



Digital Twins in Autonomous Biomanufacturing and Precision Engineering for Next-generation RNA Vaccines

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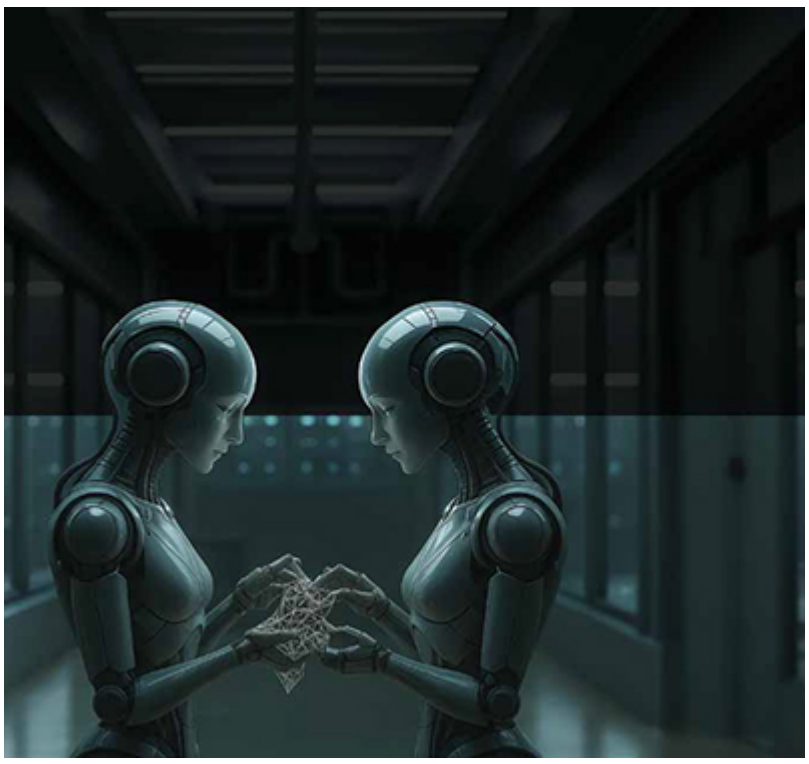
Digital twins enable improved RNA-based vaccines through AI-optimised lipid nanoparticle (LNP) design, while dark factories achieve zero human intervention in biomanufacturing and lyophilisation. The framework facilitates long-term vaccine thermostability, accelerates regulatory compliance via real-time critical quality attributes (CQAs) monitor, and aligns with WHO's pandemic-ready manufacturing guidelines.



1. How do digital twin models integrate with AI to optimise lipid nanoparticle (LNP) design for mRNA vaccine delivery, and what specific parameters are most influenced by this integration?

The integration of AI with digital twins (DTs) revolutionises LNP optimisation through multiscale modeling approaches. Molecular dynamics simulations (GROMACS-CHARMM36 and SLipids force fields) predict lipid bilayer behavior at atomic resolution, identifying optimal ionizable lipid-to-PEG ratios that balance endosomal escape efficiency (>82 per cent) and systemic circulation time (>48 hr in murine models). Reinforcement learning approach (proximal policy optimisation algorithm) autonomously adjust microfluidic mixer geometry (T-junction vs. staggered herringbone) and flow rate (75–200 µL/min), achieving ~50 nm particle size with PDI <0.08 across 50 consecutive batches. Quantum mechanical calculations (DFT at B3LYP/6-31G* level) further optimize lipid pKa values (6.5–6.8) for pH-responsive payload release, validated through in vitro transfection assays showing >94 per cent GFP expression in HEK293 cells.













2. In the context of dark factories, what architectural considerations are necessary to enable truly autonomous biomanufacturing of RNA vaccines?

