Available online **www.ijpras.com**

International Journal of Pharmaceutical Research & Allied Sciences, 2026, 15(1):191-201

https://doi.org/10.51847/ibQluQxeqy



Original Article

ISSN : 2277-3657
CODEN(USA) : IJPRPM

The Biological Afterlife of Nanocarriers: A Mass-Balance Framework for Connecting Material Degradation, Persistent Fragments, Repeat-Dose Accumulation, and Long-Term Pharmaceutical Safety

Laura Bianchi[1]*, Michael Tan[2], Julia Weber[3]

[1]Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy.
[2]Department of Pharmaceutical Sciences, National University of Singapore, Singapore.
[3]Department of Nanotechnology, Free University of Berlin, Berlin, Germany.

***Email:** laura.bianchi@unibo.it*

ABSTRACT

Nanocarrier pharmacology is commonly characterized through circulating particles, delivered payloads, tissue signals, or therapeutic responses. These observables can disappear or change while constituent material undergoes redistribution, fragmentation, or biological transformation. Their time courses therefore provide incomplete answers to long-term material-fate questions. To develop a nonempirical, material-conserving theory explaining how carrier degradation, compartmental residence, immune history, and repeated exposure can follow interacting but non-synchronous clocks. Published studies of nanocarrier transport, chemical degradation, reporter stability, biodistribution, and repeat-dose immunity provide mechanistic constraints for a prospective accounting scheme. A chemically indexed constituent balance separates physical particle integrity from molecular persistence and analytical visibility. A multiple-clocks account links this balance to tissue residence and dose-history-dependent responses without presuming that residual fragments cause toxicity. The theory predicts different outcomes when chemical cleavage, tissue redistribution, immune priming, and successive administrations overlap. It requires orthogonal constituent measurements and comparisons against conventional pharmacokinetic and immune-mechanistic explanations. Durable safety claims demand evidence about what chemical material remains, where it resides, what has been recovered, and how the biological response changes with dosing history; the theory itself supplies testable propositions, not new longitudinal safety findings.

Key words: *Nanocarrier material fate, Constituent mass balance, Lipid nanoparticle degradation, Persistent fragments, Repeat-dose pharmacokinetics, Analytical non-detection*

Received: 21 July 2026; **Revised:** 24 August 2026; **Accepted:** 29 August 2026

INTRODUCTION

Nanocarriers can alter drug solubility, tissue accessibility, cellular entry, and therapeutic performance. Their biodistribution and clearance are therefore central translational concerns, although outcomes depend strongly on material design and host physiology [1]. Precision-delivery engineering further illustrates that success at one biological barrier does not resolve what subsequently occurs to the assembled vehicle [2]. The chemical constituents of a carrier may have an exposure history that continues after its initial pharmaceutical task has ended. This mismatch is consequential for nucleic-acid formulations. Lipid nanoparticles combine ionizable lipids, helper lipids, cholesterol, surface polymers, and RNA, each with a distinguishable chemical and biological fate [3]. Cancer nanomedicine similarly exposes the limits of interpreting delivery from tumor enrichment alone: access

© 2026 The International Journal of Pharmaceutical Research and Allied Sciences (IJPRAS). **Open Access** - This article is under the **CC BY NC SA** license (https://creativecommons.org/licenses/by-nc-sa/4.0).

and effective targeting depend on heterogeneous biological conditions [4]. Transvascular transport determines one part of that trajectory, but residence, intracellular processing, and eventual recovery of material require different observations [5].

The question considered here is whether a constituent-conserving account, coupled to several interacting temporal processes, explains apparently discordant fate and redosing signals more usefully than conventional, chemically resolved pharmacokinetics. This article is an original theoretical analysis, not a systematic evidence synthesis, exposure experiment, or validated toxicological model. Its propositions are intended to be vulnerable to competing mechanisms and future prospective tests.

