result of the system's tendency to achieve the greatest thermodynamic stability by minimizing the number of

ordered water molecules required to surround hydrophobic portions of the amphiphilic molecules.

2.1.3 *Electrostatic interaction*

Forces that originate from electrostatic interactions are another example of common forces that have a strong effect on many self-assembly processes. The electrostatic interaction, which can be either an attractive or a repulsive force, occurs between two charged atoms, ions, or molecules, leading to Coulomb interaction. The Coulomb interaction is a strong (500 – 1000 kJ/mol) and long-range force (up to ~ 50 nm). Perhaps one of the most popular examples of synthetic nanoparticles in which electrostatic interactions play a significant role is that of the cationic molecule–DNA complex system [49-52]. Specifically, when cationic molecules such as cationic polymers or lipids are mixed with negatively charged DNA (or RNA), they form multiple intermolecular electrostatic interactions, resulting in the formation of condensed nanoparticles. When addressing the interactions between charged particles in a liquid medium, it is necessary to consider the total force acting on colloidal objects rather than the simple Coulomb force. In such cases, the famous DLVO (Derjaguin-Landau-Verwey-Overbeek) theory can be used to understand the stability and phase behavior of colloidal dispersions.

In addition to the non-covalent forces described above, other types of non-covalent interaction, such as hydrogen bonding, π-π staking interactions, steric and depletion forces, and solvation and hydration forces can work in concert to control the self-assembly process. Readers interested in more in-depth treatments of these issues are referred to several recent reviews and books [4,26,48,53-59].

2.2 Self-assembly into filamentous nanostructures

Filamentous nanostructures include cylindrical micelles [60,61], nanotubes [62-64], nanoribbons [57,65-68] and nanobelts [69,70]. These nanostructures can be classified into more broad term 'nanofibers' or 'filamentous nanostructures'. To fabricate filamentous nanostructures, it is necessary to control finely the molecular structure of a constituent building block. This section describes the characteristics of the building blocks mostly widely used for filamentous nanostructure formation.

2.2.1 *Filamentous nanostructures from amphiphilic building blocks*

Perhaps one of the best-known examples of amphiphilic building blocks in a biological system is that of phospholipid molecules. Phospholipids are self-assembled largely by hydrophobic interactions to form the cellular membranes. In the context of synthetic molecules, amphiphilic building blocks include block copolymers [6-10], rod-coil molecules [23-26], Gemini surfactants [71] and peptide amphiphiles [60,72,73]. Such amphiphilic molecules contain a region that is polar (and/or charged) and a region that is nonpolar. When amphiphilic building blocks are mixed with water, the polar, hydrophilic regions interact favorably and tend to dissolve in the solvent, whereas the nonpolar, hydrophobic region tends to avoid contact with water. Therefore, the nonpolar regions of the molecules cluster together to minimize exposure of the hydrophobic area to the aqueous solvent, whereas the polar regions arrange to maximize their interaction with the solvent.

In general, one of the most critical factors in determining the morphology or shape of self-assembled nanostructures is the relative volume fraction between the hydrophobic and the hydrophilic segments (packing parameter). A quantitative description of the packing parameter was proposed by Israelachvili by using surfactant molecules as models [74]. The packing parameter can be defined as $P = v/a_0 l_c$, where v is the volume of the hydrocarbon chain of the surfactant monomer in the micelle, a_0 is the area occupied by the head group on the micelle surface and l_c is the apparent length of the hydrocarbon chain of the surfactant monomer in the micelle (**Figure 2**). The packing parameter rule is derived from the picture of the force balance between the attractive driving force between the hydrophobic segments and the repulsive opposition force between the head groups. This concept has had a tremendous impact on the quantitative understanding of the formation and change of surfactant and amphiphilic polymer micelles. According to the packing parameter concept, the typical evolution of morphology by the surfactant self-assembly is as follows: spherical micelle → cylindrical micelle → vesicle → planar bilayer.

2.2.2 *Filamentous nanostructures from β-sheet peptides*

β-Sheet peptides are of such building block with a high propensity to form fibrous nanostructures. In designing β-sheet peptides, a great deal of inspiration can derive from the structures of natural proteins and β-amyloid fibers [75,76]. Polypeptide chains are nearly fully extended in β-sheet structure in which regular hydrogen bonds form between the peptide backbone amide protons and carbonyl oxygen groups of adjacent chains. The adjacent β-strands can lie either a parallel or antiparallel fashion. In both types of β-sheet, the β-strands have conformations pointing alternating amino acid side chains to opposite sides of the sheet. Contributions from electrostatic and hydrophobic forces between amino acid side chains on the same face of the sheet can also stabilize the sheets.

When sheets are composed of two layers of β-strands, it is called as β-sandwich motif in natural proteins [77]. The layers in the sandwich can be aligned either with respect to each other or orthogonally. Conformations of β-strands in artificial β-sheet nanoribbons are similar to those in the β-sandwich. The β-sheet nanoribbons are organized such that each β-strand runs perpendicular to the one-dimensional nanoribbon axis, called a cross-β structure. The design principle for nanoribbons is alternating placement of charged (or polar) and hydrophobic amino acids in β-sheet peptides. This type of arrangement promotes proper β-sheet hydrogen bonding