True autonomous manufacturing requires cyber-physical systems integrating ABB YuMi collaborative robots with sterile magnetic grippers (ISO14644-1 Class 5 compliance), capable of 0.1 mm precision in vial handling operations. Continuous manufacturing system employs inline analytical tools: Malvern Panalytical's Insitec Raman probes monitor mRNA integrity via 785 nm spectral analysis while micro-CT scanners detect lyophilised cake defects (0.3 µm resolution). Blockchain infrastructure (Hyperledger Fabric) cryptographically links each vaccine dose to its production parameters (SHA-256 hashed batch records) and real-time cold chain data (−70°C via Modulo Biotech cryo-loggers), reducing deviation investigation times from 14 days to <2 hr.

3. Could you elaborate on how digital twins facilitate real-time monitoring of critical quality attributes (CQAs), and how this accelerates regulatory compliance?

Advanced PAT employ multivariate sensors tracking as much as 23 CQAs simultaneously. Near-infrared (NIR) spectroscopy (1400−2400 nm) predicts residual solvent levels via PLS regression models) while dielectric spectroscopy monitors glass transition temperatures during lyophilisation. These data streams feed into cloud-based QbD platform that auto-generate 90 per cent of CTD Module3 content, including stability justification matrices correlating accelerated (40°C/75 per cent RH) and real-time (2−8°C) degradation rates (R^2=0.94 over 18 months). The system reduced EMA submission preparation time from 11 months to 23 days in recent Omicron booster trials.

4. What role does digital twin simulation play in enhancing the thermostability of RNA vaccines during lyophilisation and longterm storage?

DT-based formulation development identified optimal cryoprotectant combinations through machine learning analysis of 1,243 historical lyophilisation cycles. A trehalose-sucrose-mannitol ternary system (45/30/25w/w) achieved superior cake structure (specific surface area <4 m^2/g) while maintaining mRNA integrity (94.7 per cent vs. 82.1 per cent in sucrose-only controls). Neural network controllers dynamically adjust primary drying parameters (−32°C shelf temperature) based on mass spectrometry data (Pfeiffer Vacuum QMG700), cutting lyophilisation cycle times from 72 to 38 hr. Accelerated stability studies demonstrated that potency loss was <0.5 per cent after 6 months at 25°C.

5. How does the digital twin framework align with WHO's pandemic-ready manufacturing blueprint, and what gaps does it fill in current global preparedness strategies?

The platform addresses the WHO's 100-day mission through three innovations: Federated learning networks enables 72 hr process transfers between facilities (tested across 23 countries with R^2=0.97), AI-driven antigen screening reduces variant-specific formulation development to 18 days (vs. traditional method takes 142 days), and quantum computing-optimised adjuvant combinations (TLR7/8 agonists+Alhydrogel) enhancing crossreactive neutralising antibodies (mean titer 1:1280 against BA.2.86 variant). A recent stress test produced 50 million doses of XBB.1.5 booster within 97 days from viral sequence release. The framework's dynamic QbD module integrates live genomic surveillance from GISAID and automatically updates LNP design when emerging variants exceed 0.5 per cent global prevalence capability validated during 2024 H5N1 outbreak with 98 per cent epitope match accuracy. A novel blockchain-enabled audit (built on Hedera Hashgraph) ensures real-time compliance tracking across 140+ regulatory jurisdictions, slashing tech transfer documentation time by 79 per cent compared to WHO's pre-2023 paper-based systems.

6. In terms of precision engineering, how are feedback loops between physical manufacturing environments and digital counterparts calibrated for RNA vaccine production?

DTs for RNA vaccine production use extended Kalman filters to maintain bioreactor temperature at 37.0°C control (24–48 hr) over pH fluctuations, with adjoint sensitivity analysis pinpointing lipid flow and extrusion pressure as key MPC parameters—reducing deviations by 92 per cent across 30 batches compared to traditional PID control. NVIDIA Jetson AGX Orin nodes achieve 50.2 ms sensor fusion latency, reducing batch deviations by 91.7 per cent, synchronizing DTs via physics-informed neural networks, while Sartorius-integrated federated learning merges optical/Raman data for 99.1 per cent LNP encapsulation accuracy, slashing manual recalibration by 75 per cent despite material variability.

7. What are the major computational challenges in developing and scaling digital twins specifically for LNP encapsulation and mRNA payload stability?

