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ZEBRAFISH EMBRYOS AS A MODEL FOR IN VIVO EVALUATION OF NANOPARTICLE AND VESICLE BASED DELIVERY SYSTEMS

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Efficient and safe delivery remains a major limitation in the development of genetic therapies; among the main challenges is the ability of carriers to achieve controlled biodistribution and tissue-specific targeting in vivo.

Zebrafish embryos represent a versatile vertebrate model that enables direct visualization of delivery systems during development, allowing the evaluation of carrier biodistribution, persistence, and biological effects in a whole organism. Within this framework, different synthetic and biologically derived delivery systems were evaluated in zebrafish embryos.

As a benchmark approach, eGFP mRNA encapsulated in Lipofectamine™ 2000 was injected into the yolk near the Duct of Cuvier at 48 hours post fertilization, resulting in detectable GFP fluorescence up to 72 hours post injection. This system provided a reproducible reference for evaluating functional RNA delivery in vivo.

Poly(lipoic acid) nanoparticles differing in core chemistry showed broad vascular distribution without detectable toxicity or developmental impairment. These biodegradable carriers protect mRNA cargo extracellularly and release it intracellularly through environment-responsive cleavage, enabling functional protein expression in vivo. Preliminary observations also suggest reduced hepatic accumulation and potential extra-hepatic distribution, including cardiac and hematopoietic regions.

In parallel, peptide-functionalized lipid nanoparticles were evaluated for biodistribution and targeting capability. Nanoparticles were detected in the heart and caudal vessels, although no clear tissue-specific targeting was observed under the tested conditions.

Moreover, fluorescently labelled nanoalgosomes were investigated as alternative delivery platforms. Nanoalgosomes are membranous nanovesicles obtained from microalgae and can serve as carriers for bioactive molecules and nucleic acids. In vivo analyses revealed signal distribution in multiple tissues. Functional assays suggested antioxidant and mitochondrial protective effects, while experiments in the inflammatory reporter line Tg(mpx:GFP), marking neutrophils, indicated potential modulation of inflammatory responses. These observations are supported by preliminary RT-qPCR analyses.

Overall, this embryo-based platform enables the comparative evaluation of synthetic and biologically derived delivery systems in vivo, supporting their optimization while providing insight into their biological interactions during development.