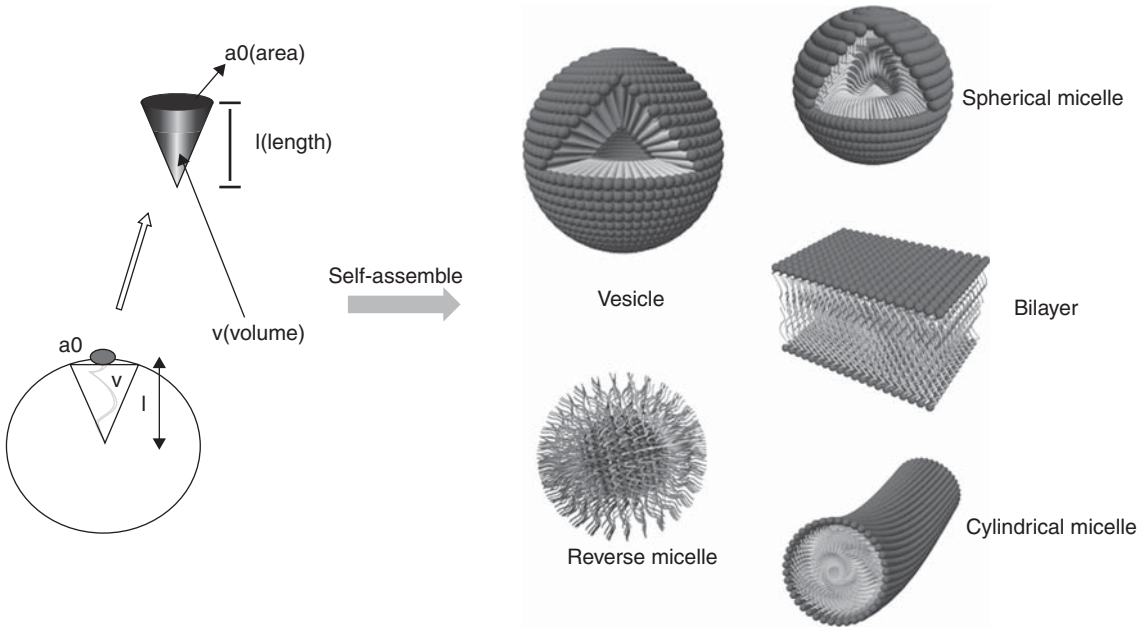

Figure 2. The relationship between the packing parameter and the self-assembled morphology. $p < 1/3$, spherical micelles; $1/3 < p < 1/2$, cylindrical micelles; $1/2 < p < 1$, vesicles; $p = 1$, planar bilayers; $p > 1$, reverse micelles.

arrangement between hydrogen donors and acceptors. When one face of the one-dimensional β-sheet consists predominantly of hydrophobic side chains, the tendency to remove hydrophobic side chains from contact with water drives two one-dimensional sheets to associate into a nanoribbon bilayer structure (**Figure 3**).

In addition to the cylindrical micelles and nanoribbons, other types of filamentous nanostructure such as nanotubes, nanobelts, collagen-type fibers and nanofibers of α-helix bundles can be fabricated with appropriately designed building blocks. An in-depth description of these structures is the beyond the scope of this review, and interested readers are advised to consult related articles on these subjects [62-64,69,70,78-80].

3. Filamentous (rodlike or fibrous) nanostructures for drug and gene delivery

Careful molecular manipulation of supramolecular building blocks can render the shape of nanostructures controllable. Given the abillity to control the nanostructural morphology, it is necessary to consider the potential advantages of filamentous structures in biological applications. Indeed, considering several viruses such as tobacco mosaic virus (TMV), Ebola and influenza A (H5N1), being filamentous in shape probably has some intrinsic merit.

Geng and co-workers recently reported that cylindrical micelles of amphiphilic diblock copolymers, which they termed filomicelles, persist ~ 10 times longer than their spherical counterparts of similar chemistry in the bloodstream [81,82]. To investigate the *in vivo* circulation behavior of such filaments for comparison with spheres of a very similar surface chemistry, they prepared block copolymers possessing the hydrophilic chain of polyethyleneglycol (PEG) and the hydrophobic chain of polyethylethylene or biodegradable polycaprolactone. As blood in circulation is in a state of rapid flow and is constantly sheared, vehicles *in vivo* interact with phagocytic cells under fluid dynamics rather than static conditions. Such *in vivo* effects were directly assessed *in vitro* by steady flow of nanostructures past phagocytes. When small spherical micelles and vesicles contact cells, they adhere and appear to be taken up; however, hydrodynamic shear forces tend to flow-align cylindrical filomicelles and pull them off phagocytes as they come into contact (**Figure 3**). Thus, although a nanofragment of a filomicelle might break off and be taken up by a cell, the strong hydrodynamic force of these long and flexible structures appears easily to overwhelm the cells responsible for filtration and clearance in the body, thereby accounting for the long circulation time of filomicelles. *In vivo* studies have shown that intravenous injection of filomicelles loaded with paclitaxel nearly doubles the maximum tolerated dose of paclitaxel in normal mice compared with paclitaxel-loaded spherical micelles. In tumor-bearing mice, the higher dose of paclitaxel produces greater and more sustained tumor shrinkage and tumor cell apoptosis. Real-time biodistribution studies have revealed that filomicelles are shown to persist in the blood circulation for at least 24 h and to delay the rapid clearance by the mononuclear phagocytic system (MPS) of the liver and spleen that is typical of nanoparticles.

