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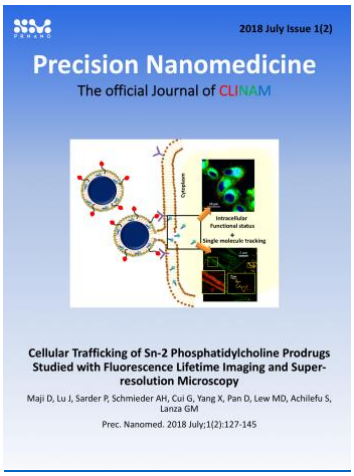

About the cover: *Fluorescence lifetime imaging microscopy (FLIM) and single-molecule super-resolution microscopy (SRM) illustrate the intracellular fate of Sn-2 phosphatidylcholine prodrugs.*

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Featured Research Article

Cellular Trafficking of Sn-2 Phosphatidylcholine Prodrugs Studied with Fluorescence Lifetime Imaging and Super-resolution Microscopy

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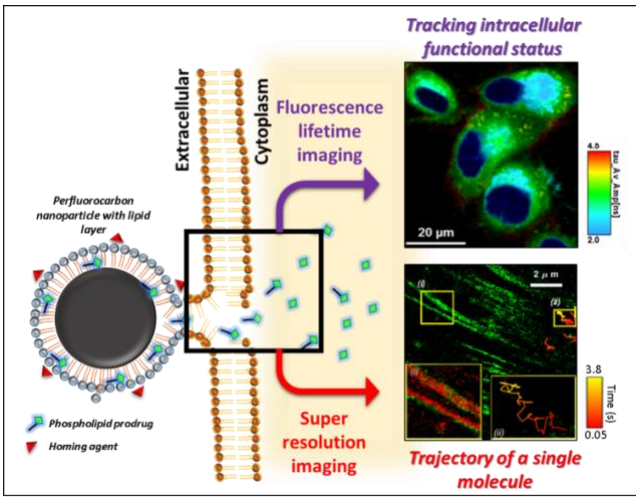

While the *in vivo* efficacy of Sn-2 phosphatidylcholine prodrugs incorporated into targeted, non-pegylated lipid-encapsulated nanoparticles was demonstrated in prior preclinical studies, the microscopic details of cell prodrug internalization and trafficking events are unknown. Classic fluorescence microscopy, fluorescence lifetime imaging microscopy, and single-molecule super-resolution microscopy were used to investigate the cellular

handling of doxorubicin-prodrug and AlexaFluor™-488-prodrug. Sn-2 phosphatidylcholine prodrugs delivered by hemifusion of nanoparticle and cell phospholipid membranes functioned as phosphatidylcholine mimics, circumventing the challenges of endosome sequestration and release. Phosphatidylcholine prodrugs in the outer cell membrane leaflet translocated to the inner membrane leaflet by ATP-dependent and ATP-independent mechanisms and distributed broadly within the cytosolic membranes over the next 12 h. A portion of the phosphatidylcholine prodrug populated vesicle membranes trafficked to the perinuclear Golgi/ER region, where the drug was enzymatically liberated and activated. Native doxorubicin entered the cells, passed rapidly to the nucleus, and bound to dsDNA, whereas DOX was first enzymatically liberated from DOX-prodrug within the cytosol, particularly in the perinuclear region, before binding nuclear dsDNA. Much of DOX-prodrug was initially retained within intracellular membranes. *In vitro* anti-proliferation effectiveness of the two drug delivery approaches was equivalent at 48 h, suggesting that residual intracellular DOX-prodrug may constitute a slow-release drug reservoir that enhances effectiveness. We have demonstrated that Sn-2 phosphatidylcholine prodrugs function as phosphatidylcholine mimics following reported pathways of phosphatidylcholine distribution and metabolism. Drug complexed to the Sn-2 fatty acid is enzymatically liberated and reactivated over many hours, which may enhance efficacy over time.

From the Clinical Editor: Sn-2 phosphatidylcholine prodrugs have been shown to be effective systemically for the delivery of a variety of drugs. Nonetheless, little is known about the pharmacokinetics in terms of cell uptake release and activation. Here, the authors have carried out experiments utilizing fluorescence microscopy, fluorescence lifetime imaging, and single-molecule super-resolution imaging techniques and show that the series of events that occur after the prodrugs had contacted with cellular membranes. The data has given valuable new understanding of prodrug pharmacokinetics.

