2C) with linear growth rates of <80 μm/s. In the experimental process, the sample holder in the reaction chamber can be moved in any space direction (Fig. 2C). New material is always deposited on the fiber tip. Almost any three-dimensional structure consisting of fiber segments can be made, such as that shown in Fig. 1. The electrical properties of a structure can then be changed by metallization.

The commercial potential of endless LCVD fibers, for prototyping versus fabricating, is best illustrated with an example (6) from the aerospace composites community. New inorganic reinforcing fibers, such as hafnium boride or tantalum carbide, are needed to perform satisfactorily for longer periods of time at much higher in-use temperatures than possible with incumbent silicon carbide or sapphire fibers. However, a very costly and time-consuming development would be required to merely fabricate a small amount of a suitable test specimen by known processes. In contrast, LCVD promises to yield suitable prototype samples quickly and with minimum cost. Once a prototype fiber is identified, it can be commercially developed on the basis of cost and performance by way of LCVD or an existing commercial process.

The issues are the same with regard to microsprings (coiled fibers) and highly complex microstructures (Fig. 1). First of all, no other suitable technology is currently available that facilitates the fabrication of strong microsprings. If a specific need arises (7, 8), LCVD will be the preferred choice. In addition, several methods, including lithography techniques and the formation of microscopic molds, are available for the fabrication of three-dimensional microstructures, but they are derived from planar processes. Thus, structural variations in the direction perpendicular to the direction must be achieved in multiple, time-consuming steps (1, 9). Also, these methods require the production of photomasks before processing. Finally, rapid prototyping processes based on fast chemical reactions are industrially available but only with submillimeter resolution (1). In contrast, LCVD facilitates rapid prototyping with micrometer resolution.

Laser-assisted chemical vapor deposition is on the threshold of commercial exploitation. Fibers fabricated by LCVD are already commercially accessible (5), and commercial uses can be envisioned for simple and complex LCVD microstructures. Some of these structures are still seeking a powerful market pull; others satisfy a need that already exists in the market. The use of the LCVD as a rapid prototyping process should therefore be of interest to a wide range of scientists and technologists, in particular specialists who may eventually become the users of the technology, both in terms of research and commerce.

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# Liposomes Revisited


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Liposomes—self-assembling colloidal particles in which a lipid bilayer encapsulates a fraction of the surrounding aqueous medium—have now successfully negotiated the crucial passage from basic research to clinical practice. This transition has relied on technical breakthroughs in the control of liposome stability and reactivity, producing a virtual renaissance in the field.

Thirty years ago Alec Bangham discovered that phospholipids in water form closed vesicles; the physicochemical properties of these liposomes were then characterized, including their ability to serve as a model for cell membranes (1). Initially liposomes were heralded as optimal drug carrier systems, but further research proved disappointing and led to a period of skepticism among some scientists in the field of drug delivery (2). The medical utility of what are now called conventional liposomes (CLs) is limited by their rapid uptake by phagocytic cells of the immune system, predominantly in liver and spleen [although this has fortuitously led to some clinically important applications in antiparasitic treatment of phagocytes and in vaccine formulations (4, 5)]. This uptake is due to the characteristic nonspecific reactivity of CLs, which results in their largely uncontrollable properties upon administration in vivo.

Interest in liposomes as drug carriers was rejuvenated by the introduction of new ideas from membrane biophysics, and this multidisciplinary approach has enhanced prospects for their use in medicine (2–5). Liposomes can now be designed rationally, resulting in nonreactive (sterically stabilized) liposomes (SLs), as well as polymorphic (catonic, fusogenic) liposomes. The SLs can also be designed to exhibit specific reactivity (targeting), while polymorphic liposomes can exhibit high reactivity to nucleic acids and cell membranes. Because of their reduced recognition and uptake by the immune system, these newly sophisticated liposomes have been referred to as "stealth" liposomes (6), and are proving useful in cancer chemotherapy. The polymorphic liposomes provide a promising approach to gene therapy because they greatly improve transfection by exogenous DNA. In parallel with these developments, more efficient loading and retention of drugs within liposomes (7) (based on active "accumulation" through ionic gradients) has contributed to the ultimate utility of this new generation of liposomes (Fig. 1A).

Sterically stabilized liposomes were created when it was realized that neither mechanical nor electrostatic stabilization could provide liposomes with enough stability in a biological environment such as the systemic circulation. Thus, in SLs (3), the lipid bilayer contains glycolipids or, more recently, lipids conjugated with ethylene glycol, which provide a steric barrier outside the membrane (8). SLs remain in the blood for up to 100 times longer than conventional liposomes and can thus increase the pharmacological efficacy of encapsulated agents (2, 3, 8). Furthermore, SLs revived the feasibility of ligand-dependent targeting to specific cells (9), because they are much less subject to nonspecific uptake than are CLs. SLs bearing attached antibodies or other ligands are accumulated much more readily in targeted cells than are CLs (9). This approach is presently limited to the vasculature and to internalizing receptors (4, 6).

