Transforming Transfection of Mammalian Cells with Pulzar® Technology

White paper | Pulzar® Technology

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Summary

Transfection, broadly defined as the introduction of exogenous biomolecules into eukaryotic cells, is a foundational technique in molecular biology. While transfection can encompass delivery of a range of molecular cargos, including proteins and other macromolecules, it is most commonly applied to the delivery of DNA or RNA to drive gene expression. These approaches support applications ranging from gene function analysis to protein production and therapeutic development. Despite its widespread use, existing transfection methods often suffer from limited efficiency, variability across cell types, and cellular stress or toxicity, constraining reproducibility and scalability.

Pulzar® technology is designed to address these challenges by enhancing the performance of nucleic acid delivery workflows without altering their core biological or chemical components. By modulating the physical environment associated with transfection, Pulzar aims to improve plasmid–reagent complex behaviour, increase cellular receptivity, and support more consistent and efficient gene expression. Together, these improvements position Pulzar as a broadly compatible technology for enhancing transient transfection and vector production workflows relevant to research and gene therapy development.

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Introduction

Transfection remains a foundational tool in molecular and cellular biology, enabling the delivery of DNA or RNA into eukaryotic cells for transient or stable gene expression. It is widely used across research and bioprocessing applications, including gene function studies, recombinant protein production, and emerging therapeutic development. However, despite its broad utility, current approaches continue to face important practical and biological constraints.

Cells are inherently resistant to the introduction of foreign genetic material, and effective delivery must overcome multiple barriers, including the plasma membrane, intracellular degradation pathways, and endosomal entrapment. In practice, this results in variable uptake and inconsistent expression across cell populations. Transfection performance is further influenced by cell type, culture conditions, and the properties of the delivery vector, often requiring extensive optimisation for each system. Many commonly used methods also introduce cytotoxicity or stress responses, which can negatively impact cell viability and downstream biological function.

As a result, transient transfection workflows frequently suffer from trade-offs between efficiency, reproducibility, and cellular health. These limitations continue to present a significant bottleneck in both research and translational applications, particularly where robust, scalable, and high-quality gene delivery is required[1,2].

Technology Overview

Pulzar® Technology, PulzFector, and Pulzar Reagent Conditioner Overview

Pulzar® is a patented technology based on the transmission of very-low-power pulsed microwave signals centred at 2.45 GHz within the Industrial, Scientific, and Medical (ISM) band of the radio-frequency spectrum, as defined by the International Telecommunication Union (ITU) Radio Regulation[3]. The signal consists of a digitally generated, randomised sequence distributed across three channels (Figure 1). It is characterised by narrow pulse widths and comparatively long rest periods, resulting in an exceptionally low duty cycle of less than 2%.

Pulzar signals are delivered via two compact, lightweight, standalone, rechargeable electronic devices: the PulzFector (Figure 2A) and the Pulzar Reagent Conditioner (Figure 2B). Both devices share a common underlying Pulzar signal-generation architecture and operate as flat-bed platforms, but are configured for use at different stages of the nucleic acid delivery workflow.

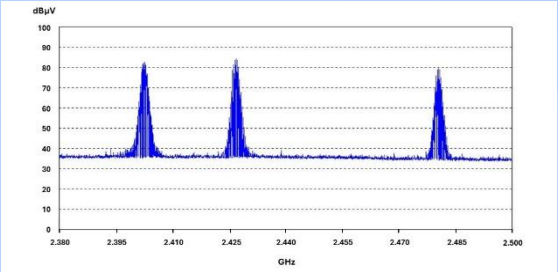

Figure 1. Anechoic Chamber Recording of Emissions from a Pulzar® Prototype Device.

Frequency-domain spectrum recorded in an anechoic chamber over an acquisition period of 4 minutes from a single-chip Pulzar prototype device. Distinct, discrete emission peaks are observed within the 2.38–2.50 GHz range against a stable background noise floor. Signal amplitude is reported in dBµV as a function of frequency (GHz). Measurements were performed under controlled conditions to characterise the electromagnetic output profile of the Pulzar device.

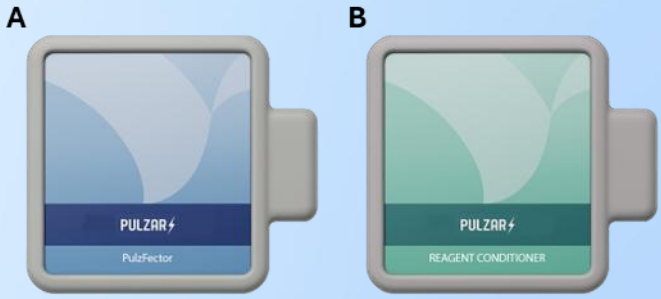

Figure 2. Pulzar® Prototype Devices.

Photographs of the PulzFector (A) and Pulzar Reagent Conditioner (B) prototype devices that house the Pulzar electromagnetic field-generating components and are designed to sit directly underneath cell culture vessels (A) or reagent preparation vessels (B), enabling field exposure through the vessel base or wall during operation.

The PulzFector (Figure 2A) is designed for the treatment of cells within a controlled cell culture environment. Operating as a flat-bed platform, the PulzFector supports standard plastic culture vessels placed directly onto its surface. The device incorporates a simple on/off toggle switch and a non-slip adhesive mat surface to securely hold shake flasks and other standard culture vessels. Multiple PulzFector units can be deployed within standard CO_2 incubators without modification to existing equipment. In this configuration, mammalian cell cultures are exposed non-invasively to Pulzar through the base of the culture vessel. Typical exposure durations range from 3 to 48 hours, depending on experimental or process requirements.