Perspective

Skin biosensing and bioanalysis: what the future holds

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Wearable skin biosensors have important applications in health monitoring, medical treatment and theranostics. There has been a rapid growth in the development of novel biosensing and bioanalytical techniques in recent years, much of it underpinned by recent advancements in nanotechnology. As the two related disciplines continue to co-evolve, we take a timely look at some notable developments in skin biosensing/bioanalysis, scan the horizon for emerging nanotechnologies, and discuss how they may influence the future of biosensing/bioanalysis in the skin.

Review

A coming era of precision diagnostics based on nano-assisted mass spectrometry

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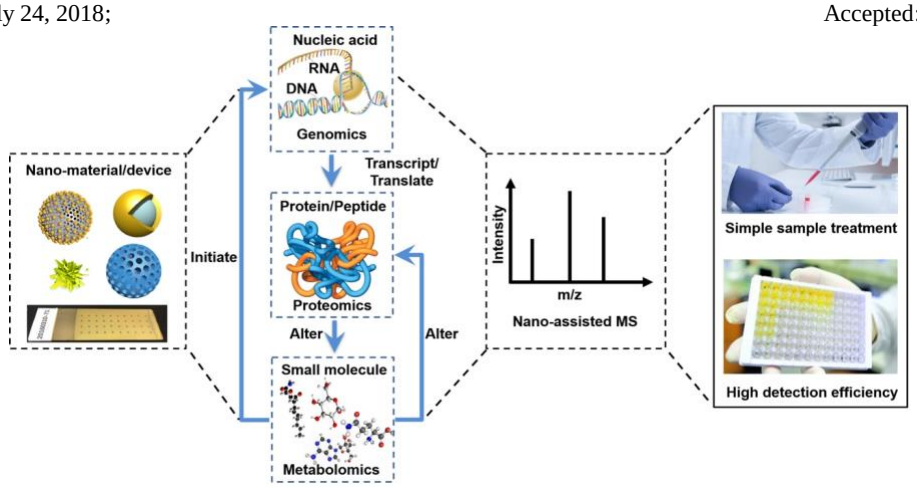

Precision diagnostics relies on omics analysis by mass spectrometry to overcome the limitation in accuracy by an individual biomarker, due to the complex nature of diseases. Recent development in nanotechnology markedly enhanced sample treatment and detection efficiency of this method. Herein, we foresee a coming era of precision diagnostics based on nano-assisted mass spectrometry. Some important progress in the field includes detection of (1) nucleic acids for genetic analysis; (2) proteins/peptides for proteomic analysis; and (3) small molecules for metabolic analysis. We anticipate that this review will be a reminder for both young and experienced researchers about the future of diagnostics and call for attention worldwide.

Research Articles

Rational Design of a siRNA Delivery System: ALOX5 and Cancer Stem Cells as Therapeutic Targets

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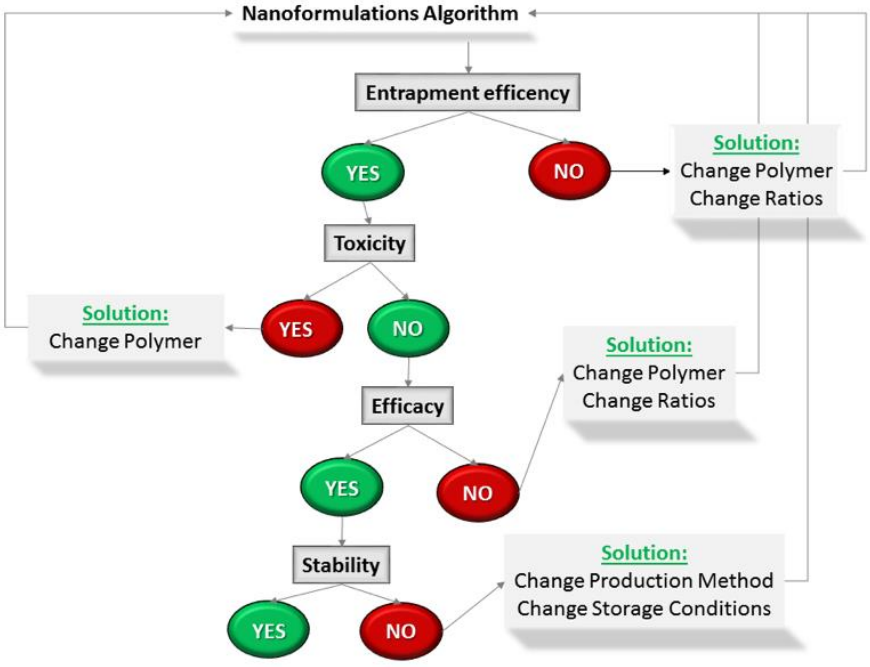