Defining nanocarrier material fate beyond therapeutic payload delivery

The administered formulation is an assembly, whereas the conserved objects are specified constituents or appropriately stable tracers. An intact vehicle, a released polymer segment, a metabolized lipid, an encapsulated drug, and free drug occupy different chemical states. Transport across biological interfaces must therefore be distinguished from transformation of molecular identity. Experimental tumor studies have challenged an exclusively passive account of nanoparticle entry [6]; later work has resolved further endothelial transport pathways [7]. Neither type of observation, by itself, identifies which administered atoms eventually leave the organism.

Blood residence constitutes one operational clock, defined by the measured concentration of a specified species over sampling time. The biological identity encountered by that species can change through protein-corona formation, with experimentally observed consequences for polymeric nanoparticle pharmacokinetics [8]. Cellular capture is another process: corona-associated ligands can support liver-macrophage uptake [9]. Entry into a macrophage marks a new anatomical and intracellular state, and subsequent breakdown, transfer, or excretion must each be established independently.

For this analysis, *material persistence* means continued presence of an administered constituent, or chemically assignable descendant, within the body at a specified time. *Structural persistence* means retention of a defined assembly or molecular architecture. *Analytical persistence* means detectability by a particular method. These definitions prevent prolonged fluorescence from being assumed to represent intact particles, or disappearance from plasma from being equated with whole-body elimination. They also allow degradation and retention to coexist without treating such coexistence as necessarily harmful.

Existing evidence on material degradation, persistence, and biological clearance

Hepatic trafficking provides a direct warning against collapsing particle and payload histories. In male mice, Kupffer cells capture PEGylated liposomal doxorubicin and participate in the subsequent transfer of released drug toward hepatocytes; free doxorubicin follows a different intrahepatic route [10]. Organ drug concentration consequently need not track intact liposome abundance. For RNA formulations, altered lipid chemistry can independently affect organ-associated mRNA accumulation and translation [11]. Degradable branched lipidoids further illustrate that linker cleavage and effective RNA delivery can be investigated together, while the longer-term disposition of each resulting chemical species remains a separate question [12].

Analytical method is central to this distinction. Radioactive-polymer size-exclusion chromatography reveals polymer degradation that conventional particle-size measurements may not establish [13]. Dual-labelled polymeric nanoparticles show that fluorophores can separate from their anchors or yield fluorescence artefacts, complicating in vivo localization [14]. Chemical transformation can therefore proceed without an easily visible change in hydrodynamic diameter; conversely, a bright reporter can remain after losing its original carrier association. A valid material-fate claim identifies the molecule or tracer measured, validates its attachment or conservation, and states the assay's recovery and uncertainty.

Material design also influences biological residence. Polymer chemistry and macrophage-related interventions change circulation and tissue tropism in experimental poly(amine-co-ester) nanoparticles [15]. Ionizable polymeric micelles achieve siRNA delivery in particular cellular and disease contexts, but functional gene silencing does not determine every carrier constituent's elimination [16]. Imaging-based pharmacokinetic studies of mesoporous silica demonstrate additional particle-property dependence [17]. These material classes should not be assigned a shared degradation constant solely because each is called a nanocarrier.

Improved spatial observation does not solve chemical speciation automatically. Whole-animal imaging at single-cell resolution can map labeled nanocarrier-associated signals [18]. Multiplexed RNA barcodes reveal organ- and cell-dependent delivery kinetics [19]. Both approaches are useful within their measured molecular scopes; neither

automatically closes a balance for unmeasured lipids, polymers, or metabolites. The contrasting evidence requirements of the investigated systems are condensed in **Table 1.**

Table 1. Material-state transformations and the evidentiary limits of nanocarrier fate measurements