The packing parameter theory has been applied in constructing controlled peptide nanostructures [72]. As peptides

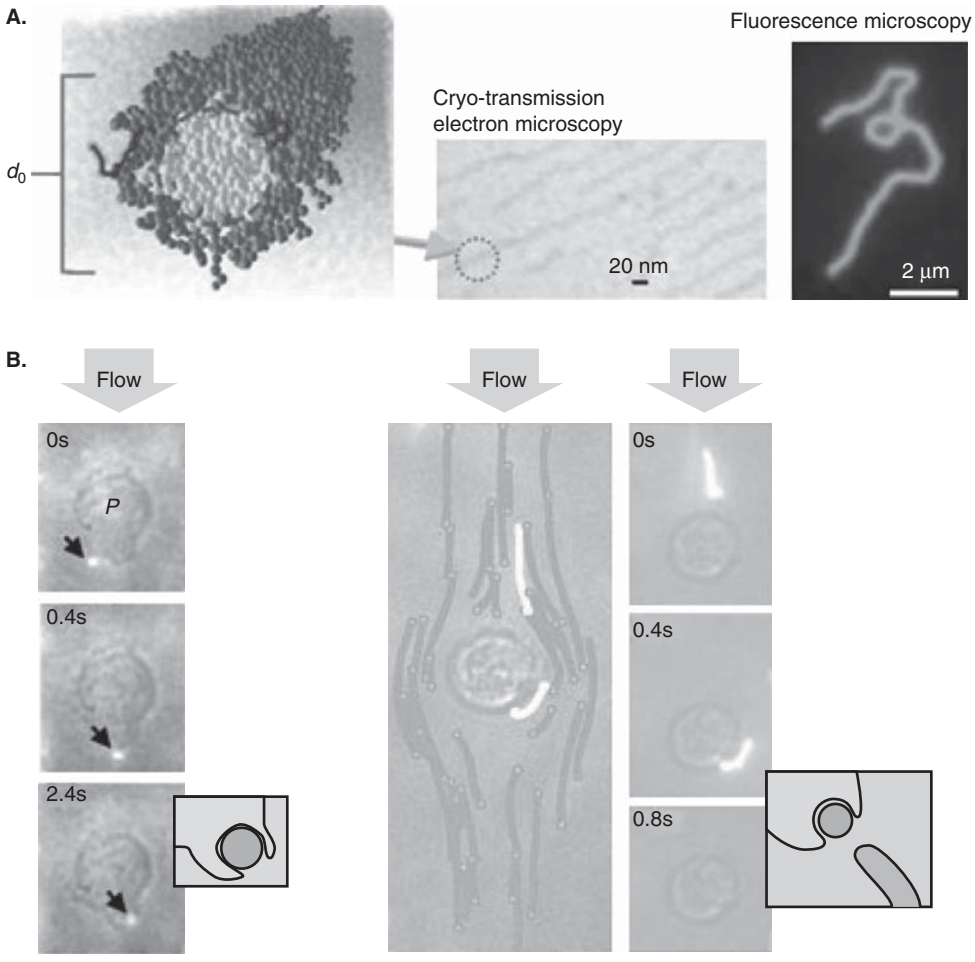

Figure 3. Interaction between filomicelles and phagocytes in flow condition. A. Filomicelles self-assembled from amphiphilic diblock copolymers. Yellow/green in cross-section indicates the hydrophobic segment and orange/blue is the hydrophilic segment. **B.** In a flow chamber with immobilized phagocytes, long filomicelles (right) flow past the cells, and occasionally leave a fragment, but smaller micelles and vesicles are captured (left) by one bite.
Reproduced with permission from [81].

have numerous biological functions, shape-controlled peptide nanostructures, if combined with multiple coatings of bioactive peptides, may provides ample opportunities to explore the myriads of biological events mediated by peptides. To study systematically the effect of the relative volume fraction of hydrophobic blocks in cell-penetrating peptide (CPP) Tat-based amphiphilic peptides, the number and length of fatty acid chains attached to the N terminus of Tat-CPP were dendritically increased from one to eight. Using this strategy, construction of spherical micelles, short and long cylindrical micelles, all decorated multivalently with Tat-CPP, has been successfully demonstrated. Further investigation of the nanostructural properties of the above-mentioned micelles revealed that it is possible not only to control the shape of the peptide-coated nanostructures, but also to control stability by this a molecular manipulation approach. It is notable that the shape of such nanostructures does not follow the packing parameter theory exactly. Therefore, it is likely that the self-assembly behavior of amphiphilic building blocks containing a highly charged hydrophilic segment such as Tat-CPP does not follow the packing parameter theory or the classical volume fraction theory exactly [17]. Indeed, an intracellular delivery experiment following encapsulation of hydrophobic guest molecules showed that cell delivery was highly efficient and cytotoxicity was minimal when nanostructural properties such as shape and stability were appropriately controlled. In the experiment described above, the cargo molecules were delivered to both the nucleus as well as the cytoplasmic compartment. Considering that Tat-CPP has nucleus localization activity [83-85], this result implies the potential to fabricate tailor-made bioactive nanostructures in which their biological activity is dependent on the properties of decorated bioactive peptides.

Recently, Lim *et al.* reported the construction of CPP-decorated nanoribbons for drug delivery [86]. The supramolecular building block, TβP, is a diblock copolypeptide consisting of a CPP Tat [85] (a hydrophilic segment) and a