The search for an ideal gene delivery system is a long and laborious process in which several factors from the first idea to final formulation, including main challenges, peaks and troughs, should be deeply taken into consideration to ensure adequate biological safety and in vivo efficacy endpoints. Arachidonate 5-lipoxygenase (ALOX5), a crucial player related with cancer development and in particular with cancer stem cells malignancy. In this work we describe the process behind the development of a small interfering RNA (siRNA) delivery system to inhibit ALOX5 in cancer stem cells (CSC), as a model target gene. We started by screening chitosan polyplexes, among different types of chitosan in different complexation conditions. Due to the low silencing efficacy obtained, chitosan polyplexes were combined with Pluronic®-based polymeric micelles with recognized advantages

regarding gene transfection. We tested different types of polymeric particles to improve the biological efficacy of chitosan polyplexes. Nevertheless, limited transfection efficiency was still detected. The well-established polyethylenimine (PEI) cationic polymer was used in substitution of chitosan, in combination with polymeric micelles, originating PEI-siRNA-Pluronic® systems. The presence of Pluronic® F127 in the formulation showed to be of utmost importance, because not only the silencing activity of the polyplexes was improved, but also PEI-associated toxicity was clearly reduced. This allowed to increase the amount of PEI inside the system and its overall efficacy. Indeed, different types of PEI, N/P ratios and preparation methods were tested until an optimal formulation composed by PEI 10k branched-based polyplexes at an N/P ratio of 50 combined with micelles of Pluronic® F127 was selected. This combined micelle presented adequate technological properties, safety profile and biological efficacy, resulting in high ALOX5 gene silencing and strong reduction of invasion and transformation capabilities of a stem cell subpopulation isolated from MDA-MB-231 triple negative breast cancer cells.

From the Clinical Editor: The use of gene-silencing technique for cancer therapy has been met with problems like cellular uptake, degradation, and clearance. The authors here test two different types of polymeric particle formulations as vectors in an attempt to improve the biological efficacy and describe a general algorithm for testing. The data obtained provide more knowledge of how to further progress the field for the benefits of selected cancer patients.

Retinal Multipotent Stem-Cell Derived "MiEye" Spheroid 3D Culture Model for Preclinical Screening of Non-viral Gene Delivery Systems

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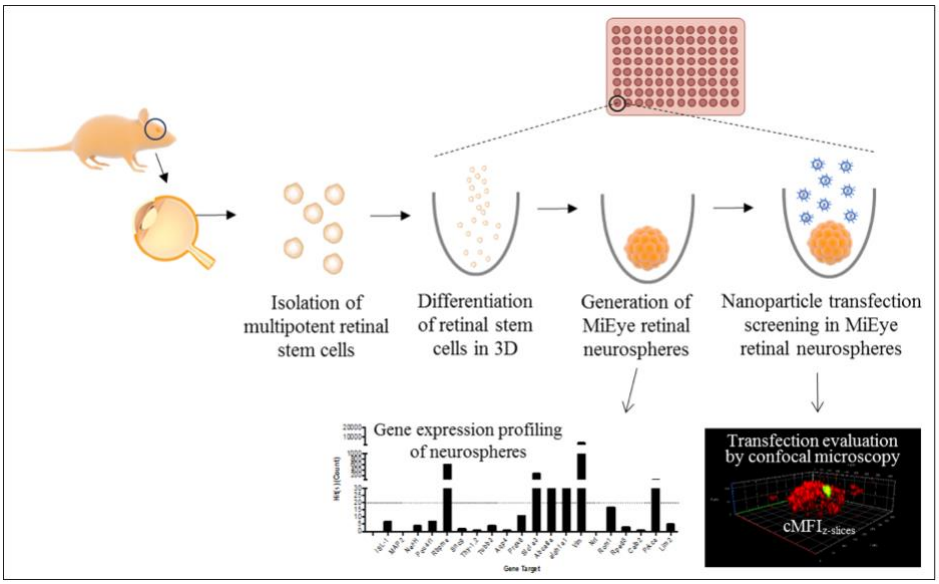