Measured pharmaceutical entity	Demonstrated observation	Material-fate distinction	Conditional inference / unresolved species	Proposed accounting test
Liposomal doxorubicin and released drug	Kupffer-cell capture and altered hepatic drug distribution	Carrier uptake versus payload transfer	Remaining liposomal lipids not inferred from drug kinetics	Parallel lipid and drug species sampling
Degradable branched lipidoid LNP	Linker design and chemical cleavage alongside RNA activity	Molecular transformation versus delivery success	Fragment identity and cumulative recovery require separate verification	Trace parent lipid and cleavage products
Radiolabelled biodegradable polymer	Size-exclusion evidence of molecular degradation	Polymer integrity versus hydrodynamic size	Particle diameter cannot establish polymer preservation	Pair chemical-size separation with colloidal sizing
Dual-labelled polymer nanoparticle	Dye dissociation and optical artefacts	Reporter persistence versus carrier persistence	Optical signal alone is conditional on label attachment	Orthogonal isotope and fluorescence tracking
Polymer, micelle, silica or barcoded LNP	Formulation-dependent tissue or functional readouts	Organ entry versus whole-body material clearance	Unmeasured material classes cannot inherit one clearance clock	Matched chemical and spatial readouts

Table note. Entries identify published evidence categories and original proposed comparisons, not newly measured outcomes. "Carrier," "label," and "payload" refer to analytically different material entities.

Unresolved questions in material-fate accounting and repeated exposure

The first unresolved issue concerns temporal ordering. A carrier can cross a membrane, release a therapeutic agent, lose colloidal identity, and leave chemical descendants in the same cell. Work on RNA–LNP intracellular barriers differentiates endosomal processing from the later occurrence of cytosolic RNA activity [20]. These events have different molecular observables. It remains uncertain, across formulations, which transformed carrier constituents persist after functional delivery has ended and whether their persistence changes subsequent exposure.

A second issue is the meaning of an altered response to another dose. Immune reactivity to LNP components is documented across experimental and therapeutic contexts [21]. Specific ionizable lipid headgroups can engage innate and antigen-presenting pathways, including TLR4 and CD1d, in mechanistic experiments [22]. Such findings establish that biological response may be modified by molecular structure without requiring accumulated intact nanoparticles. They also leave open whether chemical descendants influence later immunological or tissue responses.

Third, repeated-dose performance depends on formulation history. Experimental replacement of PEG-associated surface lipids with poly(2-ethyl-2-oxazoline) alternatives changes measured immune and delivery outcomes [23]. This observation is compatible with several explanations: altered recognition, circulation, cell access, or processing, potentially operating together. No response alone identifies retained material quantity. **Table 2** separates the unresolved tests according to the *history that must be measured*, preventing repeat-dose effects from being attributed automatically to fragments.

Table 2. Unresolved exposure histories that can produce divergent repeat-dose interpretations

Exposure-history unit	Observable event	Competing material or biological histories	What remains unidentified	Proposed discriminating comparison
First-dose endosomal processing	Cytosolic RNA activity and membrane perturbation	Carrier fragmentation; compartmental sequestration; membrane repair	Chemical fate of intracellular carrier components	Measure species during and after escape
First-dose immune encounter	Innate or adaptive markers	Lipid-headgroup signaling; antibody priming; tissue inflammation	Contribution of retained fragments	Pair immune perturbation with residual-material assays
Interdose organ residence	Tissue-associated carrier or label signal	Intact retention; fragment retention; reporter transfer	Chemical composition underlying a persistent signal	Serial organ speciation with validated tracers
Second-dose altered efficacy	Expression or PK response changes	Immune recognition; altered uptake; cellular adaptation; material competition	Cause of response change	Match dose intervals and antibody status with constituent burden
Delayed tissue response	Injury or recovery endpoint	Ongoing exposure; resolved exposure with durable immune effects	Chronology needed for causal attribution	Joint longitudinal chemistry and functional follow-up

Table note. The histories are analytical possibilities requiring study-specific evidence. No row asserts that residual fragments cause a particular adverse effect or supplies a universal repeat-dose interval.