The Pulzar Reagent Conditioner (Figure 2B) is designed for the treatment of transfection reagents and nucleic acid complexes prior to cell exposure. This mat-style device is operated outside the incubator at room temperature in a bench-top configuration, where transfection reagent-DNA mixtures are exposed to Pulzar for shorter, defined treatment periods aligned with workflow-specific requirements.

Together, the PulzFector and Pulzar Reagent Conditioner enable targeted application of Pulzar technology to both cellular and non-cellular components of gene-delivery workflows. The PulzFector generates a controlled pulsed electromagnetic environment at the interface between living cells and their surrounding aqueous medium, while the Pulzar Reagent Conditioner provides an equivalent environment for reagent–nucleic acid complexes prior to cell contact. In both cases, Pulzar technology is intended to act on aqueous interfacial systems, including local hydration layers surrounding cells and macromolecular complexes, thereby influencing system-level properties of the aqueous environment relevant to nucleic acid delivery and gene transfer.

Putative Mechanistic Hypothesis and Rationale

The following section outlines a putative mechanistic hypothesis intended to provide a conceptual rationale for the observed effects of Pulzar technology. This hypothesis is based on established physical principles governing electromagnetic field interactions with polar aqueous systems[4]; however, it has not been directly tested experimentally in the studies described here. The experiments conducted were designed to evaluate the effects of Pulzar, rather than to isolate or confirm a specific molecular or biophysical mechanism of action.

The proposed mechanistic basis of Pulzar technology relates to the interaction of low-energy, pulsed electromagnetic fields with polar aqueous environments and interfacial structures. Within this framework, oscillating fields in the microwave frequency range are hypothesised to influence the collective rotational behaviour of water dipoles and the transient organisation of hydrogen-bond networks at biological interfaces.

Under pulsed field conditions, repeated cycles of field application and relaxation may induce dynamic reorientation of water molecules, leading to transient perturbations in local hydrogen-bond connectivity (Figure 3). Such cycling is hypothesised to create a continuously evolving, non-equilibrium hydration

environment at interfaces, including those surrounding cells and nucleic acid delivery complexes.

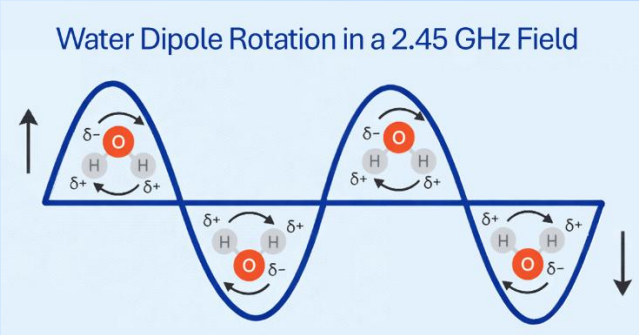

Figure 3. Schematic Illustration of Water Dipole Behaviour in an Oscillating Electromagnetic Field.

Conceptual representation of the rotational alignment of water molecular dipoles in response to an oscillating electromagnetic field at 2.45 GHz. The alternating field induces periodic reorientation of the polar water molecules, giving rise to dynamic dipole motion within the aqueous environment. This schematic is intended for illustrative purposes only and does not depict a specific molecular-scale mechanism of Pulzar action.

In systems containing reagent-DNA polyplexes, modulation of the surrounding hydration layer is hypothesised to influence colloidal stability, aggregation behaviour, and interfacial interactions with cellular membranes (Figure 4). A more uniformly distributed and dynamically equilibrated hydration environment could decrease local heterogeneity in surface interactions, potentially affecting uptake efficiency and downstream intracellular processing.

Within this proposed framework, Pulzar is not intended to act directly on genetic material or cells, but rather to establish an electromagnetic environment that may modulate solvent-mediated interactions affecting delivery system behaviour. This model is presented as a plausible system-level explanation for the observed effects, rather than as a validated mechanistic description.

Pulzar® Technology is designed to act on aqueous interfacial systems relevant to nucleic acid delivery

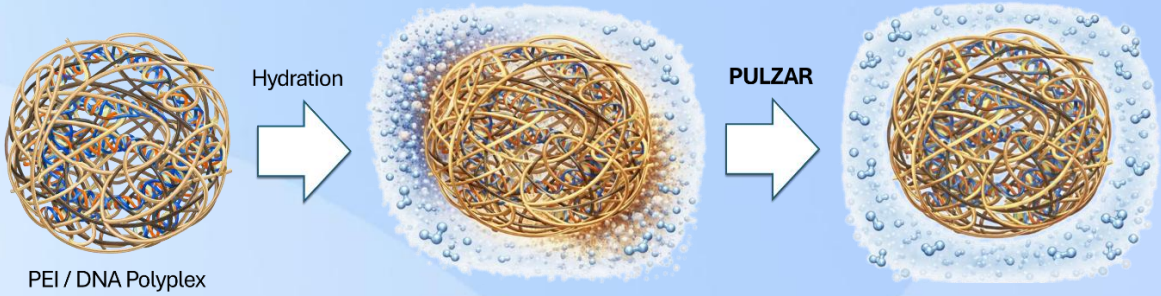

Figure 4. Conceptual Model of Pulzar®-Associated Effects on the Aqueous Hydration Environment Surrounding PEI–DNA Polyplexes.

Schematic illustration depicting a proposed system-level effect of Pulzar exposure on the aqueous hydration environment surrounding PEI–DNA polyplexes. Hydrophilic polyplex surfaces depend on a stabilising hydration layer for colloidal stability and effective transfection. In the absence of uniform hydration, irregularly distributed water layers may expose surface regions that promote polyplex coalescence, leading to larger aggregates associated with reduced transfection efficiency and increased cytotoxicity. Pulzar exposure is hypothesised to influence the local aqueous environment through periodic electromagnetic stimulation, potentially promoting a more uniform and dynamically equilibrated hydration layer around polyplexes. This schematic is illustrative and intended to describe a plausible system-level model rather than a defined molecular mechanism.