Non-viral retinal gene therapy is a promising therapeutic approach towards the management of retinal degenerative diseases especially glaucoma. Current methods of *in vitro* preclinical screening of candidate nanoparticle systems in monolayer cell cultures are not reliable in predicting *in vivo* performance. In this paper we describe the development of a multipotent stem-cell derived three-dimensional "mini-retina" culture model (MiEye) that aims to simulate an *in vivo* clinical model for more reliable gene delivery system screening. Through utilization of multiplex gene expression profiling, we have shown that retinal stem cells can be differentiated in 3D culture to generate retinal neurospheres comprising of multiple retinal cell types. The 3D cell culture model combined with confocal microscopy imaging and fluorescence profiling techniques is a powerful tool as a retinal gene and drug delivery screening model.

From the Clinical Editor: Gene therapy seems to be a promising modality for treating eye diseases. Non-viral retinal gene therapy theoretically should have an advantage over viral delivery system in terms of safety. However, non-viral system is hampered by low gene transfer efficacy in-vivo. The authors here describe a novel 3-D culture model of retinal stem cells to mimic in-vivo environment. This system should prove useful in testing the efficacy of gene transfer to retinal stem cells and predict *in vivo* performance.

Lysozyme transport to the brain by Liposomes

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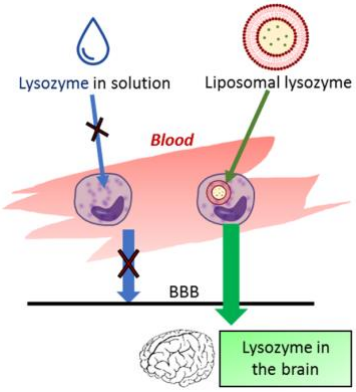

Delivery of drugs into the brain is limited due to poor penetrability of many drugs via the blood brain barrier. Previous studies have shown that the brain is kept under close surveillance of the immune system, implying that circulating phagocytic cells, such as neutrophils and monocytes, are crossing the BBB. We hypothesized that charged liposomes could be transported to the brain following their phagocytosis by circulating monocytes. In this work, we investigated whether circulating monocytes could be exploited as a drug delivery system following I.V. administration of nano-sized, positively charged and fluorescently labeled liposomes containing the protein lysozyme. Negatively charged fluorescently labeled liposomes were used for comparison. By using a modified thin film hydration technique, liposomes were made with the desired size, polydispersity index, high drug concentration, and stability. *In-vitro* results showed a significant, time-dependent uptake of positively charged liposomes by RAW264.7 cells. In vivo results revealed that circulating white blood cells (mainly monocytes) contained high levels of fluorescently-labeled liposomes. Screening of brain sections using confocal microscopy uncovered that substantial amounts of fluorescently-labeled liposomes was transported into the brain. In addition, anti-CD68 immunofluorescent staining of brain sections, demonstrated co-localization of positively charged liposomes and macrophages in different brain sections. In addition, significantly higher levels of lysozyme were detected in brain lysates from rats treated with positively charged liposomes compared to rats treated with lysozyme solution only. This confirms our hypothesis that the designed liposomes were transported to the brain following their phagocytosis by circulating monocytes.

From the Clinical Editor: The almost impenetrable blood-brain barrier (BBB) is a problem faced by many researchers developing drug delivery systems for treating brain disorders. In this context, the authors here describe a new method in utilizing the phagocytic power of monocytes in order to get entry into the brain. Liposomes with specific properties were formulated and phagocytosed by monocytes were shown to be transported into the brain. This “stealth” approach may prove quite useful clinically in the future.