Scientific requirements for a material-conserving mass-balance framework

The accounting unit must survive changes in particle assembly. For a validated constituent tracer e, administered amount must be reconciled with measured body content, recovered excreted content, and an explicit unrecovered analytical residual. The residual represents incomplete recovery, detection, or model coverage, never a hypothetical bodily reservoir. Concentration and particle number cannot substitute automatically for constituent mass. Tracer exchange, endogenous background, collection completeness, chemical derivatization, and incomplete extraction must be handled before a balance is called closed. These are requirements of a proposed experimental framework, not evidence that existing studies achieved such closure.

Material states should be indexed jointly by chemical identity, compartment, and time. Chemical conversion changes the allocation among parent molecules and daughter products; tissue redistribution changes anatomical location; loss from a particular assay changes what is visible. The resulting observation can be written conceptually as measured signal = assay mapping of material states + measurement error. If the mapping cannot distinguish two states, those states remain non-identifiable even when a curve is smooth. Radiopolymer analysis shows the need to resolve polymer species [13]. Dual-label analysis independently demonstrates the need to validate reporter attachment [14].

The framework also requires distinct treatment of immune interactions. High-density brush-shaped polymer lipids modify anti-PEG binding and repeat-dose outcomes in tested delivery systems [24]. Poly(carboxybetaine)-containing LNPs likewise alter endosomal escape and immune-associated performance relative to PEG-containing formulations [25]. These observations establish that surface chemistry can change the biological response clock. Neither study closes an elemental balance for every administered constituent, so improved redosing must not be used as a substitute for chemical elimination.

Figure 1 represents the minimum relations that a conservation-compatible fate claim must distinguish. Material may travel, transform, be retained, or be recovered in excreta, while the measurement system observes only selected states. The unresolved analytical residual remains visible so that missing signal never becomes an invented excretion pathway.

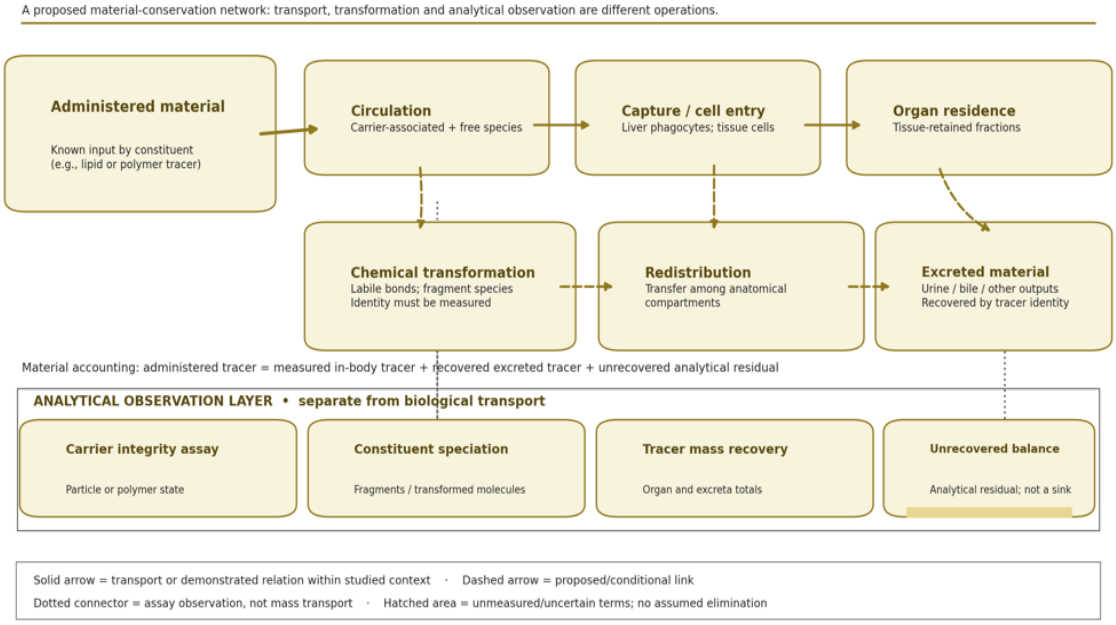


Figure 1. Nanocarrier constituents remain accountable as their chemical identities change

