

Nanodroplet Technology Targets Drug Delivery

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Dr Ackley has thirty years' experience in the areas of semiconductors, fiber-optics, MEMS, and biotechnology. He has a strong interest in applying microelectronics technology to the problems facing biotechnology companies, and has worked to pursue that interest in such diverse areas as DNA diagnostics, drug delivery, and biowarfare defense. He has written over fifty papers, two book chapters, and has over sixty issued patents in such diverse areas as biotechnology, nanotechnology, semiconductor devices and photonics.

Introduction

Nanodroplet technology uses the properties of immiscible fluids to transform continuous fluid streams into highly mono-dispersed, isolated droplets with tight control of droplet size and generation rate. The process is performed under moderate conditions of pressure-driven flow and can be combined in a low cost, microfluidic substrate with other functions such as droplet fusion, splitting and micromixing. These processing parameters can be optimised to yield sub-micron, mono-dispersed drug delivery particles. Using droplets, we can provide exquisite control of chemistry in picoliter or femtoliter volumes that may be merged or reacted downstream with other droplets, yielding the ability to tune the internal droplet chemistry and surface chemistry on the near-molecular level. An application of particular interest is the use of nanodroplets to produce designer nanoparticles for drug delivery.

Engineered Nanoparticles Target Tumours

A major problem with antibodies, peptides, and protein-based drugs is the overall fragility of the active ingredient against environmental conditions. The same difficulties arise with genetic materials such as anti-sense drugs and siRNA. As these drugs are potentially of great therapeutic value, there is a pressing need for new means to deliver them in ways effective to treat numerous conditions ranging from diabetes and obesity to a wide variety of cancers. For example, in neurological disorders, delivering drugs across the blood-brain barrier is quite difficult due to the large size of the drug particles (>1 micron). Similarly, the small and entangled vasculature within cancerous tumours can present a serious impediment to drug delivery. Encapsulated drugs on the nanoscale may provide a promising solution to these problems. In addition, the targeted delivery of powerful drugs, which have high toxicity and potentially harmful side-effects, can significantly reduce those side-effects and enhance patient health, particularly in the treatment of difficult cancers. If a nanoparticle technology can be developed with flexible

targeting, as well as optimum particle size, it could present doctors with a powerful new treatment option.

The nanodroplet technology and synthesis process is an excellent candidate to produce such nanoparticles. Applying a system that uses pressure-driven flow to generate phase separated droplets in an immiscible solvent, droplet composition, drug dosage and particle size may all be controlled in an inexpensive and disposable microfluidic device. As the process typically uses low pressures (a few psi) and very low flow rates (of the order of microliters/min), shearing of proteins and other valuable material can be minimised. By using droplets as the mechanism for forming nano-sized drug delivery 'packets', complex formulations may be produced. These particles can be polymeric or lipid-based, or a combination of both, with surfaces that can be functionalised during the droplet formation process. The droplet mechanism provides superior control of particle or liposome size distribution to ensure uniform drug uptake. For cancer treatment applications, the characteristics of small particle size and mono-dispersity has been shown to influence several aspects of liposome performance, which includes pharmacokinetics of drug delivery, circulation half-life, accumulation site in the body, delivery volume and release properties for drug vehicles (Kong *et al.*, 2000; Juliano *et al.*, 2005; Allen and Everest, 1975; Magin *et al.*, 1975; Litzinger *et al.*, 1994). Of particular interest for cancer treatment is the formation of customised liposomes that can be optimised to reach the target tumours before releasing their drug payload. For example, gene therapy using liposomal delivery systems has been demonstrated in small and large animal models for lung (Ramesh *et al.*, 2001), breast (Shi *et al.*, 2002), head and neck (Templeton *et al.*, 1997), and pancreatic cancers (Tirone *et al.*, 2001).

The targeting of the drug 'packets' to tumour cells represents the next generation of cancer treatment. Targeted treatment will be more effective and will have fewer side-effects. Numerous research groups are working to achieve targeted treatment protocols using a variety of methods based on chemical processes. For example, liposomes targeted using antibodies (Andresen *et al.*, 2005; Torchillin, 2005) have been used to demonstrate the

delivery of proteins, nucleic acids, and peptides (Torchillin, 2005). Targeted glycoproteins have been used efficiently to target liposomes to the liver (Templeton *et al.*, 1997), and breast, lung and pancreatic cancers can also be targeted using ligands. Targeting holds out the promise that drugs can be administered only to the desired cells, thus reducing compound toxicity to normal cells. The nanodroplet process adds an important new degree of freedom into the formation of targeted drug 'packets' in that the fluidic formation process can be used to control the size and structure of the packets, concurrently with the surface functionalisation process but also somewhat independently of it, providing more flexibility to optimise the particle properties. This approach will lead to advanced nanoparticles for multi-functional therapeutics.

This capability provides a flexible approach towards programmable nanoparticle synthesis that potentially can be adjusted to optimise drug delivery for individual patients, where drug treatment may be monitored by medical personnel and adjusted to provide a suitable level of efficacy. On the nanodroplet devices, since the protein environment and shear fluid are immiscible, there is minimal contact between the protein materials and the shearing fluids which minimise chemical denaturation. The microfluidic configuration may also be arranged serially to produce complex particles with multiple drug layers, which can result in tailored, complex release profiles. The nanodroplet technology also overcomes a number of practical issues that can impede the efficient delivery of drugs to the patient. Firstly, the technology provides a flexible platform that is capable of titrating particle compositions on demand. This prospect allows care gives the new ability to adjust dosage levels to meet the need of the individual according to the patient's response to initial treatments. In addition, the nanoparticles produced on the droplet platform are somewhat agnostic as to the delivery device, and are capable of being delivered by injection, by inhalation or nasal delivery, or even potentially by oral means.

