The design of delivery vehicles for transporting anticancer drugs to tumor sites has gained traction during the past few decades. Utilizing polymer-based materials has played an important role in the development of such systems, largely because of the ability to prepare polymers with tailored properties, including biocompatibility, size, structure, and functionality. Several polymer-based vehicles have been reported, including polymer particles, polymer-based micelles, polymer-drug conjugates, and polymer nanocapsules. These systems can facilitate higher payloads, prolong the circulation time of the drugs, improve drug targeting and solubility, and provide controlled-release of the therapeutics into the blood stream or the targeted tumor tissues. Among these, the polymer capsules are particularly attractive candidates for drug delivery applications.

"Layer-by-layer (LbL) assembly processes have been widely used by our group and others to prepare polymer capsules with well-defined chemical and structural properties. In LbL assembly, a nonporous sacrificial colloidal template is generally used to sequentially deposit multiple polymer layers one after another, followed by removal of the core, leading to well-defined polymer capsules with nanometer-thick walls”, Prof. Frank Caruso, Director of the Centre for Nanoscience and Nanotechnology at the University of Melbourne, Australia, explains to Nanospotlight. "Multiple assembly steps required in the LbL assembly often require the use of more than one polymer and can make the process relatively intensive and time-consuming, particularly for the synthesis of thick walled polymer capsules.”

To overcome these limitations, Prof. Caruso’s team used a novel silica particle template with a solid core and mesoporous shell (SC/MS) for polymer nanocapsules synthesis. Prof. Caruso further explains to Nanospotlight: “The use of SC/MS template allows a ‘single polymer’ to be infiltrated in the mesoporous shells of SC/MS particles in a ‘single macromolecular assembly step’ by solution adsorption, followed by cross-linking of the macromolecules in the mesoporous silica shells, and subsequent removal of the SC/MS templates, thus leading to monodispersed, single-component and thick-walled polymer nanocapsules (see Scheme 1).”

“This approach offers a number of advantages over the conventional LbL technique to prepare capsules. Firstly, uniform nanocapsules of various macromolecules are obtained by a single macromolecular assembly step of a single macromolecule type, eliminating the need for multiple polymers and/or multiple polymer adsorption steps. Secondly, the nanocapsules derived from the SC/MS templates have porous walls that are significantly thicker than those prepared by LbL assembly (e.g., more than an order of magnitude for a single adsorption step), thus offering a simple approach to regulate the physical properties (e.g., structure, permeability, payloads) of the nanocapsules.”

The SC/MS particles can be prepared with different particle size, shell thickness, and solid core composition (e.g., silica, gold and Fe₃O₄ nanoparticles). The size and thickness of the nanocapsules can be controlled by choosing the appropriate size SC/MS template and type and molecular weight of the polymers. For instance, the thickness of the capsule shells increases as the molecular weight of the PAH decreases because of more efficient adsorption of smaller species of PAH in the mesoporous shells of SC/MS templates (~45 nm and ~16 nm thick capsules with a diameter of ~400 nm size were obtained for PAH of 5 and 70 kDa, respectively). Furthermore, the macromolecules assembled in the capsules can be stabilized via engineered cleavable covalent linker (e.g.,
disulfide), which would add tunable stability and degradability characteristics to the capsules, leading to another level of control over the release properties of encapsulated substances.

The researchers have recently published their findings in *Nano Letters* (Wang et al., 2008, 8, 1741–1745) and demonstrated the general applicability of this approach by preparing nanocapsules using various macromolecules, including synthetic polyelectrolytes [polyallylamine hydrochloride (PAH)], polypeptides [poly-l-lysine (PLL), and poly-l-glutamic acid (PGA)], and polypeptide-drug conjugates [PGA-Doxorubicin (Dox)].