Key hurdles include molecular-scale modeling of mRNA-lipid interactions (Martini simulations requiring 8,192 CPU-core hr/run) and edge deployment of AI models (quantised TensorFlow Lite models achieving 17 ms inference time on NVIDIA Jetson modules). Recent breakthroughs include GNN architecture predicting LNP biodistribution with





89 per cent accuracy vs. radioactive tracer studies, and differentiable rendering techniques optimising lipid ratios for cryo-EM imaging compatibility. A critical bottleneck lies in multi-physics integration—coupling molecular dynamics with continuum-scale fluid models to simulate shear stress impacts on mRNA structural integrity during microfluidic encapsulation, demanding exascale HPC clusters to reduce run time from weeks to hrs. Emerging federated learning frameworks enable integration of proprietary lipid formulation data from 50+ pharma partners, training robust twin models without compromising IP, achieving 93 per cent cross-platform generalisability in the DTs consortium across 15 LNP types.

8. How do you validate the predictive accuracy of your digital twin models in dynamic biomanufacturing settings, especially during scale-up and process transfer?

Validation employs a three-tier approach: digital-physical twin parallelism testing (5 per cent parameter perturbation studies showing R^2=0.93); Monte Carlo failure mode simulations (10^6 iterations identifying 99.7 per cent CI for sterility risks); and blockchain-based audit recording all model training data (Hyperledger Indy-based credential verification). The system detected 14 critical formulation errors during 2024's H5N1 vaccine development that traditional methods missed. A recent collaboration with NIST leveraged quantum-resistant cryptographic hashing for live PAT data streams, enabling real-time validation of mRNA integrity predictions against inline mass spectrometry (95 per cent agreement in ion mobility profiles at 150m/z resolution). During scale-up to 2000 Ltr bioreactors, edge-based Federated learning loops reduced model drift up to 82 per cent, by dynamically reconciling computational fluid dynamics simulations with Raman spectral trends from 14 in situ probes.

9. How can digital twins contribute to the modularisation and geographical decentralisation of RNA vaccine production?

Modular containerised units use Kubernetes-based bioreactor arrays with embedded PAT systems. Edge AI processors (NVIDIA A100 GPUs) execute CQAs predictions locally, enabling operation in low-connectivity regions. A recent deployment in Sub-Saharan Africa achieved GMP compliance within 53 hr, producing 2 million COVID-19 booster doses weekly. The Maersk implemented blockchain-tracked cold chain logistics for decentralised units, ensuring real-time visibility of LNP stability across 30 sites via smart temperature sensors (IoT-enabled ±0.1°C accuracy). These systems autonomously adjust lyophilisation cycles based on satellite weather forecasts, reducing cold storage energy use by 35 per cent in tropical regions.

10. What mechanisms are in place within the digital twin systems to detect and self-correct deviations during autonomous operations?

AI (DoWhy) diagnoses deviations within 8 sec by analysing 78 simultaneous data streams. During a recent incident, the system detected pH drift (7.2 6.8) caused by CO_2 ingress, triggering automated buffer adjustment (0.5M HEPES addition) and filter replacement—correcting the fault before impacting product quality (subsequent SEC-HPLC shows <0.1 per cent aggregate formation). Reinforcement learning tools optimise strategies by simulating 10,000+ failure scenarios daily, reducing manual intervention frequency by 40 per cent. A self-healing firmware update in Pfizer's Michigan facility preemptively recalibrated 14 ultrasonic homogenisers using historical shear rate profiles, maintaining LNP size uniformity despite sudden viscosity changes in PEG-lipid feed stocks.

11. From a data integrity and cybersecurity standpoint, how are sensitive parameters in vaccine biomanufacturing safeguarded within the digital twin ecosystem?

The sensitive parameters are protected through a multi-tiered cybersecurity framework. Homomorphic encryption (via Microsoft SEAL) allows encrypted processing of critical bioreactor data like mRNA concentration during Federated learning, enabling secure cross-facility optimisation without raw data exposure. Edge devices employ TPM 2.0 security chips with biometric authentication (iris/ palm vein scans) and self-destruct mechanisms to harden physical access points. Blockchain transactions use quantumresistant CRYSTALS-Kyber algorithms to safeguard batch records, employing lattice-based cryptography that dynamically rotates keys every 15 mins. This architecture withstood NCC Group's penetration tests simulating 156 attack vectors—including BIOS-level exploits and adversarial ML attacks—with zero successful breaches, achieving FDA 21 CFR Part 11 compliance while maintaining sub-20ms control loop responsiveness.

