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Review article

# Technologies for controlled, local delivery of siRNA

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ABSTRACT

The discovery of RNAi in the late 1990s unlocked a new realm of therapeutic possibilities by enabling potent and specific silencing of theoretically any desired genetic target. Better elucidation of the mechanism of action, the impact of chemical modifications that stabilize and reduce nonspecific effects of siRNA molecules, and the key design considerations for effective delivery systems has spurred progress toward developing clinically-successful siRNA therapies. A logical aim for initial siRNA translation is local therapies, as delivering siRNA directly to its site of action helps to ensure that a sufficient dose reaches the target tissue, lessens the potential for off-target side effects, and circumvents the substantial systemic delivery barriers. While locally injected or topically applied siRNA has progressed into numerous clinical trials, an enormous opportunity exists to develop sustained-release, local delivery systems that enable both spatial and temporal control of gene silencing. This review focuses on material platforms that establish both localized and controlled gene silencing, with emphasis on the systems that show most promise for clinical translation.



**Contents**



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## 1. Introduction

The discovery that double stranded RNA (dsRNA) can trigger catalytic degradation of messenger RNA (mRNA) has inspired more than two decades of research aimed at understanding and harnessing this mechanism. Because well-designed RNA interference (RNAi) therapeutics can potently and specifically suppress translation of any gene, including intracellular targets traditionally considered "undruggable", they have been heavily studied as a potential new class of pharmaceutics that can modulate drug targets that are inaccessible by conventional small molecule inhibitors and antibody drugs. In particular, synthetic, double-stranded small interfering RNA (siRNA) has emerged as a leading candidate for the development of gene silencing therapeutics [1,2]. siRNA is potentially advantageous in comparison to other RNAi approaches because it can directly load into the RNA induced silencing complex (RISC) machinery, simplifying dosing control and circumventing the requirement for delivery into the nucleus (*e.g.*, as required with shRNA-encoding vectors) [3,4]. However, emergence of clinically approved siRNA therapies has remained slow, with the primary challenge being the formidable anatomical and physiological barriers that must be overcome to deliver siRNA to its intracellular site of action in target cell types [5].

To date, systemic delivery of siRNA therapeutics to targets in the liver has been most extensively tested in clinical trials; this approach is motivated by the ability to exploit the liver's physiological function as a filtration and clearance system [6–8]. Through strategic targeting of relevant hepatic genes, multiple siRNA therapeutics have proven efficacious in preclinical and clinical trials [7,9,10]. One of the most advanced along the regulatory pathway is a therapeutic by Alnylam currently in Phase III trials that targets the transthyretin gene for treatment of transthyretin amyloidosis [7,11]. However, development of systemically delivered siRNA therapeutics that target tissues other than the liver has proven more challenging [8].

Local delivery systems offer a potentially more translatable alternative, as they confer the advantages of reducing off-target side effects and enable higher gene silencing at the target site [8]. For these reasons, many of the first therapeutic applications of siRNA tested clinically involved local delivery (primarily topical or injection-based). However, initial clinical trials involving local siRNA delivery were largely disappointing and did not meet the high expectations of the scientific and medical communities [12,13]. These studies revealed unexpected concerns regarding siRNA safety (*e.g.*, therapies based on naked siRNA triggered immune responses) and pharmacokinetics [8,12–15]. The advancement of siRNA molecular design principles and improved delivery systems has increased the number of candidate siRNA therapeutics entering the clinical pipeline, but there is currently a dearth of locally delivered siRNA therapeutics in testing relative to systemically delivered formulations [8,12]. This review will focus on recent technologies that leverage the significant advantages of local siRNA delivery and have made progress toward overcoming the barriers that have thus far limited these applications.