The researchers also investigated the applicability of thick-walled polymer nanocapsules for tumor therapy via drug delivery. They prepared drug-loaded polymer nanocapsules according to the outlined approach by preconjugating a model anticancer drug (Dox) to a model polymer system (PGA), which is structurally related to natural proteins and is generally considered to be biocompatible, nonimmunogenic and biodegradable. The potential of Dox-loaded PGA nanocapsules in tumor therapy applications was demonstrated via in vitro capsule degradation and Dox-release studies at conditions resembling those within the living cells, nanocapsule uptake by LIM1215 human colorectal tumor cells, and delivery of the anticancer drug into the tumor cells, leading to tumor cell death.

Bansal notes that it is highly desirable for antitumor applications, that the size of the delivery vehicle is in the range capable of exploiting the ‘leaky’ nature of tumor blood vessels, which have pore diameters of between 400 and 600 nm, allowing accessibility to target tumor cells. In this study, sub-500 nm size capsules were used for this purpose. PGA-Dox nanocapsules were internalized in large numbers by LIM1215 colorectal tumor cells, with most of the internalized capsules being taken up by the lysosomes. The uptake of the PGA-Dox particles and capsules by subcellular lysosomes suggests that once internalized, hydrolytic enzymes present in the reducing environment of the lysosomes would facilitate Dox release from nanocapsules, thus causing tumor cell death.

Drug-release studies confirmed that the Dox was gradually released from PGA-Dox capsules under lysosomal conditions (pH 5.8/10 mM carboxypeptidase) with a near-linear drug release kinetics over the first 24 h. “Moreover, for a drug delivery vehicle to be highly effective, it is desirable that it should not degrade in the blood stream; however, it should be easily degraded and release its cargo after reaching the lysosomal compartments of the tumor cells”, notes Bansal: “Our control experiments showed negligible passive release of Dox from nanocapsules under physiological conditions in the absence of lysosomal hydrolases.”

The tumor cell death studies on LIM1215 human colorectal tumor cells showed that the PGA-Dox capsules were highly effective in controlling tumor cell growth (>85% cell death within 16 h). When LIM1215 tumor cells were treated with equivalent amounts of PGA-Dox polymer conjugates, insignificant tumor cell death was observed. The researchers speculate that the high negative charge of the small PGA-Dox polymer chains restricts their uptake by the negatively charged cell membranes and hence leads to reduced cell death. However, PGA-Dox loaded SCMS particles and PGA-Dox capsules can be internalized into the tumor cells via endocytosis due to their larger sizes, thus highlighting the important role that polymer capsules might play in drug delivery applications.

The researchers highlight that although free Dox was found to be as efficient as PGA-Dox capsules in causing tumor cell death, Dox is known to cause high systemic toxicity when administered into animals in its free form. The PGA-Dox capsules can provide an added advantage of controlled release, wherein Dox molecules will be released only after capsules reach the target tumor site, thus minimizing any systemic toxicity. Moreover, significantly higher amounts and more than one type of drug can be principally loaded in PGA capsules in a controlled manner, due to the presence of a large number of free –COOH groups. In addition, the remaining free –COOH groups of PGA-Dox capsules can be easily conjugated to targeting moieties to target PGA-Dox capsules to various tumors, which is the subject of further investigation.
PGA-Dox capsules shown in this study provide a unique drug delivery system: they remain stable at physiological pH and are amenable to deconstruction (by disassembly of PGA-Dox chains due to lysosomal reducing environments) and degradation (by lysosomal hydrolases) in response to chemical stimuli within living cells, thereby delivering Dox to LIM1215 human colorectal tumor cells and causing tumor cell death. The attachment of targeting ligands to the drug-conjugated capsules through established coupling protocols will further provide functional capsules for targeted drug delivery applications.

Overall, the simple, efficient, and general nature of the approach for the fabrication of a new class of monodispersed, single-component and thick-walled polymer nanocapsules, coupled with the capability to synthesize a wide range of materials with tunable properties, and the additional ability to post-functionalize the thick capsule shells, provides exciting new opportunities for designing advanced capsules for use in a range of therapeutic and diagnostic applications.

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