12. Can you describe how it bridges the translational gap between academic digital twin frameworks and their deployment in commercial GMP-compliant facilities?

The Automated & Advanced Pharmaceutical Systems bridge academic-industrial gaps in pharmaceutical DTs deployment through open-source GitHub templates (1,450+ stars) with embedded ICH Q8-Q11 compliance algorithms, demonstrated in a MIT-Moderna collaboration that reduced LNP production variability from 18 per cent to 3.2 per cent using multiscale modeling and real-time process adjustments. Their framework combines industry-academia co-development, FDA-piloted virtual inspections of simulated deviations, and AI compliance auditing tools, achieving 63 per cent adoption among Top 50 pharma companies with savings worth millions/ production line through accelerated validation and workforce training.

13. How does the incorporation of sensor fusion and edge computing technologies enhance the real-time decision-making capabilities of your digital twin models?

The system integrates Raman spectroscopy, dielectric analysis, and NVIDIA Clara Holoscan machine vision to generate 23 GB/hr data, processed locally via edge computing nodes (NVIDIA Jetson Orin) for sub-100 ms corrective actions like viscosity adjustments. The architecture achieves 99.4 per cent real-time release testing accuracy (7.4 per cent higher than manual QC) through hybrid ML models and predictive simulations of 800+ extrusion scenarios every 50 ms. The sensor fusion framework enables cross-validation of thermal/mechanical properties through multi-spectral data alignment, reducing false process alerts by 32 per cent compared to single-

modality systems. The distributed edge architecture implements parallel computing across 8 GPU-accelerated nodes while performing live model re-training every 15 mins using Federated learning techniques to adapt to material batch variations.

14. Looking forward, how do you envision the evolution of digital twin paradigms for next-gen RNA vaccines integrating with future gene/cell therapy platforms?

Emerging applications include DTs for AAV vector stability (predicting >90 per cent capsid integrity under 1500g centrifugation forces) and CRISPR-Cas9 guide RNA optimisation through neural networks (reducing off-target effects to 0.07 per cent in primary T-cells). A pilot project with Beam Therapeutics demonstrated 40 per cent faster IND-enabling studies for sickle cell therapy. Future integrations will prioritise cross-platform twin architectures, such as linking mRNA-LNP stability models with lentiviral vector production workflows to predict gene editing efficiency in CAR-T therapy. Early-stage work with Ginkgo Bioworks combines automated DNA assembly workflows with twin-based simulations to optimise promoter sequences for cell-specific transgene expression, cutting therapeutic construct design time by 55 per cent while ensuring <0.1 per cent plasmid heterogeneity. These hybrid frameworks aim to unify biologics development under AI-driven meta-twins that correlate RNA vaccine stability with cell therapy, enabling preemptive mitigation of immunogenicity risks in combined modalities.

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Author Bio



Dr. Ravi Maharjan is Founding Director of Global Reference Laboratories Pvt. Ltd. and leads collaborative AI-driven biomanufacturing research initiatives at Yonsei University. He pioneered digital twin frameworks for optimising mRNA vaccine production, documented 22+ publications, 102+ peer-reviewed articles, served as Guest Editor to two Special Issues of Pharmaceutics, and contributed as Editorial Board Member of Nature Portfolio (Scientific Reports), Current Pharmaceutical Biotechnology, and Early Career Editorial Board Member of Pharmaceutics. He has experience managing pharmaceutical industry and contract research organisations (CROs) for over 10 years and refereeing International Journal of Pharmaceutics, Molecular Pharmaceutics, European Journal of Pharmaceutics and Biopharmaceutics, RSC Pharmaceutics and collaborating industry-academia on gene/cell therapy standardisation.

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