Experimental Approach

A series of experiments was designed to evaluate the effect of Pulzar® exposure on transient gene expression, viral vector production, and downstream transduction efficiency across multiple mammalian cell systems.

All experimental work was performed by independent third-party organizations, with execution and primary data generation conducted entirely externally. All studies, with the exception of those involving lentiviral vectors, were carried out at the University of Dundee in the laboratory of Dr. Jean-Christophe Bourdon. Lentiviral vector experiments were performed through a collaboration with a UK-based contract development and manufacturing organization (CDMO), whose identity remains confidential under a non-disclosure agreement. St Andrews Pharmaceutical Technology Ltd. was involved in the design and planning of the studies and in the visualisation of results. It had no role in the execution of experiments or primary data collection.

Luciferase-Based Analysis of Transient Transfection in Suspension and Adherent Cell Lines

Transient transfection studies were conducted in Jurkat (T lymphocyte), CHO (Chinese Hamster Ovary), and HCT116 (human colorectal carcinoma) cells using firefly luciferase reporter plasmids driven by Ad (adenovirus), IGFBP3 (Insulin-like growth factor-binding protein 3), and SV40 (simian virus 40) promoters. These constructs were used to assess whether Pulzar exposure influences promoter-dependent expression across diverse transcriptional regulatory contexts in both suspension and adherent cell systems. To dissect the contribution of Pulzar treatment at both the reagent–DNA complex and cellular levels, four experimental conditions were evaluated: a control transfection performed under standard incubation conditions (Control); transfection using Pulzar-treated reagent–DNA complexes under standard cell incubation (Pulzar Reagents/DNA); transfection with control reagent–DNA complexes followed by Pulzar exposure of the cells (Pulzar Transfected Cells); and transfection using Pulzar-treated reagent–DNA complexes followed by Pulzar exposure of the cells (Pulzar Reagents/DNA and Pulzar Transfected Cells). This design enabled independent and combined assessment of Pulzar effects on reagent–DNA complexes and cells. Expression output was quantified using a luminescence luciferase reporter assay.

GFP-Based Analysis of Transient Expression in HEK293 Freestyle Cells

HEK293F cells were transfected with a green fluorescent protein (GFP)-encoding plasmid to evaluate the effects of Pulzar exposure on protein expression dynamics at the population level. Two experimental conditions were compared: a control transfection performed under standard incubation conditions (Control), and a transfection with control reagent–DNA complexes followed by Pulzar exposure of the cells (Pulzar). GFP expression was monitored using Incucyte fluorescence microscopy, enabling quantification of total green fluorescence intensity alongside the number of GFP-positive cells over time. Together, these readouts allowed distinction between changes driven by an increased proportion of transfected cells versus changes in overall transgene output across the cell population under Pulzar treatment compared with control conditions.

rAAV Production and Functional Transduction Analysis

Recombinant adeno-associated virus (rAAV) was produced in HEK293F cells by transient transfection with a three-plasmid system

comprising a cytomegalovirus (CMV)-driven GFP plasmid flanked by AAV inverted terminal repeats (ITRs), an AAV capsid plasmid, and a helper plasmid. Two experimental conditions were compared: a control transfection performed under standard incubation conditions (Control), and a Pulzar condition involving cumulative Pulzar treatment, including exposure of the cells prior to transfection, Pulzar treatment of the reagent–DNA complexes, and continued Pulzar exposure following transfection (Pulzar). Following vector production, viral particles were harvested and used to transduce HT1080 cells. Transduction efficiency was quantified through GFP expression, enabling assessment of whether Pulzar exposure during production influences downstream viral functionality.

Lentiviral Vector Production and Functional Hepatocyte Transduction

Lentiviral vectors were produced in HEK293 suspension cells using standard packaging workflows through an external collaboration and subsequently evaluated in a hepatocyte transduction assay. Four experimental conditions were assessed: a control condition with no Pulzar exposure (Control); Pulzar treatment of the transfection reagent–DNA complexes alone (Pulzar Reagents/DNA); Pulzar pre-treatment of the producer cells prior to transfection with control reagent–DNA complexes (Pulzar Cells); and a combined condition in which both the producer cells and reagent–DNA complexes were treated with Pulzar (Pulzar Reagents/DNA and Pulzar Cells). Functional performance was assessed using functional titre measurements, reported exclusively as relative, normalised values. In line with confidentiality disclosure agreement (CDA) restrictions, underlying production details, raw data, and assay-specific methodologies were not disclosed, enabling comparative assessment of Pulzar-exposed and control conditions based on normalised functional output.

Results

Pulzar® technology has demonstrated improved gene delivery efficiency across multiple systems and cell types under experimental conditions.

Pulzar® Enhances Luciferase Reporter Expression Across Cell Lines

To assess the robustness and broad applicability of Pulzar-mediated enhancement of transient transfection, a series of ten independent experiments was conducted across multiple mammalian cell types, transfection reagents, and promoter architectures. Representative experiments illustrating Pulzar performance across these experimental dimensions are presented below.

Jurkat T Cells

Pulzar performance was evaluated in Jurkat T lymphocytes, a cell type that is historically challenging to transfect. Using TransIT-mediated delivery of an adenoviral (Ad) promoter-driven firefly luciferase construct, Pulzar treatment resulted in a marked increase in reporter gene expression relative to the untreated control (Figure 5). Improvements were observed across all Pulzar conditions, with the largest enhancement occurring when both the transfection reagent–DNA complex and the cells were exposed to Pulzar. These data demonstrate that Pulzar can substantially improve transfection efficiency in difficult-to-transfect cell models.

