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Artificial Intelligence Driven Digital Twin Framework for Real-Time Monitoring and Quality Control of GMP Stem Cell Manufacturing Processes with Nonlinear Bifurcation Analysis

Khadija Sankoh[1]

[1]Department of Natural and Mathematical Sciences, University of Maryland Baltimore County, Catonsville, Maryland, USA.

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Abstract: This study develops a simulation-based artificial intelligence (AI) digital-twin framework for real-time monitoring and quality control of Good Manufacturing Practice (GMP) stem-cell manufacturing processes, with nonlinear bifurcation analysis used to identify loss-of-stability boundaries before conventional process limits are crossed. The supplied study dataset represents 60 manufacturing batches and 43,200 synchronized five-minute process states, including 14 online process variables and eight laboratory or soft-sensor critical quality attributes (CQAs). The framework combines temporal prediction, mechanistic state estimation, anomaly detection, and AUTO-07p continuation to generate a bifurcation-proximity index for quality-risk assessment. On the held-out simulation set, the integrated AI digital twin with bifurcation layer achieved 96.2% accuracy, an F1-score of 95.8%, AUROC of 0.978, and AUPRC of 0.969 for impending GMP deviation detection. Digital-twin viability prediction reached R^2 = 0.954 with an RMSE of 1.82 percentage points. Continuation analysis identified two fold points (LP1 and LP2), a Hopf point (HB1), and a periodic-orbit envelope that separated stable high-viability operation from oscillatory or undesirable regimes. The proposed system increased median warning lead time to 118 min while reducing the false-alarm rate to 2.7%, and 88% of simulated deviations were classified as avoidable under timely corrective action. These findings support bifurcation-aware digital twins as a promising research architecture for predictive quality assurance, while emphasizing that experimental and regulatory validation on real GMP manufacturing data remains necessary.

Keywords: *Artificial Intelligence, Bifurcation Analysis, Digital Twin, GMP, Process Analytical Technology, Quality Control, Stem-Cell Manufacturing, AUTO-07p.*

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I. INTRODUCTION

Cell-based therapeutics require manufacturing systems capable of producing living products with reproducible identity, purity, viability, potency, and functional activity despite intrinsic biological variability. This challenge is particularly acute for stem-cell platforms because expansion, differentiation state, metabolic condition, and phenotype can change in response to comparatively small shifts in culture pH, dissolved oxygen (DO), nutrient supply, temperature, hydrodynamic stress, and metabolite accumulation. Contemporary GMP practice therefore places strong emphasis on process understanding, material control, in-process testing, validated analytical methods, and a documented state of control. FDA process analytical technology (PAT) guidance explicitly encourages science-based approaches that improve process understanding and real-time quality assurance [1], while cellular and gene therapy guidance emphasizes product-specific potency characterization and risk-based manufacturing control [2]–[4].

Conventional monitoring remains dominated by fixed alarm limits, statistical trending, and laboratory measurements obtained at discrete sampling times. These methods are essential but can be intrinsically reactive: an alarm may be raised only after a critical process parameter (CPP) has crossed a predefined boundary or after a CQA has already deteriorated. In stem-cell culture, the onset of adverse dynamics can precede such threshold violations. For example, feedback among cell density, oxygen demand, nutrient consumption, lactate accumulation, and agitation

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may produce nonlinear transitions in which an apparently acceptable operating point loses stability and evolves toward an oscillatory or low-quality state.

Digital twins provide a means to connect the physical manufacturing process to a continuously updated virtual process model. In biomanufacturing, this concept is commonly framed as a physical-to-digital-to-physical information loop that combines data acquisition, mechanistic or hybrid models, state estimation, and decision support [6]–[9]. Machine-learning methods can complement these models by extracting high-dimensional temporal patterns and predicting difficult-to-measure quality attributes [10], [11]. However, an AI predictor alone does not necessarily distinguish a benign process drift from an approach to a nonlinear stability boundary. This motivates integration of dynamical-systems analysis into the digital-twin architecture.

The central premise of this study is that bifurcation analysis can provide a mathematically interpretable description of process resilience. Instead of asking only whether pH, DO, agitation, feed, or lactate remain within isolated limits, the digital twin also asks whether the coupled process state is approaching a fold, Hopf, or periodic-orbit boundary. AUTO-07p continuation is used to follow steady-state and periodic solution branches and to locate special points in the nonlinear model [15], [16]. A bifurcation-proximity index (BPI) then translates this mechanistic information into a real-time early-warning feature for the AI quality-control layer.

The study contributes a seven-part, simulation-based framework that: integrates synchronized CPP/CQA data with a dynamic digital twin; compares conventional machine-learning and temporal models with an AI digital twin augmented by a bifurcation layer; formulates a nonlinear stem-cell culture model suitable for continuation analysis; maps one- and two-parameter stability boundaries; visualizes viability, GMP quality, stability proximity, and potency as three-dimensional response surfaces; and evaluates early-warning performance and robustness under sensor loss and measurement perturbation. The numerical results used in this manuscript are the supplied simulation results and should not be interpreted as clinical or commercial batch-release evidence.

II. GMP STEM-CELL MANUFACTURING, DIGITAL TWINS, AND RESEARCH GAP

➢ *GMP Quality Attributes and Process Sensitivity*

Manufacturing of human pluripotent and mesenchymal stem cells must preserve therapeutic quality while achieving sufficient scale. Literature on cGMP iPSC culture emphasizes controlled sourcing, xeno-free or otherwise qualified materials, traceable handling, expansion, banking, cryopreservation, and quality-control testing [12], [14]. Reviews of stem-cell bioprocessing further show that scale-up requires simultaneous control of growth, viability, phenotype, oxygen transfer, shear environment, and culture chemistry [12], [13]. Consequently, the process cannot be represented adequately by a single end-point assay. A practical control strategy requires a hierarchy of CPPs and CQAs, together with real-time or near-real-time estimates of those quality states that are not directly measurable.

FDA potency guidance notes that cellular products are complex and that potency measurements should reflect relevant biological properties and, where possible, the mechanism of action [2]. The more recent potency-assurance framework extends this logic beyond a single release assay by linking manufacturing design, process control, material control, in-process testing, and potency testing [3]. These principles are consistent with the knowledge-management and quality-risk-management concepts in ICH Q10 [5]. The implication for an AI digital twin is that it should not operate as an isolated black-box classifier; it should be embedded within a traceable process-knowledge framework that preserves data provenance, version control, alarm rationale, and human review.