Legend. Conceptual material-conservation network, read primarily left to right. The upper region separates administered constituent material from circulation, cell entry, tissue residence, molecular transformation, redistribution, and recovered excreted material. Solid arrows denote physically justified transport operations within appropriate systems; dashed arrows denote conditional transitions requiring species-resolved verification. A lower layer distinguishes particle-integrity assays, chemical speciation, tracer recovery, and unrecovered analytical balance. Dotted connectors denote observations and do not imply physical transfer. No diagram width or arrow represents a measured quantity. NC, nanocarrier; PK, pharmacokinetics. Source-supported examples motivate the compartments, whereas the integrated balancing architecture is original and proposed.
Alt text. Wide Golden Ochre diagram with an administered-material region on the left, circulation, cellular capture and organ residence across the upper row, and chemical transformation, redistribution and excretion below. Solid or dashed arrows express transport or conditional transformation. A separate bottom assay row measures integrity, chemical species and recovery; an unrecovered residual is explicitly labeled analytical, not biological.

Development of the proposed nanocarrier biological-fate theory

A single scalar clearance time cannot represent every constituent and functional event once carrier disassembly, organ capture, and immune history overlap. The first clock tracks chemical integrity: the probability or amount of an identified parent constituent remaining before transformation. The second tracks anatomical residence: constituent quantity within named cells, tissues, or excreted fractions. A third, assay-observation clock defines the times and detection limits with which these states become knowable. A fourth tracks host response, including immune recognition, recovery, and possible changes after a subsequent dose. These clocks may be coupled, but their temporal order is empirical rather than assumed.

One discriminating prediction follows when chemistry is changed without equivalently changing the dosing schedule. Biodegradable poly(D,L-serine) surface lipids elicit limited anti-polymer IgM in tested mouse redosing experiments, while retaining measurable mRNA-delivery function [26]. This supports the plausibility of modified immune recognition, not an assertion that all surface material clears before redosing. Studies of PEG-lipid parameters likewise find repeat-administration performance sensitive to polymer-lipid architecture within tested formulations [27]. Consequently, altered efficacy should be modeled as a joint result of current carrier delivery and prior biological state, with chemically retained material included only when independently demonstrated.

A second prediction separates material burden from inflammatory mechanism. Endosomal membrane injury can recruit galectin-dependent inflammatory sensing, and experimentally modifying that signaling changes inflammation associated with LNP escape [28]. The effect need not wait for long-term accumulation. A delayed injury observation could arise from retained chemicals, persistent host response after chemical elimination, or renewed acute injury following another exposure. Distinguishing those alternatives requires measurements at each clock, plus controls that block proposed immune mechanisms without silently changing dose delivery.

The proposed theory therefore does not assign a universal "biological afterlife" duration. It predicts *discordance* where chemically resolved body burden, intact-carrier readouts, RNA expression, and immune markers decline or recur at different rates. More strongly, it predicts that matched observed residual material may coexist with different redosing responses if immune priming differs, and matched immune status may coexist with different residue kinetics when degradable chemistry differs. If ordinary constituent-specific pharmacokinetics and known immune mechanisms already predict these outcomes without additional coupling terms, the broader temporal account should be rejected or simplified.

Figure 2 sets out this competing-clock logic without assigning measured time intervals or causal priority to any process. Material persistence and immune memory are represented as alternative, possibly coexisting explanations for later outcomes, and the figure preserves the assay boundary between a missing signal and demonstrated material loss.