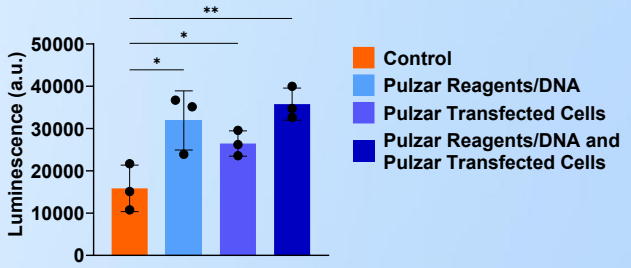

Figure 5. TransIT-Mediated Delivery of Ad-Driven Firefly Luciferase to Jurkat T Cells.

Jurkat T cells were transfected with an Ad-driven firefly luciferase reporter plasmid using TransIT and exposed to Pulzar under four conditions: untreated control, Pulzar treatment of the transfection reagent-DNA complex, Pulzar treatment of the transfected cells, or combined treatment of both. Luminescence was quantified as a measure of transfection efficiency and reporter gene expression. Pulzar exposure resulted in increases of approximately 102%, 67%, and 126% relative to control for reagent-only, cell-only, and combined treatment conditions, respectively. Data are shown as mean ± SD, with individual data points overlaid. Statistical significance is indicated (* $p < 0.05$; ** $p < 0.01$).

CHO Cells

To evaluate Pulzar performance in an industrially relevant context, transient transfection was assessed in CHO cells using Turbofect-mediated delivery of an Ad-driven firefly luciferase construct. Pulzar exposure again resulted in increased reporter gene expression compared with the control (Figure 6). Pulzar-associated improvements were detected across conditions, with the largest benefit occurring when both transfection reagent-DNA complexes and cells were exposed to Pulzar. These findings indicate that Pulzar is compatible with CHO cell systems and effectively enhances transient gene expression in a manufacturing-relevant cell background.

Pulzar® exposure is consistently associated with increased reporter gene expression across cell systems

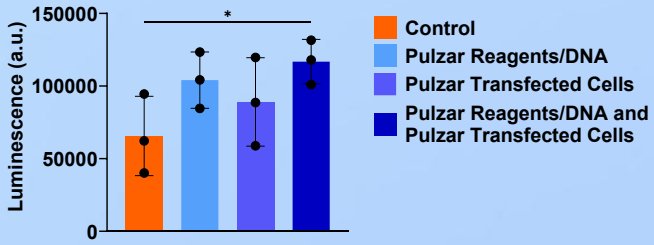

Figure 6. Turbofect-Mediated Delivery of Ad-Driven Firefly Luciferase to CHO Cells.

CHO cells were transfected with an Ad-driven firefly luciferase reporter using Turbofect under control conditions or following Pulzar treatment of the transfection reagent–DNA complexes, the cells, or both. Luminescence was quantified as a measure of transfection efficiency. Pulzar exposure resulted in increases of approximately 59%, 36%, and 78% relative to control for reagent-only, cell-only, and combined treatment conditions, respectively. Data are shown as mean ± SD with individual data points overlaid (* $p < 0.05$).

HCT Cells

The effect of Pulzar on transient transfection efficiency was also evaluated in HCT cells, a human colorectal carcinoma cell line, using PEI-mediated delivery of an Ad-driven firefly luciferase construct. Relative to the control, Pulzar exposure was associated with increased reporter gene expression across all treatment modes (Figure 7).

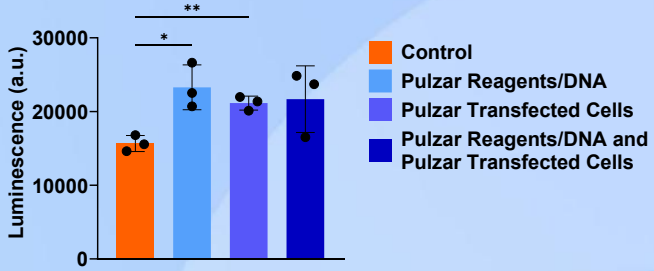

Figure 7. PEI-Mediated Delivery of Ad-Driven Firefly Luciferase to HCT Cells.

HCT cells were transfected with an Ad-driven firefly luciferase reporter using PEI under control conditions or following Pulzar treatment of the transfection reagent–DNA complexes, the cells, or both. Luminescence was quantified as a readout of transfection efficiency and reporter gene expression. Pulzar exposure resulted in increases of approximately 49%, 35%, and 38% relative to control for reagent-only, cell-only, and combined treatment conditions, respectively. Data are shown as

mean ± SD, with individual data points overlaid (* $p < 0.05$; ** $p < 0.01$).

Next, to assess whether Pulzar-mediated enhancement of transgene expression is dependent on promoter architecture, additional experiments were conducted using alternative expression drivers within the same cellular and transfection context. PEI-mediated delivery of firefly luciferase constructs driven by either the IGFBP3 promoter or the SV40 promoter was evaluated in HCT cells using a two-condition design (control versus Pulzar-treated cells).

Across both promoter contexts, Pulzar exposure resulted in comparable increases in reporter gene expression relative to control (Figure 8A-B). Together, these findings indicate that Pulzar-associated improvements in transgene expression are not restricted to a specific promoter architecture and suggest that the Pulzar effect is independent from promoter-specific transcriptional regulation.