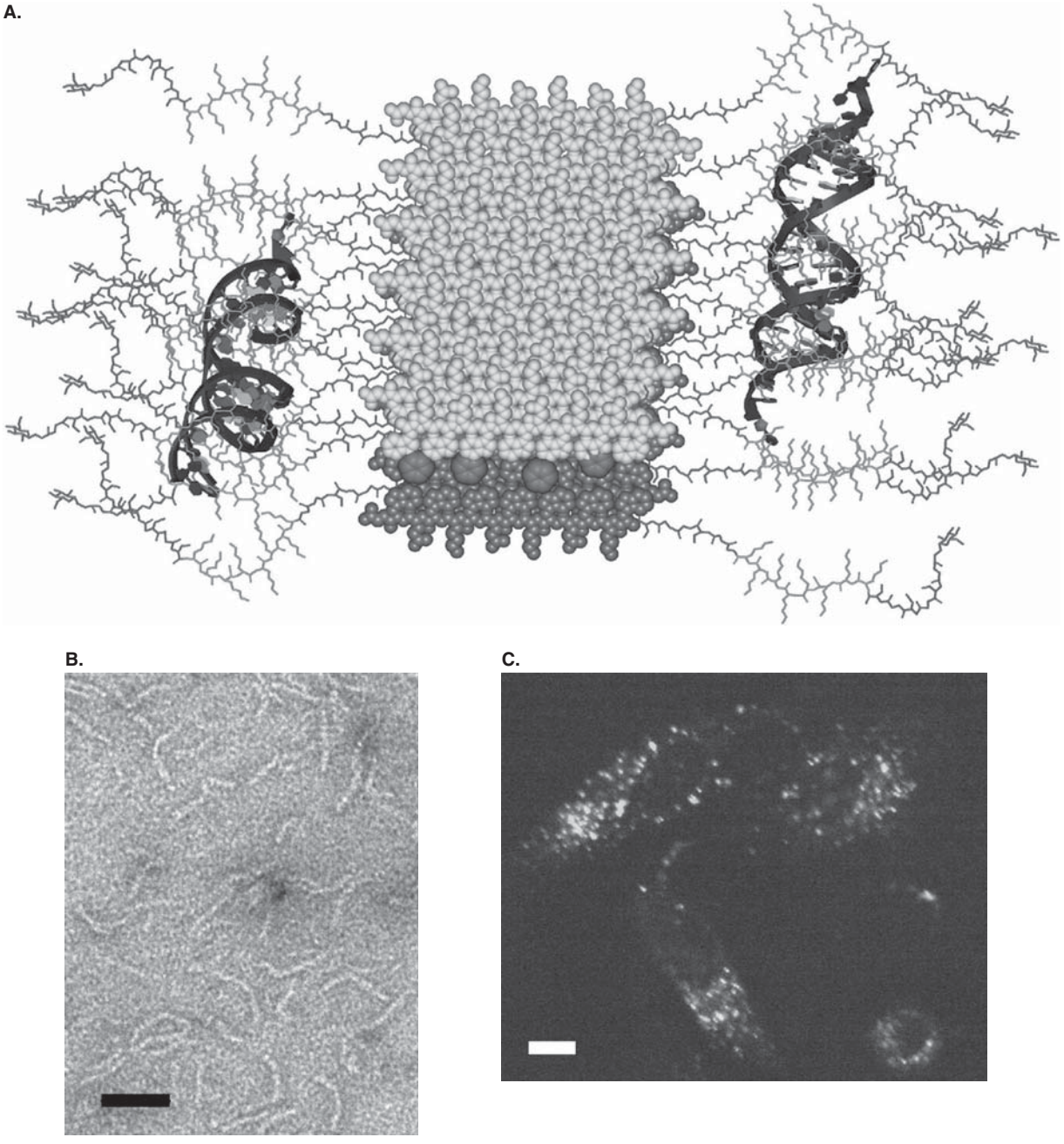

Figure 4. Filamentous artificial virus. A. The filamentous artificial virus encapsulated both with siRNAs and hydrophobic guest molecules. **B.** Transmission electron microscopy image of the filamentous artificial viruses. Scale bar: 100 nm. **C.** Intracellular distribution of filamentous artificial viruses. Scale bar: 50 μm.
Reproduced with permission from [89].

β-sheet-forming peptide (a self-assembling segment). It was found that the diblock copolypeptide formed a filamentous nanoribbon structure in which β-sheet interaction was the main driving force for the self-assembly. The nanoribbons, similar to conventional amphiphilic diblock copolymer micelles, were able to encapsulate hydrophobic guest molecules such as pyrene or Nile red within the hydrophobic space between two β-sheets, thereby demonstrating the feasibility of their use in hydrophobic drug delivery applications. It was also found that the cell penetration and intracellular delivery efficiency of the nanoribbon was much higher than that of unimolecular Tat-CPP, indicating that the multivalent coating of CPPs is advantageous in increasing cellular uptake efficiency.

To explore the possibility of developing a filamentous delivery carrier specific for certain cell types, a β-sheet peptide-based building block was synthesized to contain a cyclic RGD motif as a hydrophilic segment (cRGD-FKE) [87]. The RGD peptide can specifically bind to $\alpha_v\beta_3$ integrin receptor, expressed only on proliferating endothelial cells such as those present in growing tumors [88]. An intracellular delivery experiment revealed that cRGD-FKE filamentous nanoribbon can specifically deliver encapsulated hydrophobic guest molecules to cancer cells. This result suggests that a β-sheet peptide filamentous nanoribbon can be functionalized to become a selective intracellular nanocarrier.

Recently, Lim *et al*. reported the development of a filamentous artificial virus (**Figure 4**) [89]. The building block (Glu-KW) structure was characterized by a β-sheet-forming self-assembly segment, two flexible linker segments, an RNA-binding segment and a carbohydrate glucose segment. The artificial virus was developed as a highly stable circulating gene delivery carrier *in vivo* in order to overcome the problems associated with current gene delivery carriers, which tend to form uncontrollable nanoaggregates [49,50,90-94]. The filamentous artificial virus was highly efficient in simultaneously delivering both siRNA and hydrophobic guest molecules. This capability was attributed to its controlled shape, minimal interaction with serum proteins owing to its charge-neutral surface and enhancement in cell interactions by multivalent decoration of carbohydrate ligands. Considering the current ability to control the morphology of self-assembled nanostructures, this type of approach provides a general means to construct various repertoires of shape-controlled artificial viruses.