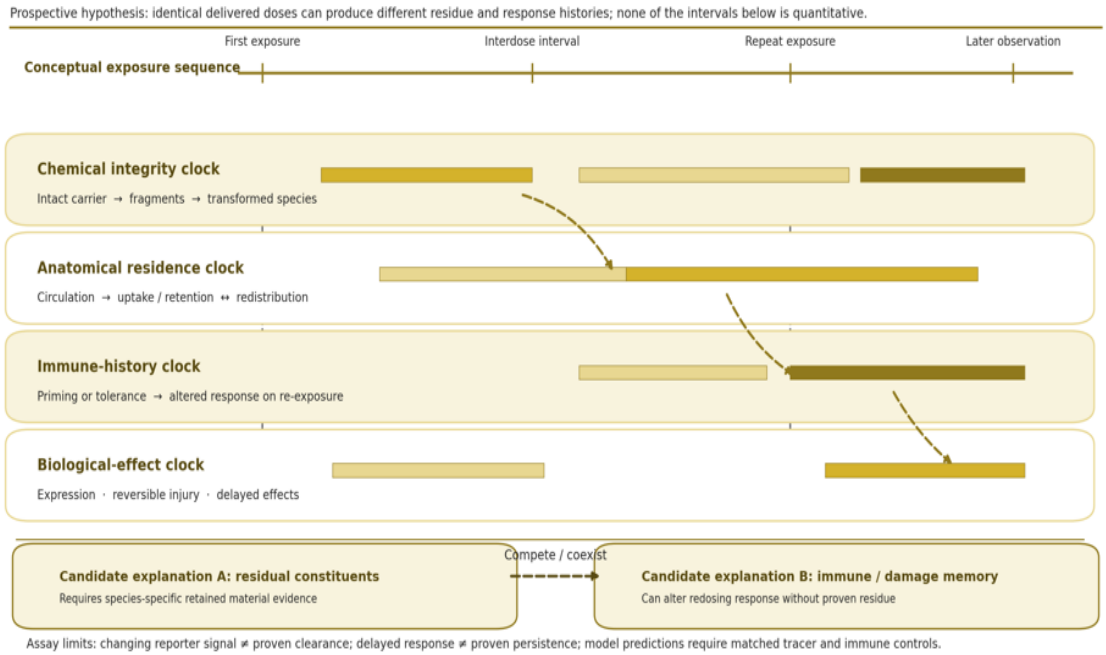


Figure 2. The biological afterlife unfolds across non-synchronous clocks

Legend. The horizontal axis denotes a conceptual progression from first exposure through an interdose period, repeat administration, and later observation; positions and band lengths are illustrative and have no quantitative meaning. Four parallel lanes distinguish structural/chemical integrity, anatomical residence, immune history, and biological effect. Dashed inter-lane arrows are proposed conditional couplings, not demonstrated causal rates. The lower competing explanations contrast residue-associated effects with immune or damage memory, which may coexist. No repeated-dose toxicity, interval threshold, or human risk magnitude is claimed. LNP, lipid nanoparticle; PEG, poly (ethylene glycol).

Alt text. Wide landscape longitudinal map with a non-scaled exposure sequence above four horizontal lanes. Short and long Golden Ochre bands differ across chemical integrity, anatomical residence, immune history, and biological effects. Dashed connectors cross between lanes. Two bottom regions identify residual-constituent and immune-memory explanations, linked by a conditional comparison. An assay caveat states that reporter decline cannot by itself prove complete clearance.

Implications for long-term pharmaceutical safety and repeat-dose assessment

Long-term assessment should follow the administered carrier constituents beyond the interval in which payload delivery is considered successful. A useful question at each sampling time is how much of a chemically specified parent component, its identifiable descendants, and its recovered excreted fraction can be accounted for, with uncertainty stated. This approach changes the interpretation of apparent persistence: a tissue-associated signal prompts chemical identification before intact-particle retention or toxicity is inferred. It also changes the interpretation of apparent clearance: disappearance from circulating blood, fluorescent imaging, or a parent-compound assay cannot, alone, establish absence of administered constituent material. For a degradable

formulation, cleavage may lower exposure to an intact assembly while creating new species whose kinetics and biological activity require separate evaluation.