➢ *Digital Twins and AI in Bioprocessing*

Digital twins in bioprocessing have evolved from offline simulation models toward cyber-physical systems that continuously assimilate plant data and return predictions or control recommendations. Gargalo et al. described data and models as the two central building blocks for bio-manufacturing twins [6]. Appl et al. demonstrated how digital twins can support control-strategy development, including substrate and metabolite control [7], and Canzoneri et al. discussed practical deployment prerequisites in biopharmaceutical environments [8]. More recent pharmaceutical reviews position digital twins as enabling infrastructure for predictive monitoring, continuous manufacturing, and AI-assisted optimization [9].

Machine learning provides complementary capabilities for nonlinear regression, anomaly detection, multivariate batch monitoring, soft sensing, and temporal forecasting. Helleckes et al. summarized applications across monitoring, control, optimization, and scale-up [10], while Mondal et al. highlighted the growing use of hybrid strategies that combine process knowledge with data-driven models [11]. A persistent constraint is data scarcity: biopharmaceutical datasets are often relatively small because batches are expensive and failure cases are intentionally rare. Recent work therefore emphasizes small-data strategies, transfer learning, online learning, and uncertainty-aware modeling [17].

➢ *Need for Bifurcation-Aware Monitoring*

A conventional multivariate alarm system identifies unusual observations relative to a learned normal region, but it does not necessarily explain whether a state is dynamically recoverable. In nonlinear systems, fold bifurcations can create abrupt switching between alternative equilibria, and Hopf bifurcations can produce self-sustained oscillations when a conjugate pair of eigenvalues crosses the imaginary axis [15], [19]. These phenomena are relevant to cell culture because oxygen-transfer limits, metabolic feedback, and growth-dependent demand can interact to generate abrupt or oscillatory changes even when individual

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variables move gradually. A bifurcation-aware digital twin therefore adds a structural question to predictive monitoring: how far is the current state from a mathematically identified loss-of-stability boundary?

The research gap addressed here is the lack of an integrated stem-cell manufacturing framework that links GMP-oriented PAT data, temporal AI, dynamic digital-twin state estimation, and numerical bifurcation continuation in one quality-control loop. The proposed approach treats AI prediction and nonlinear stability analysis as complementary rather than competing methods.

III. PROPOSED AI-DRIVEN DIGITAL TWIN FRAMEWORK

➢ *System Architecture*

The proposed architecture is organized as a closed information loop from the physical GMP process to sensing, state estimation, AI prediction, nonlinear stability analysis, and human-supervised control. Fig. 1 summarizes the six principal layers. Online measurements provide current process conditions, the digital twin estimates hidden states and CQAs, temporal AI models forecast future quality and deviation risk, and AUTO-07p-derived stability information is translated into the BPI. The decision layer combines these outputs to generate alerts or recommended corrective actions, while all decisions remain subject to GMP review and audit-trace requirements.

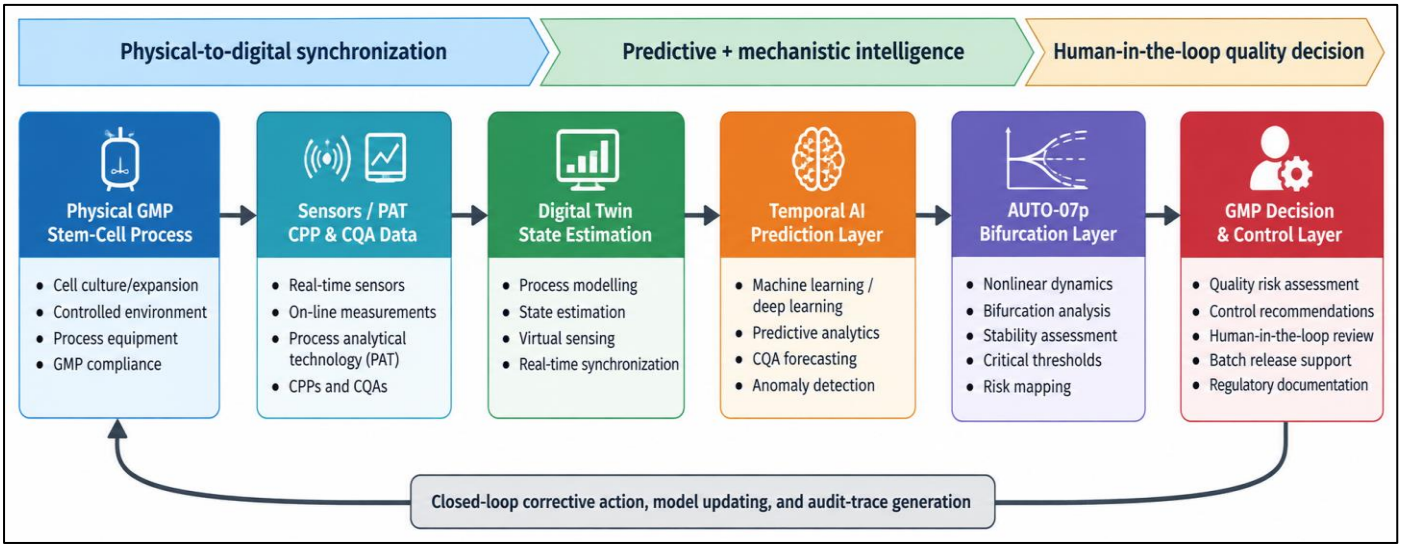

Fig 1 Proposed Closed-Loop Architecture for the AI-Driven, Bifurcation-Aware GMP Stem-Cell Manufacturing Digital Twin.

➢ *Simulation Dataset and Monitored Variables*

The supplied analytical dataset contains 60 simulated manufacturing batches and 43,200 synchronized process states sampled at five-minute intervals. Forty-two batches (70.0%) are nominal GMP-compliant trajectories, 11 (18.3%) represent warning or drift conditions, and seven (11.7%) represent simulated deviation or failure trajectories. Fourteen online variables cover pH, DO, temperature, agitation, feed, gases, and related CPPs, while eight laboratory or soft-sensor CQAs include viability, potency, purity, identity, and associated quality endpoints. These data are intended as a controlled test bed for evaluating the proposed method rather than as records from a licensed manufacturing facility.