4. Conclusions

Filamentous nanostructures have been shown to be more persistent than spherical ones under actual flow conditions. This is a remarkable advantage because most biomedical nanostructures are intended for use *in vivo*. As with the EPR effect, spherical nanostructures of certain sizes have specific preferences for tumor tissue accumulation, as compared with normal tissues. In addition, filamentous nanostructures have more variables than spherical nanostructures. Specifically, the only variable of a spherical nanostructure is its spherical radius. Conversely for filamentous nanostructures, length is the second variable in addition to fiber thickness. As such, transitioning from simple spherical nanostructures to more complex filamentous nanostructures will be complicated, especially under *in vivo* conditions. Therefore, it will not be surprising if more interesting properties of filamentous nanostructures are discovered beyond their high persistence in flow conditions.

5. Expert opinion

As the field of self-assembling nanostructures is becoming more advanced, more complex, elaborate and functional nanostructures are being developed. In terms of nanostructural shapes, there is now a diverse array of different shapes such as spheres, filaments, vesicle, sheets (layers), tubes, rings (toroids), onions, supramolecular helices and various intermediary structures. Depending on the molecular architecture of the building blocks and preparation protocols, even more complex and unique nanostructures such as undulated multicompartment cylinders [95,96] and porous capsules [97] can also be prepared. One of the most exciting self-assembled nanostructures is that of highly controlled DNA nanostructures [98,99]. In addition, hybrid nanostructures of organic and inorganic molecules also show promising potential [100]. Given the variety of choices available for developing self-assembling nanostructures, it is important to identify the best-fit self-assembled nanostructures for specific bioapplications. Nano-sized self-assembled materials are significantly different from ordinary molecules. Indeed, when considering biological use of self-assembled nanomaterials, numerous parameters such as surface functionality, stability, size, shape, stiffness, building block biocompatibility and stimuli responsiveness should be controlled in order to meet the specific requirements of their respective applications.

Ever since the 1966 Hollywood movie 'Fantastic Voyage' in which shrunken doctors in a micro- or nano-sized submarine travelled through the bloodstream of a dying patient, scientists and doctors have dreamed of a real-life Fantastic Voyage. In this vein of thought, the ideal medicine would be a nano-sized medical vehicle small enough to travel through blood vessels with the capacity to fix faulty cells in the body. Viruses have come close to achieving such functionality through evolution. However, most artificial viruses developed so far for drug/gene delivery are far from ideal, producing many problems when injected into the bloodstream. Therefore, in order to develop effective and safe drug/gene vehicles, a quantum leap in the field of self-assembling nanostructures will be necessary.

Traditionally, spherical nanostructures have been the main focus of research for developing intracellular delivery agents. With recent advances in nanostructural control, trial and error with many different types of nanostructure will provide unprecedented opportunity to develop an ideal drug/gene delivery vehicle. At present, we have only limited information with respect to the influence of nanostructural properties on the cell and the body. Filamentous nanostructures, which constitute a large proportion of current nanomaterials, are also a good initial target for developing better versions of delivery agents. As we gain more control over the self-assembly process and acquire new knowledge with respect to nanostructural property/bioactivity relationships, we approach a 'dream delivery nanomachine'.

Acknowledgment

The authors thank the National Research Foundation (NRF) of Korea for support through Grants-in-aid 2009-0066736, 2009-0076516 and 2009-0082617, and the grants from the

Translational Research Center for Protein Function Control, Yonsei University (2009-0092971), Priority Research Center (2009-0093823) and Yonsei University Research Fund (2009-7-0126).

Declaration of interest

This paper does not reflect any financial, commercial, or other relationship between the authors and any other party.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Maeda H, Wu J, Sawa T, et al. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Control Release 2000;65(1-2):271-84
2. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer-chemotherapy - mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res 1986;46(12):6387-92

• **This article provided the foundation for the EPR effect.**

3. Kiessling LL, Gestwicki JE, Strong LE. Synthetic multivalent ligands as probes of signal transduction. Angew Chem Int Ed 2006;45(15):2348-68
4. Lim YB, Moon KS, Lee M. Recent advances in functional supramolecular nanostructures assembled from bioactive building blocks. Chem Soc Rev 2009;38(4):925-34

• **A recent review on bioactive supramolecular nanostructures.**

5. Mammen M, Choi SK, Whitesides GM. Polyvalent interactions in biological systems: Implications for design and use of multivalent ligands and inhibitors. Angew Chem Int Ed 1998;37(20):2755-94