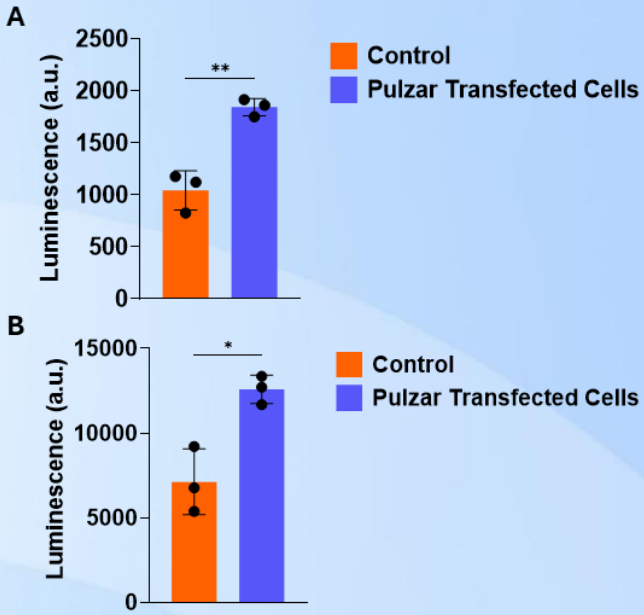

Figure 8. PEI-Mediated Delivery of IGFBP3- and SV40-Driven Firefly Luciferase to HCT Cells.

HCT cells were transfected with firefly luciferase constructs driven by either the IGFBP3 promoter (A) or the SV40 promoter (B) using PEI under control conditions or following Pulzar treatment of the transfected cells. Luminescence was quantified as a readout of transient transfection efficiency and reporter gene expression. Pulzar exposure resulted in an approximately 77% increase in reporter expression relative to control for both promoter contexts. Data are shown as mean ± SD, with individual data points overlaid (* $p < 0.05$; ** $p < 0.01$).

Pulzar® Increases GFP Expression in HEK293 Freestyle Cells

The effect of Pulzar exposure on transient transfection performance was assessed in HEK293 Freestyle suspension cells transfected with a GFP-encoding plasmid using either PEI or Fectin transfection reagents. Cells were incubated for 3 hours under either standard conditions (Control) or undergoing Pulzar treatment prior to live-cell monitoring by Incucyte imaging.

In a representative experiment using PEI, Pulzar treatment resulted in a marked increase in transfection performance without impacting cell growth. Total cell confluence remained comparable between Pulzar-treated and control cultures throughout the monitoring period, indicating no effect on overall cell number or viability (Figure 9A). In contrast, Pulzar exposure led to a pronounced increase in the number of GFP-positive cells over time (Figure 9B), alongside a substantial elevation in total population-level GFP signal intensity (Figure 9C). Quantification at 72 hours post-transfection demonstrated an ~82% increase in total GFP intensity in Pulzar-treated cells relative to control conditions (Figure 9D).

These findings are representative of three independent experiments, including two conducted using the same HEK293 cell system and PEI transfection reagent and one performed using Fectin as an alternative transfection reagent. Across all experiments, Pulzar exposure consistently increased both the number of GFP-positive cells and total GFP signal intensity, while maintaining comparable cell confluence between Pulzar-treated and control conditions. Together, these data indicate that Pulzar enhances transient transfection efficiency in HEK293F cells in a manner that is reproducible across

experimental repeats and transfection chemistries.

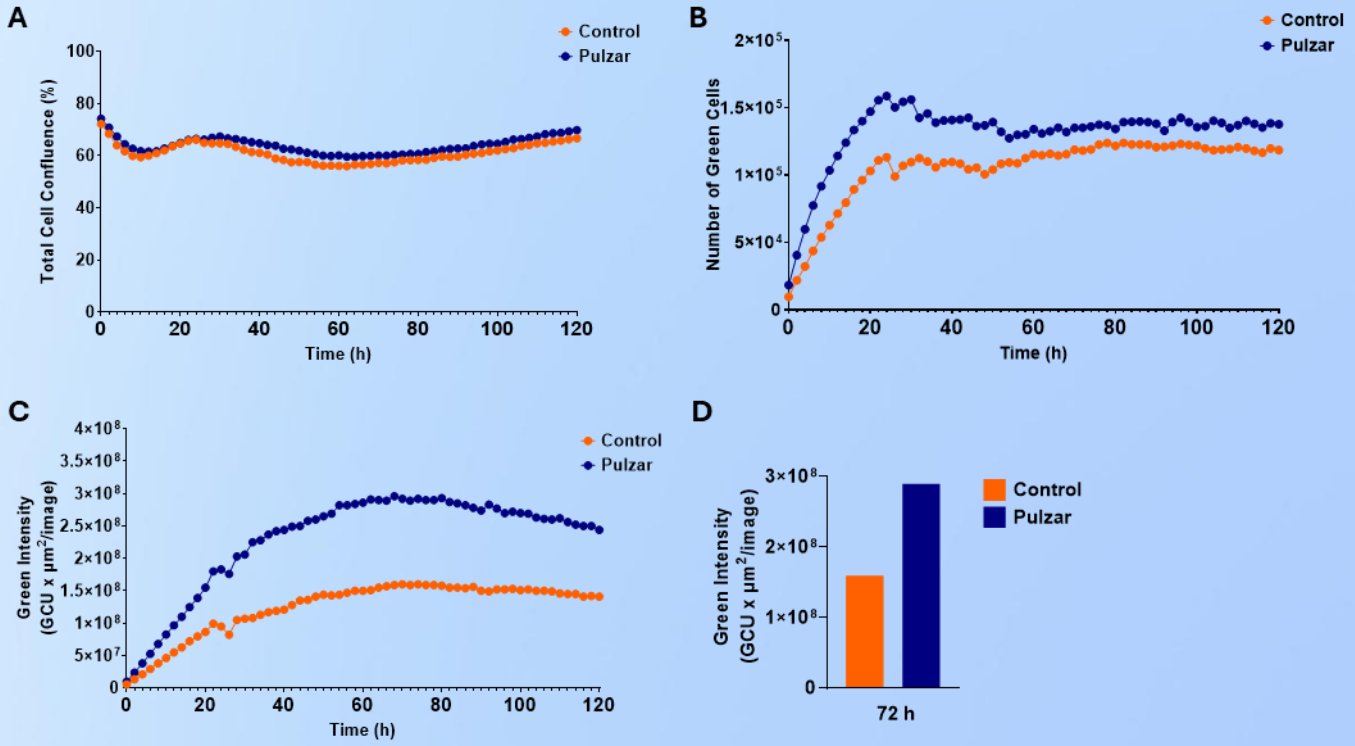

Figure 9. Pulzar® Enhances Transient Transfection Efficiency in HEK293F Cells.