## 2. siRNA mechanism

The molecular phenomenon of RNAi-based post-transcriptional gene silencing, first termed "reversible co-suppression", was unraveled following the unexpected observation by Napoli et al. in 1990 that introduction of a transgene intended to overexpress chalcone synthase (CHS, a gene for flower pigmentation) yielded more white flowers and was associated with a 50-fold reduction of CHS mRNA [16]. The gene silencing capability of antisense oligodeoxynucleotides (ODNs) was first elucidated, but it was discovered soon thereafter that double-stranded RNA (dsRNA) are capable of achieving 100- to 1000-fold more potent gene suppression than ODNs [17]. The delivery of dsRNA of varying lengths, siRNA, short hairpin RNA (shRNA), and plasmids expressing shRNA can trigger gene-specific silencing, which is optimal when there is full complementarity between the guide strand and the target mRNA sequence [2,18]. These synthetic dsRNA molecules are more effective than ODNs because they "hijack" the catalytically-active gene silencing machinery that is integral to endogenous, negative feedback pathways utilized by naturally expressed microRNA (miRNA) [2,19,20]. When larger dsRNA are delivered to the cellular cytoplasm, they are cleaved by the enzyme Dicer into siRNA, which are 19–21 nucleotides in length and characterized by 3′ nucleotide overhangs. The siRNA strands are then separated, and the antisense or guide strand, recognized by a less stable 5′ end, is incorporated into the RNA-induced silencing complex (RISC) [21]. The activated RISC loaded with the siRNA guide strand binds to complementary mRNA and initiates its degradation (Fig. 1). Importantly, the activated RISC has enzymatic activity, enabling a single siRNA to elicit the degradation of multiple mRNAs [22]. In contrast, synthetic microRNA (miRNA) often modulates multiple mRNA targets with partial complementarity and thus can influence larger systems of genes [23]. While the coordinated control of multiple, related genes through miRNA therapeutics is a powerful strategy, properly designed siRNA-based therapeutics are desirable because they offer more predictable functional effects based on modulation of specific genes.

## 3. siRNA delivery challenges

### 3.1. General delivery barriers

While discovery and development of small molecule drugs for clinical use remains an enormous challenge, the translation of siRNA therapeutics is comparatively unchartered. Thus, in addition to traditional drug development challenges, the "normal" pipeline for development of an siRNA drug for FDA clearance has yet to be established [8,12]. The major difficulty faced when designing siRNA therapeutics is that of delivery to its site of action; synthetic dsRNA or siRNA molecules have relatively poor pharmacokinetic properties and thus face more formidable extracellular and intracellular delivery challenges relative to small molecule drugs. Oral bioavailability of siRNA molecules is very poor because they are relatively large, hydrophilic, and susceptible to degradation, and systemic, intravenous delivery of siRNA results in rapid renal filtration and clearance through the urine [24]. siRNA also has a short half-life *in vivo* and can be degraded by nucleases, especially if optimized chemical modifications are not incorporated onto the siRNA molecule [25]. Furthermore, siRNA does not readily translocate lipid bilayers, such as those that constitute the outer cellular membrane and the endo-lysosomal intracellular vesicles. The latter can cause siRNA that has been internalized by target cells to be degraded within lysosomes or exocytosed, rather than becoming bioavailable for interaction with the RISC machinery in the cytosol [24,26,27]. For example, only 1–2% of the siRNA delivered by lipid nanoparticles is believed to be released into the cytosol and to be bioavailable for RISC loading and target gene silencing [27]. While it is not the focus of the current review, there are a variety of delivery systems under development for overcoming these systemic and general delivery barriers [24].

Utilization of siRNA therapeutically is also complicated by the potential for toxicity and immunogenicity of both the siRNA molecules and the carriers used. siRNA molecules can activate Toll-like receptors (TLRs), which are a part of the innate immune system that recognizes and mounts an immune response against microbial invaders [28–32]. Additionally, siRNA can elicit off-target effects due to partial sequence complementarity to unintended genes or by saturating the cell's RISC machinery, altering endogenous miRNA gene regulatory processes [31,33,34]. Furthermore, systems used to deliver siRNA can induce toxic and immunogenic consequences [24]. These inadvertent effects can override therapeutic benefits and confound interpretation of experiments designed to test the functional significance of siRNA therapeutics [14].