• **An excellent review on multivalent interactions.**

6. Hanson JA, Chang CB, Graves SM, et al. Nanoscale double emulsions stabilized by single-component block copolypeptides. Nature 2008;455(7209):85-8
7. Warren SC, Messina LC, Slaughter LS, et al. Ordered mesoporous materials from metal nanoparticle-block copolymer self-assembly. Science 2008;320(5884):1748-52
8. Schatz C, Louguet S, Le Meins JF, Lecommandoux S. Polysaccharide-block-polypeptide copolymer vesicles: towards synthetic viral capsids. Angew Chem Int Ed Engl 2009;48(14):2572-5
9. Hu J, Liu G, Nijkang G. Hierarchical interfacial assembly of ABC triblock copolymer. J Am Chem Soc 2008;130(11):3236-7
10. Kataoka K, Harada A, Nagasaki Y. Block copolymer micelles for drug delivery: design, characterization and biological significance. Adv Drug Deliv Rev 2001;47(1):113-31
11. Lim YB, Moon KS, Lee M. Stabilization of an alpha helix by beta-sheet-mediated self-assembly of a macrocyclic peptide. Angew Chem Int Ed Engl 2009;48(9):1601-5
12. Hamley IW. Peptide fibrillization. Angew Chem Int Ed Engl 2007;46(43):8128-47
13. Konig HM, Kilbinger AF. Learning from nature: beta-sheet-mimicking copolymers get organized. Angew Chem Int Ed Engl 2007;46(44):8334-40
14. Gazit E. Self-assembled peptide nanostructures: the design of molecular building blocks and their technological utilization. Chem Soc Rev 2007;36(8):1263-9
15. Klok HA. Protein-inspired materials: synthetic concepts and potential applications. Angew Chem Int Ed Engl 2002;41(9):1509-13
16. Ulijn RV, Smith AM. Designing peptide based nanomaterials. Chem Soc Rev 2008;37(4):664-75
17. Yoon YR, Lim YB, Lee E, Lee M. Self-assembly of a peptide rod-coil: a polyproline rod and a cell-penetrating peptide Tat coil. Chem Commun (Camb) 2008;(16):1892-4
18. Sharma J, Chhabra R, Cheng A, et al. Control of self-assembly of DNA tubules through integration of gold nanoparticles. Science 2009;323(5910):112-6
19. Aldaye FA, Palmer AL, Sleiman HF. Assembling materials with DNA as the guide. Science 2008;321(5897):1795-9
20. Andersen ES, Dong M, Nielsen MM, et al. Self-assembly of a nanoscale DNA box with a controllable lid. Nature 2009;459(7243):73-U5
21. Douglas SM, Dietz H, Liedl T, et al. Self-assembly of DNA into nanoscale three-dimensional shapes. Nature 2009;459(7245):414-8
22. He Y, Ye T, Su M, et al. Hierarchical self-assembly of DNA into symmetric supramolecular polyhedra. Nature 2008;452(7184):198-U41
23. Lee M, Cho BK, Zin WC. Supramolecular structures from rod-coil block copolymers. Chem Rev 2001;101(12):3869-92
24. Ryu JH, Lee E, Lim YB, Lee M. Carbohydrate-coated supramolecular structures: transformation of nanofibers into spherical micelles triggered by guest encapsulation. J Am Chem Soc 2007;129(15):4808-14
25. Kim HJ, Lim YB, Lee M. Self-assembly of supramolecular polymers into tunable helical structures. J Polym Sci A Polym Chem 2008;46(6):1925-35
26. Lim YB, Moon KS, Lee M. Rod-coil block molecules: their aqueous self-assembly and biomaterials applications. J Mater Chem 2008;18(25):2909-18
27. Gellman SH. Foldamers: a manifesto. Acc Chem Res 1998;31(4):173-80
28. Hill DJ, Mio MJ, Prince RB, et al. A field guide to foldamers. Chem Rev 2001;101(12):3893-4011
29. Gillies ER, Deiss F, Staedel C, et al. Development and biological assessment of fully water-soluble helical aromatic amide foldamers. Angew Chem Int Ed 2007;46(22):4081-4
30. Thoma G, Katopodis AG, Voelcker N, et al. Novel glycodendrimers self-assemble to nanoparticles which function as polyvalent ligands in vitro and in vivo. Angew Chem Int Ed 2002;41(17):3195-98
31. Percec V, Dulcey AE, Balagurusamy VSK, et al. Self-assembly of amphiphilic dendritic dipeptides into helical pores. Nature 2004;430(7001):764-8
32. Sanchez C, Julian B, Belleville P, Popall M. Applications of hybrid organic-inorganic nanocomposites. J Mater Chem 2005;15(35-36):3559-92

33. Yiu HHP, Wright PA. Enzymes supported on ordered mesoporous solids: a special case of an inorganic-organic hybrid. J Mater Chem 2005;15(35-36):3690-700

34. Jiang W, Kim BYS, Rutka JT, Chan WCW. Nanoparticle-mediated cellular response is size-dependent. Nat Nanotechnol 2008;3(3):145-50

35. Zhang K, Fang HF, Chen ZY, et al. Shape effects of nanoparticles conjugated with cell-penetrating peptides (HIV Tat PTD) on CHO cell uptake. Bioconjug Chem 2008;19(9):1880-7

36. Perrault SD, Walkey C, Jennings T, et al. Mediating tumor targeting efficiency of nanoparticles through design. Nano Lett 2009;9(5):1909-15

37. Raffa V, Ciofani G, Nitodas S, et al. Can the properties of carbon nanotubes influence their internalization by living cells? Carbon 2008;46(12):1600-10

38. Lim YB, Park S, Lee E, et al. Glycoconjugate nanoribbons from the self-assembly of carbohydrate-peptide block molecules for controllable bacterial cell cluster formation. Biomacromolecules 2007;8(5):1404-8

39. Wang HF, Gu LR, Lin Y, et al. Unique aggregation of anthrax (Bacillus anthracis) spores by sugar-coated single-walled carbon nanotubes. J Am Chem Soc 2006;128(41):13364-5

40. Kim BS, Hong DJ, Bae J, Lee M. Controlled self-assembly of carbohydrate conjugate rod-coil amphiphiles for supramolecular multivalent ligands. J Am Chem Soc 2005;127(46):16333-7

41. Tuzikov AB, Chinarev AA, Gambaryan AS, et al. Polyglycine II nanosheets: supramolecular antivirals? Chembiochem 2003;4(2-3):147-54