Representative Incucyte analysis of HEK293F cells transfected with a GFP-encoding plasmid and exposed to either standard incubation conditions (Control) or Pulzar treatment for 3 hours post-transfection (Pulzar). (A) Total cell confluence over time, showing no significant difference between control and Pulzar-treated conditions. (B) Number of GFP-positive cells, demonstrating an increased proportion of transfected cells following Pulzar exposure. (C) Total population-level GFP fluorescence intensity over time, indicating enhanced overall transgene output under Pulzar treatment. (D) Quantification of total GFP fluorescence intensity at 72 hours post-transfection, showing an approximately 82% increase in the Pulzar condition relative to control.

Pulzar® Improves rAAV Genome Yield and Functional Transduction Output

Pulzar effects on rAAV production and downstream functional transduction of target cells were evaluated in a single, preliminary experiment. rAAV was produced in HEK293F cells under control or Pulzar conditions and subsequently used to transduce HT1080 fibroblast cells, with equal volumes of viral supernatant applied across conditions.

Pulzar exposure during rAAV production resulted in a 63% increase in average rAAV genome copy number relative to control conditions (Figure 10). This indicates enhanced vector yield at the level of viral genome production in HEK293F producer cells.

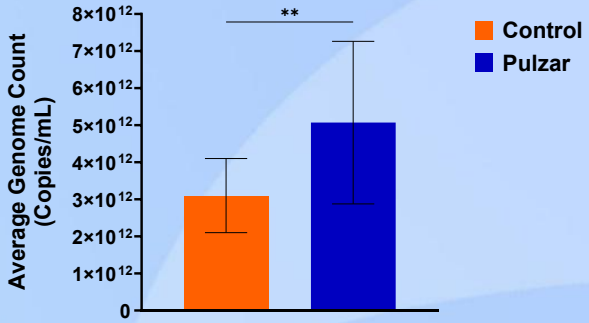

Figure 10. Increased rAAV Genome Yield During Production.

Average rAAV genome copy number produced in HEK293F cells under control conditions or following Pulzar exposure during vector production. Pulzar treatment resulted in an approximately 63% increase in rAAV genome copy number relative to control, indicating enhanced viral genome yield in producer cells. Genome copies are expressed as copies per millilitre. Data are

shown as mean ± SD, with statistical significance indicated (** $p < 0.01$).

Viral supernatants derived from Pulzar-treated producer cells drove a marked increase in GFP-positive HT1080 cells over time compared to control-derived vectors (Figure 11A). When averaged across the infection time course, this corresponded to an approximately 2.7-fold (or 270%) increase in apparent infectivity for the Pulzar condition (Figure 11B).

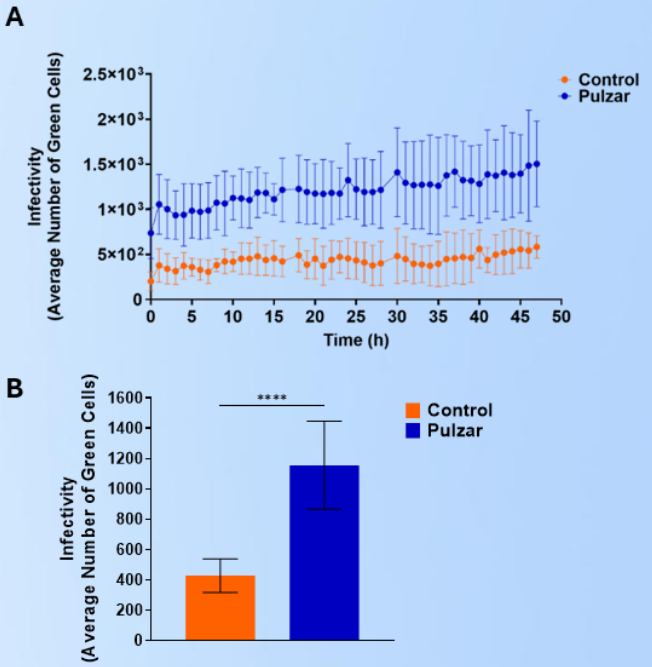

Figure 11. Enhanced Apparent Transduction of HT1080 Cells by rAAV Produced Under Pulzar® Conditions.

Live-cell monitoring by Incucyte of HT1080 fibroblast cells transduced with equal volumes of GFP-expressing rAAV-containing supernatant derived from control or Pulzar-treated HEK293F producer cells. (A) Time-course analysis showing a higher number of GFP-positive HT1080 cells following exposure to Pulzar-derived rAAV compared with control-derived material. (B) Average number of GFP-positive cells across the monitoring period, demonstrating an approximately 2.7-fold increase in apparent infectivity for the Pulzar condition relative to control. Data are shown as mean ± SD, with statistical significance indicated (**** $p < 0.0001$).

Importantly, viral genome copy number was not normalised prior to transduction, and identical volumes of rAAV-containing supernatant were used across conditions. Consequently, HT1080 cells infected with Pulzar-derived material were exposed to a higher total rAAV genome load, consistent with the observed increase in genome production during vector manufacture. Notably, the magnitude of the increase in GFP-positive cells (~2.7-fold) exceeded the corresponding increase in rAAV genome copy number (~63%), suggesting that Pulzar exposure may additionally enhance the functional quality or infectivity of the produced rAAV particles. Further studies employing genome-normalised dosing will be required to directly assess infectivity per-particle.