The simulated design space spans 35.0–39.0 °C, pH 6.80–7.60, DO 20–65%, agitation 25–95 rpm, glucose-feed rate 0.3–1.8 g L^{-1} h^{-1}, lactate 0.5–6.0 mM, viable cell density (VCD) 0.5–5.0 ×10^6 cells mL^{-1}, and viability 60–96%. Preferred operating regions are narrower and correspond to 36.5–37.2 °C, pH 7.10–7.35, DO 35–55%, agitation 45–70 rpm, glucose feed 0.8–1.4 g L^{-1} h^{-1}, lactate below 2.5 mM, VCD 1.8–3.8 ×10^6 cells mL^{-1}, and viability at or above 85%.

➢ *Digital-Twin State Estimation and Temporal Prediction*

Let x(t) denote the synchronized process state and u(t) the manipulated inputs. The digital twin is represented by a nonlinear state model augmented with data-driven state correction:

$$\dot{x}(t) = f(x(t), u(t), \theta) + w(t), \quad y(t) = h(x(t)) + v(t) \qquad (1)$$

Where θ is the parameter vector, y(t) contains measured CPPs and CQAs, and w(t) and v(t) represent process and measurement disturbances. A temporal predictor receives a rolling window of synchronized observations and generates a future CQA vector and a probability of impending GMP deviation:

$$\hat{y}(t+\Delta) = F\Theta(x(t-W{:}t), u(t-W{:}t)), \; pdev(t+\Delta) = \sigma(g\Theta(\cdot)) \qquad (2)$$

The comparative modeling set includes logistic regression, random forest, XGBoost, long short-term memory (LSTM), and Transformer temporal models. The integrated model adds digital-twin states and the BPI to the temporal feature representation. Performance is evaluated using accuracy, F1-score, AUROC, AUPRC, sensitivity,

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and specificity, while continuous CQA predictions are evaluated with R^2, RMSE, MAE, and mean bias.

➢ *Quality-Risk Fusion and Decision Logic*

The decision layer fuses statistical prediction with mechanistic stability information. A generic quality-risk score is written as:

$$RQ(t)=w1\ pdev(t)+w2\ BPI(t) + w3\ AS(t) + w4\ DCQA(t) \quad (3)$$

Where AS is an anomaly score and DCQA is a normalized distance from the desired CQA region. The weights are intended to be calibrated during validation rather than fixed a priori. Importantly, the framework is conceived as decision support: it can recommend adjustments in oxygenation, agitation, nutrient feed, pH, or temperature, but the GMP control strategy must define which actions are automatic, which require operator confirmation, and which require quality-unit approval.

IV. NONLINEAR PROCESS MODEL AND BIFURCATION ANALYSIS

➢ *Reduced Dynamic Model*

To provide a tractable mechanistic core for continuation analysis, the culture is represented by five coupled states: viable cell concentration X, limiting substrate S, inhibitory metabolite L, cellular stress H, and a normalized quality or potency state Q. The model is deliberately reduced; its purpose is to represent dominant feedback needed for stability analysis rather than to replace a product-specific mechanistic model. The governing equations are:

$$dX/dt = [\mu(S,DO,pH,T) - kd(H,L)]X(1 - X/K) \quad (4)$$

$$dS/dt = Fin(Sin - S)/V - qS\ X \quad (5)$$

$$dL/dt = qL\ X - Fin\ L/V - kL\ L \quad (6)$$

$$dH/dt = \alpha L\ \varphi L(L) + \alpha O\ \varphi O(DO) + \alpha A\ \varphi A(A) - kH\ H \quad (7)$$

$$dQ/dt=\beta X\psi X(X)+\beta S\ \psi S(S) - \beta H\ H - \beta L\ L - kQ(Q - Q0) \quad (8)$$

The specific growth term μ depends on substrate availability and culture conditions, while kd increases under metabolic or hydrodynamic stress. The nonlinear penalty functions φL, φO, and φA activate as lactate, oxygen limitation, or agitation move away from favorable regions. This formulation allows the same model to represent a high-viability equilibrium, a low-quality equilibrium, and oscillatory responses under adverse control intensity.

➢ *Equilibria, Local Stability, and Special Points*

Equilibria satisfy $f(x^*,u,\theta)=0$. Local stability is determined from the Jacobian $J = \partial f/\partial x$ evaluated at x^*. A steady state is locally asymptotically stable when all eigenvalues have negative real parts. A fold or limit point occurs when a real eigenvalue passes through zero and the equilibrium branch turns with respect to a continuation parameter. A Hopf bifurcation occurs when a complex-conjugate eigenvalue pair crosses the imaginary axis, creating or destroying a periodic solution [15]. These definitions provide a direct mathematical interpretation of the LP and HB labels reported by AUTO-07p.

➢ *AUTO-07p Continuation and Bifurcation-Proximity Index*

AUTO-07p is used to continue equilibria and periodic orbits with respect to selected control parameters and to locate fold, Hopf, and other special points [16]. Candidate one-parameter continuations include DO, agitation, glucose-feed rate, and a composite control-intensity index. Two-parameter continuations map stability boundaries in the DO-agitation, pH-temperature, glucose-feed-VCD, lactate-DO, and stress-potency planes. The BPI converts the distance from the current normalized operating point z(t) to the nearest identified stability boundary B into a bounded warning signal:

$$BPI(t) = 1 - \min\{\ d(z(t),B) / dmax\ ,\ 1\ \} \quad (9)$$

Thus BPI approaches zero in the interior of a stable operating region and approaches one as the current state approaches the nearest bifurcation boundary. The index does not replace a process specification; it provides an additional dynamic-risk indicator whose value depends on the validated model and the selected normalization.

V. RESULTS

➢ *Analytical Dataset and Operating Envelope*

Table 1 summarizes the supplied simulation dataset. The class structure deliberately includes both benign drift and explicit failure trajectories so that the monitoring problem is not reduced to simple normal-versus-failure discrimination. Table 2 reports the preferred operating regions and wider simulation ranges used to challenge the digital twin beyond the nominal design space.