The enhanced biological stability of SLs is a result of the inhibition of interactions with plasma proteins (such as opsonins and lipoproteins) and cell surface receptors by


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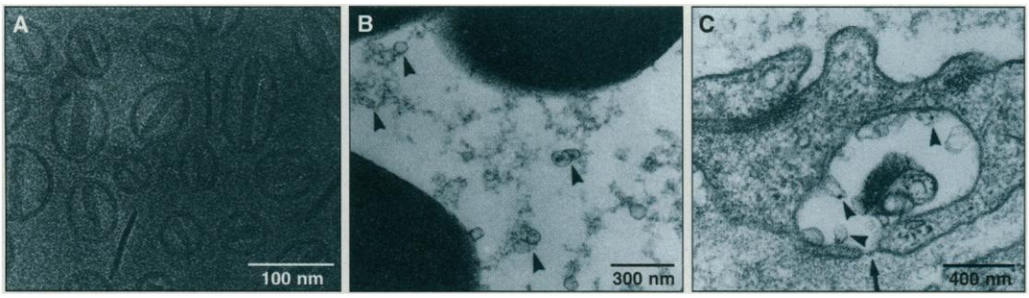


**Fig. 1. Stealth liposomes invade a lesion.** (**A**) SL containing the precipitated drug doxorubicin, as seen by cryoelectron microscopy. [Photo courtesy of P. M. Frederik, Limburg University, Maastricht, Netherlands] (B and C) SLs containing colloidal gold accumulate in Kaposi sarcoma–like dermal lesions of transgenic mice bearing the human immunodeficiency virus *tat* gene. (**B**) Liposomes containing colloidal gold particles (arrowhead) near extravasated erthrocytes within the lesion. (**C**) Gold-labeled liposomes (arrowhead) in a large vesicle of an endothelial cell in the lesion. Arrow, fenestra [Reprinted with permission from Huang *et al.* in (*11*)]

the steric barrier above the bilayer. Measurements by the osmotic stress technique and surface force apparatus have shown drastically increased repulsive pressure above the bilayer of the SL with surface-attached polyethylene glycol.

In contrast to CLs, which are cleared from the blood in a dose-dependent manner with saturation at higher lipid concentrations, SLs show dose-independent kinetics of blood clearance (*3*). And most important, fewer SLs are accumulated in the liver and more are accumulated in, for example, implanted rodent tumors than CLs. The prolonged presence of liposomes in blood allows them to extravasate into sites where the vasculature is leaky—often within tumors. Indeed, in animals and humans, more than 10 times the amount of drug is concentrated in tumors by SLs than by administration of the free drug (*3*, *6*). Such liposomes are found intracellularly within the Kupffer cells in the liver, but extracellularly around tumor cells after extravasation (*10*). They also accumulate to a certain extent in the skin, extravasating beyond the endothelium of postcapillary venules, sometimes traversing the endothelial cells through large caveollae (Fig. 1, B and C).

The colon carcinoma C26 solid tumor in mice is practically insensitive to treatments with doxorubicin, either free or in CLs. However, the administration of SLs containing doxorubicin (SL-Dox) results in complete remission of tumors after early treatment and in significant improvements after delayed treatment. In a mammary carcinoma tumor model, SL-Dox was substantially more effective than CL in curing mice with implanted tumors and in reducing the incidence of metastases from these tumors. SL-Dox can arrest the growth of human lung tumor cell xenographs in severe combined immunodeficient (SCID) mice, whereas equivalent doses of doxorubicin, either free or encapsulated in CL, were not effective (*11*).

These very encouraging preclinical results have been strengthened by clinical studies in humans. More than 1000 patients with AIDS-related Kaposi sarcoma have been treated with SL-Dox and show good responses with minimal toxicity. SL-Dox was selectively accumulated in Kaposi sarcoma lesions, with SL delivering 11.4 times more doxorubicin than free drug administration (*6*). These initial clinical trials have been expanded to include phase III comparison with standard therapy and to some solid tumors (*6*). Finally, SL can also be used in the treatment of infectious diseases and inflammation, conditions also characterized by leaky vasculature (*6*).

Liposomes have proven useful for gene therapy. The successful encapsulation of a whole virus and then DNA into large, negatively charged CLs opened exciting opportunities in genetic engineering by enhancing DNA introduction into mammalian cells (*12*). However, the cumbersome and inefficient procedures for encapsulation often did not sufficiently improve transfection, and electroporation emerged as an alternative in vitro method. This field was revived by the discovery that cationic lipids can condense DNA and increase transfection yields in vitro by several orders of magnitude (*13*). Subsequently, reports on transfections in vivo stimulated intense interest in the use of liposomes for gene therapy (*13*).

In the DNA–cationic liposome complex, the nucleic acids or short, single-strand antisense oligonucleotides are not encapsulated but are simply complexed with small unilamellar vesicles by electrostatic interactions. Intricate topological rearrangements occur, including DNA condensation, liposome aggregation, and fusion (*14*). This supramolecular complex is then either added to cells in vitro, injected parenterally, or aerosolized for pulmonary applications. Although neither the physicochemical properties of the complex nor its interaction with cells are clearly understood, the process yields reasonably efficient transfection of a variety of cells and tissues in vivo. In addition to increased adsorptivity, fusion between liposomes attached to DNA and various cell membranes after endocytosis may facilitate the nuclear localization of DNA.

In vivo transfection of cells with foreign genes is a rapidly developing field, with promising prospects for a variety of diseases, most notably cystic fibrosis, cancer, and cardiovascular diseases. The liposome delivery has advantages over viral vectors, which are limited in the size of the foreign gene carried and can have undesirable effects from the viral components.

Future efforts should improve the specificity of drug- and gene-carrying liposomes and optimize the release kinetics for their cargo. Useful applications in medicine and other fields will continue to emerge from the synergy between theoretical and experimental studies on liposomes.

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