Collectively, these preliminary data indicate that Pulzar treatment increases rAAV genome yield and may also improve downstream functional performance beyond what can be explained by genome copy number alone.

Pulzar® Enhances Functional Lentiviral Gene Delivery in Hepatocyte Models

The impact of Pulzar exposure on HEK293 suspension producer cells was first evaluated to assess potential upstream effects relevant to lentiviral production. Exposure of HEK293 cells to Pulzar for 48 hours prior to vector production resulted in enhanced cell growth, reflected by a higher maximum VCD (Figure 12A) and a reduced population doubling time compared with untreated controls (approximately 19 hours versus 22 hours, data not shown). Pulzar exposure during this pre-production phase had no detrimental effect on cell viability (Figure 12B).

Pulzar® Technology enhances gene delivery performance without altering core workflow components

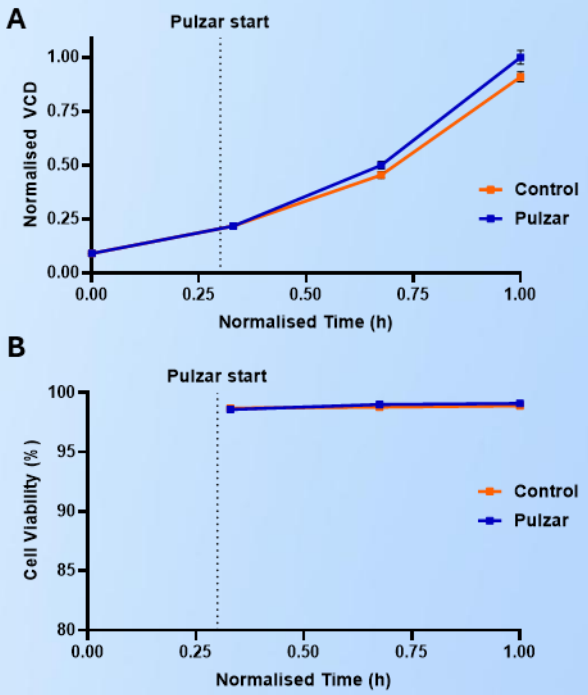

Figure 12. Pulzar® Pre-Treatment of HEK Producer Cells Increase Maximum Viable Cell Concentration Without Affecting Viability.

HEK293 suspension producer cells were pre-treated with Pulzar for 48 hours prior to vector production and compared with untreated control cells. (A) Normalised viable cell density (VCD) over time, showing increased cell growth and a higher maximum VCD following Pulzar pre-treatment (Pulzar P4) relative to control. (B) Cell viability over the same time course, demonstrating comparable viability profiles between Pulzar-treated and control conditions. Data are shown as mean ± SD; Control: n = 6; Pulzar: n = 8. The dashed line indicates the initiation of Pulzar exposure.

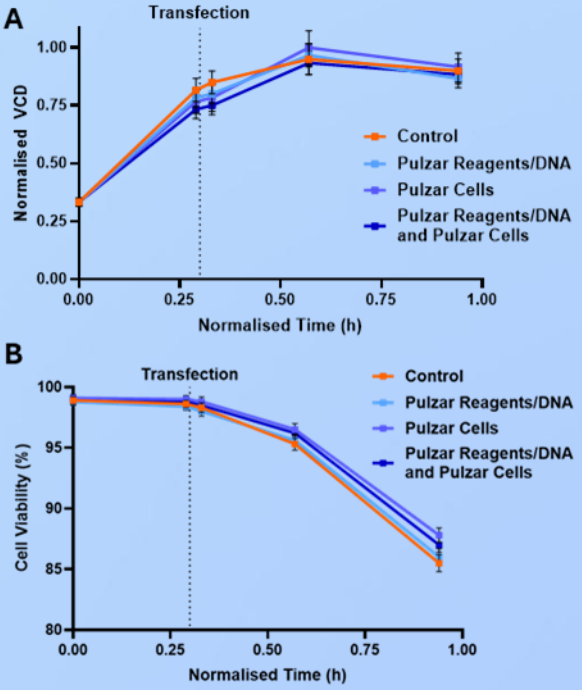

Figure 13. HEK293 Producer Cell Growth and Viability During Lentiviral Vector Production.

HEK293 suspension producer cells were monitored during lentiviral vector production under four Pulzar exposure conditions: untreated control, Pulzar treatment of the transfection reagent-DNA complexes (Pulzar Reagents/DNA), Pulzar treatment of the cells prior to production (Pulzar Cells), or combined treatment of both transfection complexes and cells (Pulzar Reagents/DNA and Pulzar Cells). (A) Normalised VCD over the production time course, demonstrating comparable growth profiles across all conditions. (B) Cell viability over the same time course, showing similar viability trends between Pulzar-treated and control cultures. The dashed line indicates the timing of transfection (TFX). Data are shown as mean ± SD.

During the lentiviral vector production phase, HEK293 producer cells exposed to Pulzar (either via treatment of the cells, the transfection reagent–DNA complexes, or both) exhibited comparable VCD and viability profiles to control cultures across the production time course (Figure 13A-B). These data indicate that Pulzar does not compromise producer cell health during lentiviral manufacture and supports its compatibility with suspension-based production workflows.

Pulzar® exposure supports healthy cell conditions

Lentiviral preparations generated under the four experimental conditions (Control, Pulzar Reagents/DNA, Pulzar Cells, and combined treatment Pulzar Reagents/DNA and Pulzar Cells) were subsequently evaluated for functional gene delivery in hepatocyte systems.