Droplet Technology Can Engineer Nanoparticles

The formation of nanodroplets on a microfluidic device was first introduced by Thorsson *et al.* 2001 in a T-junction device, followed by work from Nisisako *et al.* (2002) and Song and Ismagilov (2003) in similar structures. This work was quickly followed by results from Anna *et al.* (2003), and Tan *et al.* (2004) using devices with various cross channel configurations. Nanotrope is working closely with Professor Abraham Lee's group at the University of California Irvine to advance the droplet technology for all the applications described here. The Lee group has worked extensively on the mechanisms of droplet generation and manipulation, as well as well as applications of the technology to different types of nanochemistry.

An application of the droplet process to the formation

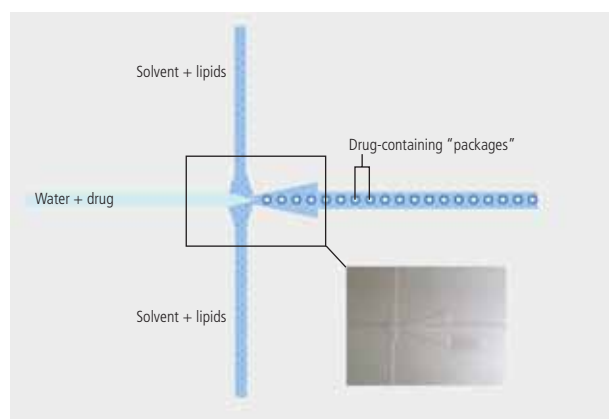


Figure 1 – A schematic depiction of the formation of lipids in a droplet device. The inset shows a still micrograph of a droplet generation device in operation.

of drug containing liposomes is schematically depicted in Figure 1. An aqueous solution of drug is pumped into the central channel of the device. An immiscible solvent containing the particle forming chemistry (in this case, a mix of lipids optimised for cellular uptake) is flowed down the two side channels. Through a mix of flow-focusing and viscous shear, droplets are formed at the channel junction from the continuously flowing liquids. By varying the channel geometry and relative flow rates of the fluid streams, the size of the droplets may be varied over a wide range, with diameters that can vary from hundreds of nanometers to hundreds of microns. Since the droplet size is determined by the flow rates, which can be made extremely consistent, the droplets are substantially mono-dispersed, and the droplet size can be varied continuously in a controlled manner for applications that demand size variation.

The formation of liposomes on the droplet platform may be visualised using a high speed camera (Figure 2). In this experiment, liposomes were formed from a complex lipid mixture which incorporated surface functionalisation moieties. These liposomes are attached to functionalised plates for analysis, and are precursors to drug-containing packages, which can be targeted at a wide variety of tumours using generalised surface ligands that can attach specific entities targeted at cellular receptors.

A major advantage of the droplet platform in forming

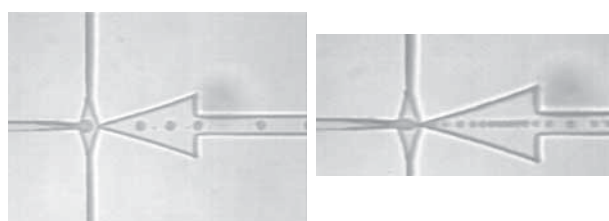


Figure 2 – High-speed camera image of the generation of surface functionalised liposomes, at different generation rates. Liposomes can be formed at a rate as high as 1000/sec in a single device. The platform is readily scaled to large numbers of devices operating in parallel.

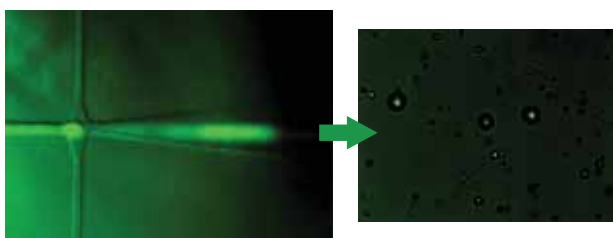


Figure 3 – Formation of droplets containing fluorescent proteins, along with a fluorescent micrograph of protein-containing liposomes formed using the droplet process.

nanoparticles is the ability to encapsulate proteins without damage. Many techniques currently used or investigated for drug encapsulation use high pressure or flow rates, or generate high shear forces, which can damage the structure of proteins or peptides. To demonstrate that capability, fluorescent proteins have been encapsulated in liposomes of various sizes. Since the droplet size is determined by relative flow rates of solvent and water in the device (which essentially varies the droplet-forming shear forces), we can ensure that the fragile proteins remain undamaged over a wide range of shear forces and particle sizes. Bright fluorescence is observed from the fluorescent protein prior to, during, and subsequent to the droplet formation process, suggesting that the proteins remain unperturbed throughout the droplet process (Figure 3). Liposomes encapsulating fluorescent protein are seen in the figure as well. These liposomes have the capability to be dried down and rehydrated while maintaining the fluorescence of the encapsulated proteins. Dry-down is an important component of stabilising drugs for delivery by a variety of methods.

Conclusion

The nanodroplet technology has the potential to make an important impact on the way drugs are delivered in the near future. By providing a platform for the engineering of complex nanoparticle drug delivery packets, and coupling that to the ability to engineer contrast agents for a variety of imaging techniques, the droplet technology will enable the visualisation of drug therapies as they are administered, and the precision targeting of tumours to improve drug efficacy and reduce side effects.

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