42. Kostarelos K. The long and short of carbon nanotube toxicity. Nat Biotechnol 2008;26(7):774-6

43. Poland CA, Duffin R, Kinloch I, et al. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat Nanotechnol 2008;3(7):423-8

44. Connor EE, Mwamuka J, Gole A, et al. Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity. Small 2005;1(3):325-7

45. Goodman CM, McCusker CD, Yilmaz T, Rotello VM. Toxicity of gold nanoparticles functionalized with cationic and anionic side chains. Bioconjug Chem 2004;15(4):897-900

46. Pernodet N, Fang XH, Sun Y, et al. Adverse effects of citrate/gold nanoparticles on human dermal fibroblasts. Small 2006;2(6):766-73

47. Pan Y, Neuss S, Leifert A, et al. Size-dependent cytotoxicity of gold nanoparticles. Small 2007;3(11):1941-9

48. Lee YS. Self-assembly and nanotechnology - a force balance approach. Wiley, New York; 2008

49. Pack DW, Hoffman AS, Pun S, Stayton PS. Design and development of polymers for gene delivery. Nat Rev Drug Discov 2005;4(7):581-93

50. Mastrobattista E, van der Aa MAEM, Hennink WE, Crommelin DJA. Artificial viruses: a nanotechnological approach to gene delivery. Nat Rev Drug Discov 2006;5(2):115-21

51. Han M, Bae Y, Nishiyama N, et al. Transfection study using multicellular tumor spheroids for screening non-viral polymeric gene vectors with low cytotoxicity and high transfection efficiencies. J Control Release 2007;121(1-2):38-48

52. Xu PS, Li SY, Li Q, et al. Virion-minnicking nanocapsules from pH-controlled hierarchical self-assembly for gene delivery. Angew Chem Int Ed 2008;47(7):1260-4

53. Goodsell DS. Bionanotechnology-lessons from nature. WILEY-LISS, New Jersey; 2004

54. Gazit E. Plenty of room for biology at the bottom-An introduction to bionanotechnology. Imperial College Press, London; 2007

55. Nesloney CL, Kelly JW. Progress towards understanding beta-sheet structure. Bioorg Med Chem 1996;4(6):739-66

56. Konig HM, Kilbinger AFM. Learning from nature: beta-sheet-mimicking copolymers get organized. Angew Chem Int Ed 2007;46(44):8334-40

57. Lim YB, Lee M. Nanostructures of beta-sheet peptides: steps towards bioactive functional materials. J Mater Chem 2008;18(7):723-7

58. Brunsveld L, Folmer BJB, Meijer EW, Sijbesma RP. Supramolecular polymers. Chem Rev 2001;101(12):4071-97

59. Shimizu T, Masuda M, Minamikawa H. Supramolecular nanotube architectures based on amphiphilic molecules. Chem Rev 2005;105(4):1401-43

60. Hartgerink JD, Beniash E, Stupp SI. Self-assembly and mineralization of peptide-amphiphile nanofibers. Science 2001;294(5547):1684-8

• **An exploration of cylindrical micelles of peptides in biomineralization.**

61. Wang X, Guerin G, Wang H, et al. Cylindrical block copolymer micelles and co-micelles of controlled length and architecture. Science 2007;317(5838):644-7

62. Ghadiri MR, Granja JR, Milligan RA, et al. Self-assembling organic nanotubes based on a cyclic peptide architecture. Nature 1993;366(6453):324-7

• **A rationally designed peptide nanotube.**

63. Chen X, Tam UC, Czlapinski JL, et al. Interfacing carbon nanotubes with living cells. J Am Chem Soc 2006;128(19):6292-3

64. Oda R, Artzner F, Laguerre M, Huc I. Molecular structure of self-assembled chiral nanoribbons and nanotubules revealed in the hydrated state. J Am Chem Soc 2008;130(44):14705-12

65. Aggeli A, Nyrkova IA, Bell M, et al. Hierarchical self-assembly of chiral rod-like molecules as a model for peptide beta -sheet tapes, ribbons, fibrils, and fibers. Proc Natl Acad Sci USA 2001;98(21):11857-62

66. Zhang S, Marini DM, Hwang W, Santoso S. Design of nanostructured biological materials through self-assembly of peptides and proteins. Curr Opin Chem Biol 2002;6(6):865-71

67. Lim YB, Park S, Lee E, et al. Glycoconjugate nanoribbons from the self-assembly of carbohydrate-peptide block molecules for controllable bacterial cell cluster formation. Biomacromolecules 2007;8(5):1404-8

68. Lim YB, Park S, Lee E, et al. Tunable bacterial agglutination and motility inhibition by self-assembled glyco-nanoribbons. Chem Asian J 2007;2(11):1363-9

69. Shi HT, Qi LM, Ma JM, Wu NZ. Architectural control of hierarchical nanobelt superstructures in catanionic reverse micelles. Adv Funct Mater 2005;15(3):442-50

70. Cui H, Muraoka T, Cheetham AG, Stupp SI. Self-assembly of giant peptide nanobelts. Nano Lett 2009;9(3):945-51

71. Menger FM, Keiper JS. Gemini surfactants. Angew Chem Int Ed Engl 2000;39(11):1906-20

72. Lim YB, Lee E, Lee M. Controlled bioactive nanostructures from self-assembly of peptide building blocks. Angew Chem Int Ed 2007;46(47):9011-4

73. Moon KS, Lee E, Lim YB, Lee M. Bioactive molecular sheets from self-assembly of polymerizable peptides. Chem Commun (Camb) 2008;(34):4001-3