Functional performance was assessed using normalised functional titre measurements, provided exclusively as relative values. Notably, physical vector measurements were generated by the external collaborator and used to normalise vector input prior to transduction, ensuring that hepatocyte infections were

performed using equivalent vector doses across conditions. However, the underlying physical vector data are not disclosed under the terms of the CDA.

Across conditions, Pulzar exposure was associated with a clear enhancement of functional gene delivery relative to control. Treatment of either the transfection reagent–DNA complexes (Pulzar Reagents/DNA) or the producer cells alone (Pulzar Cells) resulted in intermediate increases in normalised functional titre, while the combined Pulzar condition (Pulzar Reagents/DNA and Pulzar Cells) produced the greatest effect, corresponding to an approximately two-fold increase in functional gene delivery efficiency in hepatocyte models (Figure 14). The additive pattern observed across conditions suggests that Pulzar acts through both reagent-level and cell-level mechanisms to enhance lentiviral functional performance.

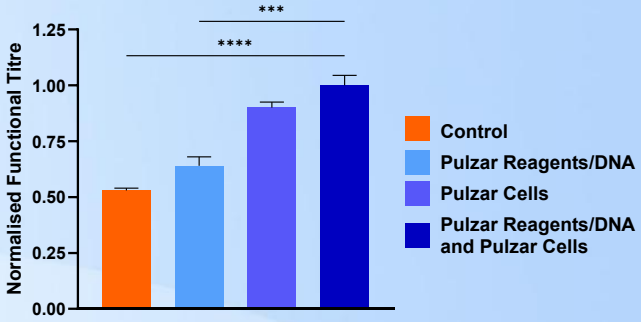

Figure 14. Normalised Functional Titre Following Transduction of Hepatocytes.

Functional gene delivery was assessed in hepatocyte systems following transduction with lentiviral vectors produced in HEK293 suspension cells under four Pulzar exposure conditions: untreated control, Pulzar treatment of the transfection reagent–DNA complexes (Pulzar Reagents/DNA), Pulzar pre-treatment of the producer cells (Pulzar Cells), or combined treatment of both cells and transfection complexes (Pulzar Reagents/DNA and Pulzar Cells). Functional titre is reported as normalised values, enabling relative comparison across conditions. Pulzar treatment resulted in significant increases in functional titre relative to control, with the largest enhancement observed under the combined Pulzar Reagents/DNA and Pulzar Cells condition. Data are shown as mean ± SD, with statistical significance indicated (*** $p < 0.001$; **** $p < 0.0001$).

Together, these results demonstrate that Pulzar favourably influences HEK293 producer cell growth characteristics without adversely affecting viability and is associated with a significant enhancement of normalised functional lentiviral transduction in hepatocyte systems. As functional titres were assessed following vector dose normalisation, these findings support a role for Pulzar in improving lentiviral vector potency rather than reflecting differences in vector input. Collectively, the data highlight Pulzar as a promising adjunct for enhancing lentiviral gene delivery performance within manufacturing-relevant workflows.

Pulzar® Technology may offer practical benefits across gene delivery workflows by improving efficiency and consistency without introducing additional biological or chemical complexity, with potential value in both research and therapeutic manufacturing settings

"Pulzar has become the device of choice for hard-to-transfect cell types, including primary, stem, and cancer cells, across both suspension and adherent systems."

Dr Jean-Christophe Bourdon, University of Dundee

Discussion

Across a diverse set of experimental systems, Pulzar® exposure was consistently associated with improved functional gene delivery outcomes. Enhancements were observed in non-viral transfection assays using luciferase and GFP reporters, as well as in viral vector workflows involving both rAAV and lentivirus production. These effects spanned multiple cell types, including suspension and adherent cells, were compatible with a range of transfection reagents, and were observed across distinct promoter architectures, suggesting a broadly applicable rather than system-specific effect. Improvements were frequently additive when Pulzar was applied to both transfection complexes and producer cells, while no detrimental effects on cell growth or viability were observed.

Notably, the observed increases in reporter expression and vector productivity suggest the potential to achieve equivalent protein output using lower amounts of input DNA. Such a decrease in DNA requirements could offer practical advantages, including decreased reagent consumption, decreased cytotoxicity associated with high DNA or transfection reagent loads, and improved scalability and cost-efficiency in both research and manufacturing workflows[5]. While systematic DNA-titration studies were not performed at this stage, the consistency of enhanced expression at fixed DNA inputs supports this possibility and warrants further investigation.

Although some datasets reflect exploratory studies and varying experimental frameworks, particularly for viral vector analyses, the overall consistency of the observed trends supports the potential of Pulzar to contribute to more efficient and consistent gene delivery across vector systems relevant to gene therapy workflows.

Applications and Potential Use Cases

The results presented in this white paper suggest that Pulzar® may offer practical benefits across a range of gene delivery workflows by improving efficiency and consistency without introducing additional biological or chemical complexity. In research laboratory settings, Pulzar could be used to enhance transient transfection performance in both suspension and adherent cell lines, including cell types that are traditionally difficult to transfect. By increasing reporter expression and reducing variability across experiments, Pulzar may improve data robustness and reduce the need for repeated optimisation or increased reagent input.

In the context of gene and cell therapy development, Pulzar may provide value within upstream vector production workflows. The observed improvements in viral genome yield, functional transduction, and normalised lentiviral titre, together with the absence of negative effects on producer cell viability or growth, suggest that Pulzar could support more reliable and potentially more efficient vector manufacture. Such improvements may contribute to better process consistency and functional output, which are critical considerations during process development and scale-up.

Across both research and translational settings, Pulzar is positioned as a non-invasive, workflow-compatible technology that can be integrated into existing transfection and vector production processes with minimal procedural change. This flexibility supports its potential use as an enabling technology across a broad range of gene delivery applications.

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