Table 1 Analytical Dataset and Process-Monitoring Composition

Item	Value	Purpose
Manufacturing batches	60	Complete batch trajectories used for model development and validation
Total synchronized time points	43,200	One multivariate process state every 5 min across all batches
Nominal GMP-compliant batches	42 (70.0%)	No critical quality excursion
Warning/drift batches	11 (18.3%)	Approach to CPP/CQA limits without full failure
Deviation/failure batches	7 (11.7%)	Simulated quality or stability excursion
Online process variables	14	pH, DO, temperature, agitation, feed, gases, and related CPPs
Laboratory/soft-sensor CQAs	8	Viability, potency, purity, identity, and related quality endpoints

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Table 2 Illustrative Process Ranges Used to Generate the Simulation Results

Process variable	Preferred operating region	Simulation range	Digital-twin relevance
Temperature	36.5–37.2 °C	35.0–39.0 °C	Thermal stress and growth kinetics
pH	7.10–7.35	6.80–7.60	Metabolic activity and phenotype stability
Dissolved oxygen	35–55%	20–65%	Oxygen-transfer adequacy and oxidative stress
Agitation	45–70 rpm	25–95 rpm	Mixing/oxygen transfer versus shear stress
Glucose feed	0.8–1.4 g L^{-1} h^{-1}	0.3–1.8 g L^{-1} h^{-1}	Nutrient availability and lactate formation
Lactate	<2.5 mM	0.5–6.0 mM	Metabolic-stress indicator
Viable cell density	1.8–3.8 ×10^6 cells mL^{-1}	0.5–5.0 ×10^6 cells mL^{-1}	Expansion performance
Viability	≥85%	60–96%	Principal release-relevant CQA

➢ *Deviation-Detection Performance*

Table 3 compares the predictive models on the held-out simulation set. Performance improved progressively from logistic regression through ensemble methods to temporal deep-learning models. The Transformer achieved an AUROC of 0.963, whereas the integrated AI digital twin with bifurcation layer reached 0.978. Relative to the Transformer, the integrated framework improved accuracy by 2.1 percentage points and sensitivity by 2.7 percentage points, while retaining high specificity. These results support the hypothesis that mechanistic state and stability information can add discriminative value to purely temporal prediction in this simulated setting.

Table 3 Held-Out Performance for Detection of Impending GMP Quality Deviations

Model	Accuracy (%)	F1-score (%)	AUROC	AUPRC	Sensitivity (%)	Specificity (%)
Logistic regression	81.4	78.9	0.842	0.801	76.3	84.2
Random forest	87.6	85.9	0.904	0.879	84.8	88.7
XGBoost	90.8	89.6	0.931	0.912	89.1	92.0
LSTM temporal model	92.9	92.1	0.952	0.939	92.4	93.3
Transformer temporal model	94.1	93.5	0.963	0.951	94.0	94.2
AI digital twin + bifurcation layer	96.2	95.8	0.978	0.969	96.7	95.6

➢ *Digital-Twin Prediction of CPPs and CQAs*

The digital twin showed strong agreement with the supplied target trajectories. Viability prediction produced R^2 = 0.954, RMSE = 1.82 percentage points, MAE = 1.34 percentage points, and a small mean bias of +0.21 percentage points. VCD and glucose concentration achieved R^2 values of 0.962 and 0.975, respectively. Potency was more difficult to predict than online chemistry variables but still reached R^2 = 0.931 with an RMSE of 0.041 normalized units. The pattern is consistent with the expected hierarchy of observability: directly sensed or strongly constrained process states are easier to predict than complex biological CQAs.

Table 4 Digital-Twin Accuracy for Selected CPPs and CQAs

Predicted quantity	R^2	RMSE	MAE	Mean bias
Viability (%)	0.954	1.82 percentage points	1.34 percentage points	+0.21 pp
Viable cell density	0.962	0.21 ×10^6 cells mL^{-1}	0.16 ×10^6 cells mL^{-1}	−0.03 ×10^6
Glucose concentration	0.975	0.18 mM	0.13 mM	+0.02 mM
Lactate concentration	0.949	0.24 mM	0.18 mM	−0.04 mM
Potency score	0.931	0.041 normalized units	0.031 normalized units	+0.006
Purity (%)	0.938	1.55 percentage points	1.16 percentage points	−0.18 pp

➢ *Nonlinear Stability and AUTO-07p Results*

Continuation analysis identified a lower fold LP1 at control index 0.312 and viability fraction 0.742, an upper fold LP2 at 0.586 and 0.908, and a Hopf point HB1 at 0.793 and 0.884. The interpretation of LP1 is that the low-quality equilibrium loses persistence as effective oxygen-transfer capacity increases, while LP2 marks accessibility of the stable high-viability branch. HB1 indicates that further control intensification can destabilize the steady state and generate sustained oscillation. The periodic-orbit envelope reached a reported maximum at control index 0.914 with viability fraction 0.817, representing large-amplitude oscillations and increased quality variability.

Table 5 Auto-07p Special Points for a Representative Stem-Cell Manufacturing Trajectory

Label	Control index	Viability fraction	Dynamic interpretation	GMP/process meaning
LP1	0.312	0.742	Fold / lower switching threshold	Low-quality equilibrium loses persistence as oxygen-transfer capacity increases
LP2	0.586	0.908	Fold / upper switching	Stable high-viability branch becomes fully accessible

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			threshold	
HB1	0.793	0.884	Hopf bifurcation	Stable state transitions to sustained oscillation under excessive control intensity
PO-max	0.914	0.817	Periodic-orbit envelope	Large oscillations increase quality variability and reduce process desirability

Table 6 Two-Parameter GMP Operating Regions

Parameter plane	**Primary favorable range**	**Secondary favorable range**	**Interpretation**
DO × agitation	35–55% DO	45–70 rpm	Broad stable high-viability zone; instability rises at low DO/high agitation
pH × temperature	7.10–7.35	36.5–37.2 °C	Highest predicted GMP quality index near central operating region
Glucose feed × VCD	0.8–1.4 g L^{-1} h^{-1}	1.8–3.8 ×10^6 cells mL^{-1}	Stable nutrient-supported growth without excessive metabolite accumulation
Lactate × DO	0.5–2.5 mM	35–55% DO	Quality maintained when metabolic stress remains low and oxygenation is adequate
Stress index × potency	0.00–0.35	0.80–0.98 potency fraction	Predicted potency deteriorates rapidly beyond the pre-bifurcation warning region

➢ *Three-Dimensional Digital-Twin and Stability Landscapes*

The response surfaces supplied with the study provide a compact visualization of the joint process interactions. Fig. 2 shows a broad high-viability plateau at moderate-to-high DO and intermediate glucose-feed rate, followed by reduced performance toward low oxygen or extreme feed conditions. Fig. 3 shows a central GMP quality maximum around the preferred pH-temperature region. Fig. 4 converts DO-agitation combinations into BPI, illustrating a low-risk basin surrounded by increasing proximity to the nonlinear stability boundary. Fig. 5 shows a monotonic loss of predicted potency as lactate concentration and cellular stress increase together.