74. Israelachvili JN. Intermolecular & surface forces. Academic Press, New York; 1992

75. Binder WH, Smrzka OW. Self-assembly of fibers and fibrils. Angew Chem Int Ed Engl 2006;45(44):7324-8

76. Mimna R, Camus MS, Schmid A, et al. Disruption of amyloid-derived peptide assemblies through the controlled induction of a beta-sheet to alpha-helix transformation: application of the switch concept. Angew Chem Int Ed Engl 2007;46(15):2681-4

77. Nesloney CL, Kelly JW. Progress towards understanding beta-sheet structure. Bioorg Med Chem 1996;4(6):739-66

78. Gore T, Dori Y, Talmon Y, et al. Self-assembly of model collagen peptide amphiphiles. Langmuir 2001;17(17):5352-60

79. Papapostolou D, Bromley EH, Bano C, Woolfson DN. Electrostatic control of thickness and stiffness in a designed protein fiber. J Am Chem Soc 2008;130(15):5124-30

80. Strzalka J, Xu T, Tronin A, et al. Structural studies of amphiphilic 4-helix bundle peptides incorporating designed extended chromophores for nonlinear optical biomolecular materials. Nano Lett 2006;6(11):2395-405

81. Geng Y, Dalhaimer P, Cai SS, et al. Shape effects of filaments versus spherical particles in flow and drug delivery. Nat Nanotechnol 2007;2(4):249-55

- **This showed longer persistence of filamentous nanostructures in flow condition.**

82. Christian DA, Cai S, Garbuzenko OB, et al. Flexible filaments for in vivo imaging and delivery: persistent circulation of filomicelles opens the dosage window for sustained tumor shrinkage. Mol Pharm 2009;6(5):1343-52

83. Frankel AD, Pabo CO. Cellular uptake of the tat protein from human immunodeficiency virus. Cell 1988;55(6):1189-93

84. Schwarze SR, Hruska KA, Dowdy SF. Protein transduction: unrestricted delivery into all cells? Trends Cell Biol 2000;10(7):290-5

85. Futaki S. Membrane-permeable arginine-rich peptides and the translocation mechanisms. Adv Drug Deliv Rev 2005;57(4):547-58

86. Lim YB, Lee E, Lee M. Cell-penetrating-peptide-coated nanoribbons for intracellular nanocarriers. Angew Chem Int Ed Engl 2007;46(19):3475-8

87. Lim YB, Kwon OJ, Lee E, et al. A cyclic RGD-coated peptide nanoribbon as a selective intracellular nanocarrier. Org Biomol Chem 2008;6(11):1944-8

88. Janssen ML, Oyen WJ, Dijkgraaf I, et al. Tumor targeting with radiolabeled alpha (v)beta(3) integrin binding peptides in a nude mouse model. Cancer Res 2002;62(21):6146-51

89. Lim YB, Lee E, Yoon YR, et al. Filamentous artificial virus from a self-assembled discrete nanoribbon. Angew Chem Int Ed Engl 2008;47(24):4525-8

- **The first example of filamentous artificial virus development.**

90. Aoyama Y, Kanamori T, Nakai T, et al. Artificial viruses and their application to gene delivery. Size-controlled gene coating with glycocluster nanoparticles. J Am Chem Soc 2003;125(12):3455-7

91. Lim YB, Choi YH, Park JS. A self-destroying polycationic polymer: Biodegradable poly(4-hydroxy-L-proline ester). J Am Chem Soc 1999;121(24):5633-9

92. Lim YB, Kim CH, Kim K, et al. Development of a safe gene delivery system using biodegradable polymer, poly [alpha-(4-aminobutyl)-L-glycolic acid]. J Am Chem Soc 2000;122(27):6524-5

93. Lim YB, Kim SM, Lee Y, et al. Cationic hyperbranched poly(amino ester): a novel class of DNA condensing molecule with cationic surface, biodegradable three-dimensional structure, and tertiary amine groups in the interior. J Am Chem Soc 2001;123(10):2460-1

94. Lim YB, Kim SM, Suh H, Park JS. Biodegradable, endosome disruptive, and cationic network-type polymer as a highly efficient and nontoxic gene delivery carrier. Bioconjug Chem 2002;13(5):952-7

95. Cui H, Chen Z, Zhong S, et al. Block copolymer assembly via kinetic control. Science 2007;317(5838):647-50

96. Fang B, Walther A, Wolf A, et al. Undulated multicompartment cylinders by the controlled and directed stacking of polymer micelles with a compartmentalized corona. Angew Chem Int Ed Engl 2009;48(16):2877-80

97. Kim JK, Lee E, Lim YB, Lee M. Supramolecular capsules with gated pores from an amphiphilic rod assembly. Angew Chem Int Ed Engl 2008;47(25):4662-6

98. He Y, Ye T, Su M, et al. Hierarchical self-assembly of DNA into symmetric supramolecular polyhedra. Nature 2008;452(7184):198-201

99. Douglas SM, Dietz H, Liedl T, et al. Self-assembly of DNA into nanoscale three-dimensional shapes. Nature 2009;459(7245):414-8

100. Rosi NL, Mirkin CA. Nanostructures in biodiagnostics. Chem Rev 2005;105(4):1547-62

Affiliation

Jun-hyung Lee[1], Young Joo Choi[2] & Yong-beom Lim[†1] PhD
[†]Author for correspondence
[1]Yonsei University,
Translational Research Center for Protein Function Control,
Department of Materials Science and Engineering,
Seoul 120-749, Korea
Tel: +82 2 2123 5836; Fax: +82 2 365 5882;
E-mail: yblim@yonsei.ac.kr
[2]Seoul National University,
Institute of Health and Environment,
School of Public Health,
Seoul 110-460, Korea