**Ensuring Safety and Efficacy in mRNA Therapeutics through Affinity FPLC Purification**

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**Background and aims.** The clinical promise of mRNA therapeutics depends fundamentally on delivering highly pure mRNA that supports efficient protein expression and minimizes unwanted immune responses. However, widely used purification methods such as LiCl precipitation and silica-based spin columns often leave behind impurities (including double-stranded RNA and truncated transcripts) that can compromise both efficacy and safety, especially in vivo. This study aims to address these challenges by evaluating affinity-based fast protein liquid chromatography (FPLC) with oligo-deoxythymidine (oligo-dT) resin as an alternative purification strategy.

**Methods.** Three representative mRNAs with different lengths (1,006 nt, 1,925 nt, and 3,264 nt) were in vitro transcribed and we compare the performance of affinity-based FPLC with oligo(dT) resin to the traditional purification methods. The purity and integrity of each mRNA sample were assessed by gel electrophoresis and dsRNA-specific assays. Functional validation was performed by transfecting mammalian cells and measuring both protein expression and immune activation in vitro.

**Results.** Preliminary results indicate that affinity FPLC yields mRNA with markedly higher purity compared to LiCl precipitation and spin column methods. mRNAs purified by affinity FPLC supported stronger protein expression and triggered lower immune activation in cell-based assays.

**Conclusion/Discussion.** These findings highlight the critical role of advanced purification methods in enabling the safe and effective clinical translation of mRNA therapeutics. Affinity-based FPLC with oligo-dT resin shows clear promise for producing high-purity mRNA, and ongoing work will further validate these results and explore the implications for in vivo applications.

**Acknowledgement:** This work was supported by the National Research Foundation (NRF) grant funded by the Korean Government (MSIT) (RS-2022-NR070844 and RS-2024-00343765).

**References:**

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