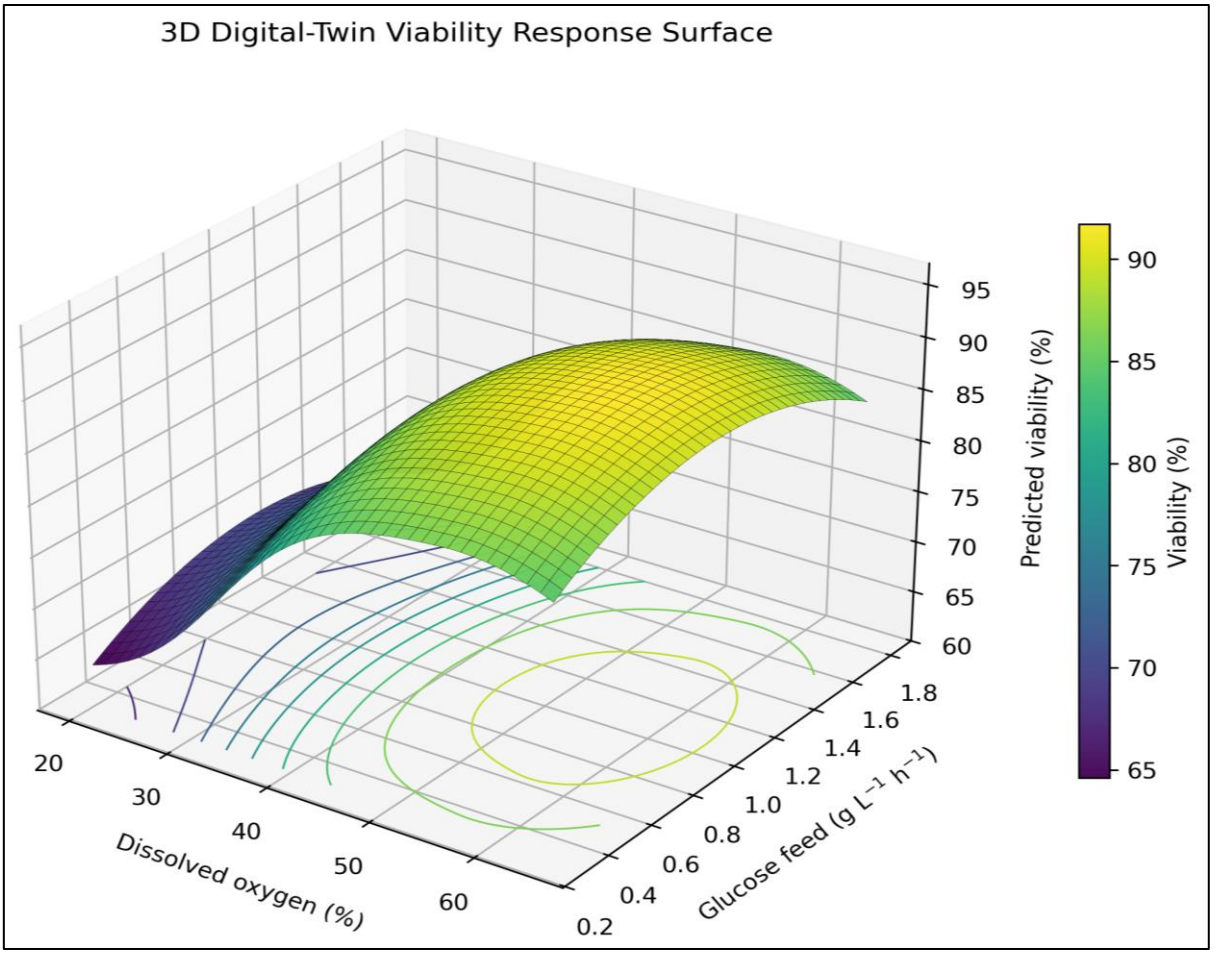

Fig 2 SimCafe-Style 3D Digital-Twin Response Surface Showing Predicted Stem-Cell Viability as a Function of Dissolved Oxygen and Glucose Feed.

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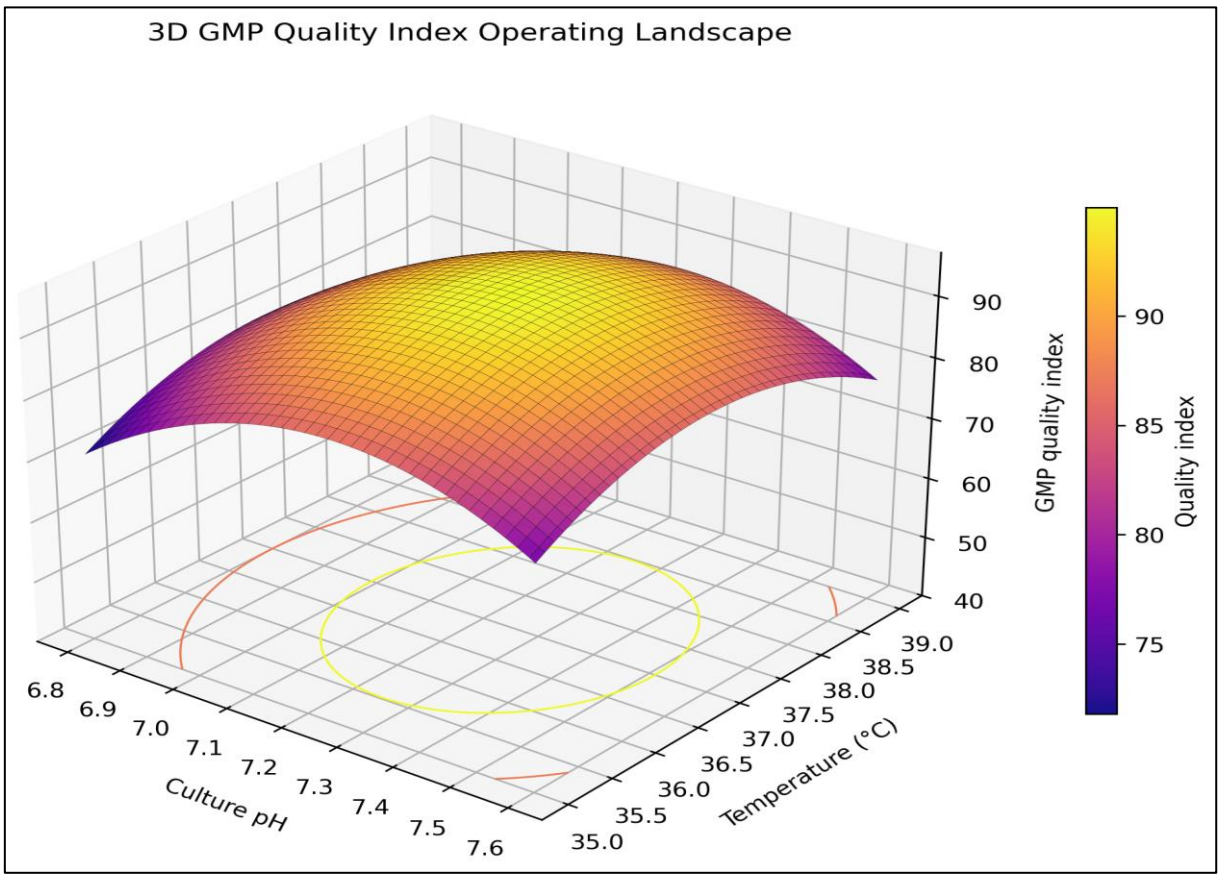

Fig 3 SimCafe-Style 3D GMP Quality-Index Surface Across Culture pH and Temperature.

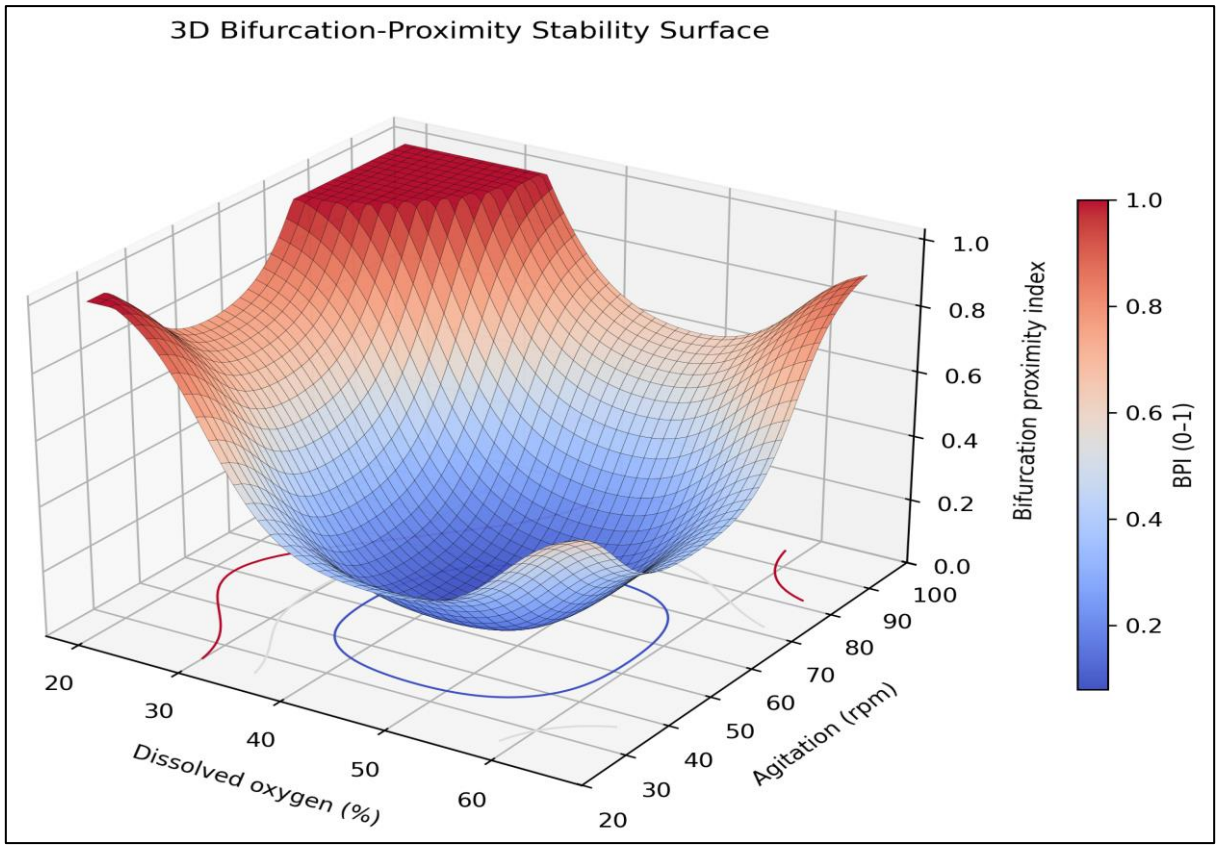

Fig 4 SimCafe-Style 3D Bifurcation-Proximity Surface Across Dissolved Oxygen and Agitation. Higher BPI Indicates Operation Closer to a Nonlinear Stability Boundary.

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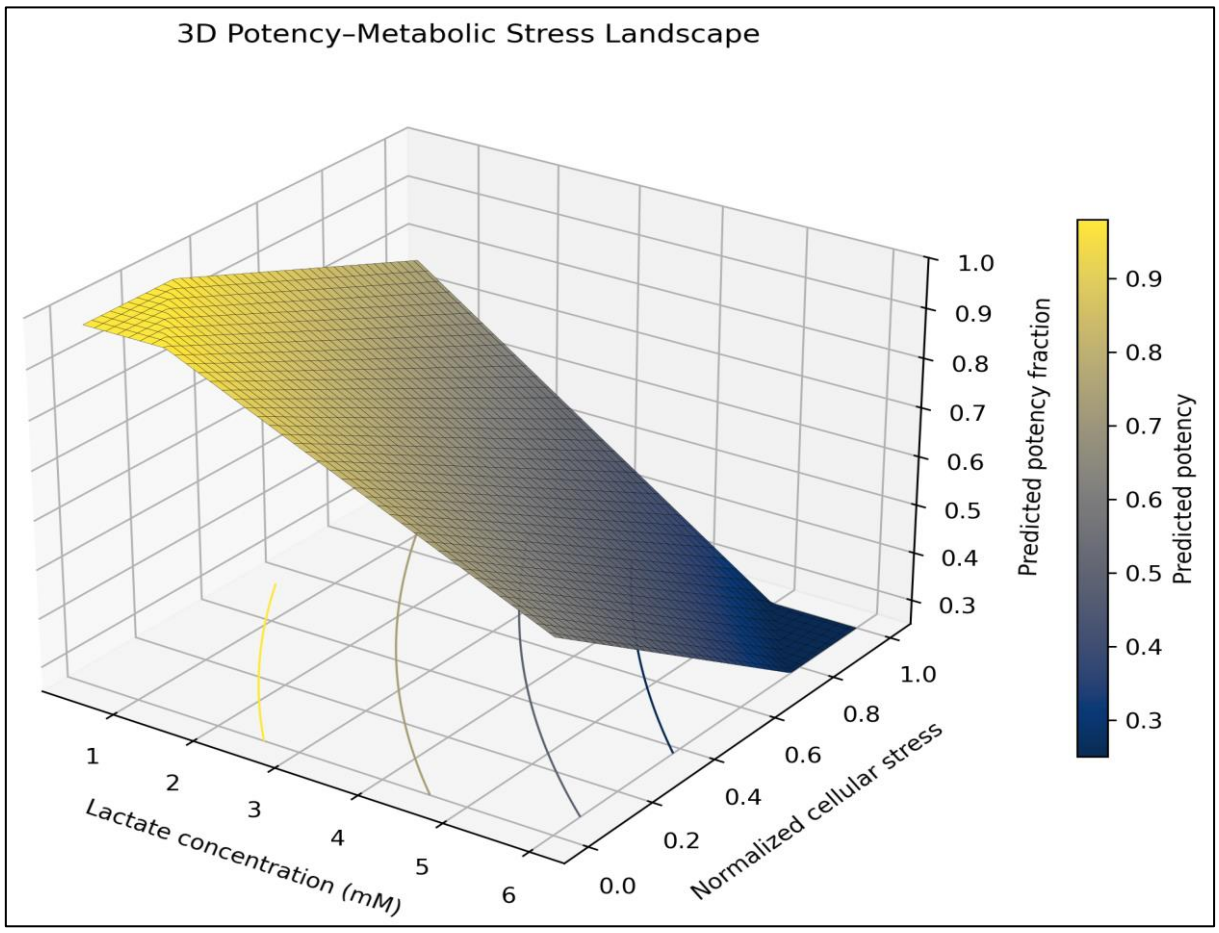

Fig 5 SimCafe-Style 3D Potency Landscape Showing the Joint Effect of Lactate Accumulation and Normalized Cellular Stress on Predicted Product Potency.

➢ *Early-Warning and Robustness Performance*

Bifurcation-aware monitoring provided the longest warning horizon in the supplied simulations. Fixed GMP limits produced a median lead time of 22 min, statistical process control 46 min, standalone temporal AI 73 min, and the AI digital twin plus BPI 118 min. At the same time, false alarms fell from 8.9% for fixed limits to 2.7% for the integrated method, and the proportion of deviations classified as avoidable increased from 38% to 88%. This result is important because an early-warning system is useful only if the warning horizon is sufficiently long for a feasible process intervention and if the alarm burden remains manageable.

Robustness tests show graceful degradation rather than catastrophic failure. Removing advanced assay inputs reduced AUROC from 0.978 to 0.965. Removal of the DO sensor reduced AUROC to 0.948, while removal of the pH sensor reduced it to 0.951, indicating that oxygen and acid-base state carry substantial information. Five percent Gaussian sensor noise produced only a 1.6-point AUROC reduction; 10% noise and 15% missing time points reduced AUROC by 3.7 and 4.2 points, respectively.

Table 7 Early-Warning and Quality-Control Performance

Monitoring approach	Median lead time (min)	False-alarm rate (%)	Avoidable deviations (%)	Interpretation
Fixed GMP limits	22	8.9	38	Reactive; deviation detected close to limit crossing
Statistical process control	46	7.2	52	Detects sustained drift but not nonlinear transitions
Standalone temporal AI	73	5.0	71	Predictive but lacks explicit stability boundary
AI digital twin + BPI	118	2.7	88	Earliest warning with bifurcation-aware process context

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Table 8 Robustness Under Missing Measurements and Perturbation

Robustness condition	AUROC	Change (points)	Effect
Full model	0.978	0.0	Reference
Without metabolomics/advanced assay inputs	0.965	−1.3	Minor degradation; online CPPs remain informative
Without DO sensor	0.948	−3.0	Moderate degradation; oxygen-state estimation becomes uncertain
Without pH sensor	0.951	−2.7	Moderate degradation; metabolic-state confidence decreases
5% Gaussian sensor noise	0.962	−1.6	Minor degradation
10% Gaussian sensor noise	0.941	−3.7	Moderate degradation
15% missing time points	0.936	−4.2	Performance remains usable with imputation/state estimation

Table 9 Consolidated Outcomes of the Simulation Study

Outcome	Result	Interpretive significance
Deviation-detection AUROC	0.978	Integrated temporal, mechanistic, and stability information
Digital-twin viability R^2	0.954	High agreement between virtual and target quality state
Median advance warning	118 min	Earlier intervention than fixed-limit monitoring
False-alarm rate	2.7%	Improved alarm specificity
Avoidable simulated deviations	88%	Potential quality-control benefit after timely corrective action
Primary nonlinear outputs	LP1, LP2, HB1, periodic orbit	Explicit mapping of stable and unstable process regimes

VI. DISCUSSION

➢ *Value of Combining Temporal AI with Dynamic Stability Information*

The principal result is not simply that a more complex model produced a higher AUROC. The substantive difference is the type of information incorporated into the decision. Temporal AI estimates what is likely to happen next from patterns in historical trajectories, whereas bifurcation analysis evaluates whether the current nonlinear system can remain near its operating equilibrium as a control parameter changes. The integrated framework therefore combines predictive evidence with a mechanistic indicator of resilience. In the simulation, that combination produced both better discrimination and longer warning lead time.

The gain is especially visible when comparing the Transformer and the integrated framework. The Transformer already achieved AUROC 0.963 and sensitivity 94.0%, leaving limited headroom for pure classification improvement. Yet adding the digital-twin and bifurcation layer raised sensitivity to 96.7% and, more importantly, supported a 118-min median warning horizon. This suggests that stability information may be most valuable not at the final classification boundary but earlier, when a process is still technically within conventional operating limits but is moving toward a low-resilience region.

➢ *Interpretation of the Bifurcation Structure*

The LP1-LP2 pair implies a region of multistability or switching behavior in the reduced model. In practical terms, the same control variable may be associated with qualitatively different cell-culture states depending on the trajectory by which the system arrived there. This creates the possibility of hysteresis, meaning that simply returning a manipulated variable to its previous value may not immediately restore the former high-quality state. The HB1 result is equally important because oscillatory culture behavior can increase quality variance even when average values remain near nominal targets. A monitoring system based only on pointwise specifications may therefore understate risk during the approach to a Hopf boundary.

The two-parameter operating maps reinforce this interpretation. Stable high-viability operation occurs over combinations rather than single-variable thresholds. For example, DO of 35–55% is favorable in conjunction with agitation of 45–70 rpm, but the simulation indicates increasing instability when low DO is paired with high agitation. This interaction is physically plausible as a modeling concept because agitation simultaneously influences oxygen transfer, mixing, and shear. Nevertheless, the precise numerical boundaries in this manuscript are simulation outputs and must not be transferred to a real stem-cell process without product-specific experiments and model identification.

➢ *GMP and Regulatory Implications*

The proposed system aligns conceptually with PAT and pharmaceutical-quality-system principles because it uses process understanding to support a state of control rather than replacing GMP specifications [1], [5]. It also fits the risk-based direction of current cellular and gene therapy guidance, which links product quality to manufacturing design, controls, in-process testing, and potency assurance [2]–[4]. However, a regulatory-grade implementation would require considerably more than predictive accuracy. Model inputs must be traceable to qualified instruments, data transformations must be controlled, software versions and model parameters must be governed under change control, and alarm or recommendation logic must be validated for the intended use.

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A practical implementation should therefore distinguish three levels of automation. First, the digital twin may operate in passive monitoring mode to generate soft-sensor estimates and BPI trends without changing the process. Second, it may provide operator advisory recommendations that require human confirmation. Third, only after extensive validation might selected low-risk adjustments be considered for closed-loop automation. The latter would require a documented control strategy, fail-safe states, cybersecurity controls, model-performance monitoring, and clear procedures for reverting to conventional control if the digital twin becomes unreliable.

- *Robustness, Limitations, and Threats to Validity*

The robustness analysis indicates that the framework can tolerate moderate missingness and noise, but it also identifies the DO and pH channels as influential. This finding has two implications: virtual sensing may provide temporary resilience during sensor failure, but it should not become an excuse to operate with chronically degraded instrumentation; and uncertainty bounds should widen when critical sensors are unavailable. In a GMP setting, confidence or uncertainty should be presented alongside point predictions so that the operator can distinguish a high-risk state from a low-confidence state estimate.

The most important limitation is that the study is simulation-based. The dataset contains simulated warning and failure trajectories, and the four 3D surfaces are generated from the supplied numerical design space rather than independent experimental batches. As a result, the reported AUROC, R^2, warning lead time, and avoidable-deviation rate demonstrate internal consistency of the proposed framework but do not establish external clinical or manufacturing validity. A second limitation is model reduction: the five-state nonlinear system captures feedback among growth, nutrient, metabolite, stress, and quality but omits cell-line-specific signaling, microcarrier dynamics, morphology, spatial gradients, and assay uncertainty. Third, class balance and failure mechanisms in simulation may differ from real GMP operations, where true critical deviations are rare and data can be censored by operator intervention.

Future validation should therefore proceed through staged evidence generation: parameter identification from historical engineering batches; retrospective replay against known drift and deviation events; prospective shadow-mode deployment; challenge studies under approved design-space excursions; external validation across cell lines, operators, media lots, and bioreactor scales; and formal assessment of data integrity, explainability, cybersecurity, and human factors. Only after these steps should the system be considered for process-control claims beyond research decision support.

VII. CONCLUSION AND FUTURE WORK

This paper presented an AI-driven digital-twin framework for real-time monitoring and quality control of GMP stem-cell manufacturing, augmented with numerical bifurcation analysis. The simulation dataset comprised 60 batch trajectories and 43,200 synchronized process states. The integrated AI digital twin with bifurcation layer achieved 96.2% accuracy, F1-score 95.8%, AUROC 0.978, and AUPRC 0.969 for impending deviation detection, while viability prediction reached $R^2 = 0.954$. AUTO-07p analysis identified LP1, LP2, HB1, and a periodic-orbit envelope, providing explicit stability boundaries that were converted into a BPI. Relative to fixed-limit monitoring, the integrated approach increased median warning lead time from 22 to 118 min, reduced false alarms from 8.9% to 2.7%, and increased the share of simulated deviations classified as avoidable from 38% to 88%.

The key research contribution is the integration of predictive AI with dynamical-systems stability analysis inside a GMP-oriented digital-twin loop. The simulation suggests that this architecture can detect not only whether a process is drifting but also whether it is approaching a qualitative loss of stability. Future work should replace the illustrative nonlinear model with cell-line- and bioreactor-specific parameterizations, quantify uncertainty around the bifurcation boundaries, validate the BPI against real deviation mechanisms, and evaluate the framework prospectively in a qualified manufacturing environment. Until such validation is completed, the numerical thresholds and performance values reported here should be regarded as proof-of-concept simulation results rather than manufacturing acceptance criteria.

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