Repeat-dose decisions introduce another dimension because the previous administration can change the biological system that receives the next one. Repeated-delivery studies show that surface-polymer substitutions and lipid architecture alter experimentally measured response, while inflammation may depend on intracellular membrane damage or immune recognition [23]. Chemical persistence, immune priming, and functional recovery therefore need their own observation schedules. A proposed comparison would vary interdose interval while separately measuring residual chemically assigned material and immune state before each administration. Divergent effects under matched measured material burdens would favor a history-dependent biological explanation; effects varying with chemically resolved retained burden after immune conditions are controlled would strengthen a material-mediated account. Neither pattern, by itself, identifies a clinical toxicity threshold.

The implications depend on route and diseased-tissue physiology. Lung-directed RNA formulations that modify intracellular handling and inflammatory responses have been studied preclinically [29]. Work on cancer nanomedicine likewise shows that host barriers and target accessibility differ across biological contexts [30]. These results constrain extrapolation: a pulmonary exposure, a tumor-associated deposit, and a liver-captured polymer population may share constituent chemistry while exhibiting different transport and response clocks. Pharmaceutical development could consequently prioritize matched repeated-dose chemistry, organ-specific sampling, and clinically relevant functional endpoints over a single generic clearance surrogate. The decision remains conditional on demonstrated exposure and harm, not on an assumption that any persistent fragment is intrinsically unsafe. A practical preclinical protocol would record administered constituent amounts separately from administered particle counts, report recovery for each tissue and excretion route, and retain a time-stamped record of every intervention that might alter immune state. It would also specify which assays recognize intact assemblies, which detect transformed molecules, and which observe only a therapeutic reporter. These distinctions make negative findings interpretable and allow toxicity signals to be aligned with the material actually present, while preserving uncertainty when samples or chemical products remain uncharacterized.

Evidence requirements for testing material-fate predictions

A decisive study must distinguish a molecule's physical whereabouts from the reporting method used to see it. Serial sampling should quantify an independently validated conserved constituent or stable isotope across the administered formulation, named tissues, blood, and collected excreta. Where chemically feasible, orthogonal separation and mass-spectrometric methods should identify parent components and daughter species; particle-integrity and fluorescence assays should be retained as different measurements. Radio-polymer chromatography and dual-label investigations demonstrate why chemical transformation and reporter attachment cannot be assumed from hydrodynamic size or optical intensity [13]. Imaging at high spatial resolution can map localization, but its chemical meaning still depends on the label and what is actually detected [18].

Biological uptake introduces a separate experimental alternative. Genome screens implicate specific receptors in recognition of protein-corona-coated nanoparticles [31]. Receptor perturbation could therefore help distinguish altered cellular capture from faster chemical degradation, provided the intervention's effects on delivery and host physiology are evaluated. Optical reporters require equally explicit controls: afterglow nanoparticle research demonstrates that photophysical emission can have its own time course [32]. Thus prolonged emission or signal decay must be cross-checked against chemical identity rather than equated directly with molecular residence or excretion. Matching sampling windows to anticipated kinetics matters as much as selecting orthogonal instruments, because an unsampled intermediate can make a multi-step process appear instantaneous.

Delivery route constitutes an additional falsification condition. Nebulized therapeutic mRNA has required formulation optimization distinct from systemic LNP administration [33]. An experiment that transfers an intravenous tissue-distribution parameter to pulmonary delivery without accounting for airway deposition, local barriers, and mucociliary or cellular handling cannot validate a general fate theory. **Table 3** specifies what each proposed test must resolve before its outcome can legitimately enter a material-balance or long-term safety argument.

Table 3. Proposed discriminating measurements for constituent recovery and repeated-dose fate

Experimental comparison unit	Necessary simultaneous observables	Sampling and control requirement	Result favoring a constituent-persistence explanation	Result favoring a rival explanation
Parent carrier versus chemical descendants	Intact-assembly marker; constituent identity; candidate products	Validated tracer stability; extraction recovery; tissue and excreta sampling	Identified descendants remain in tissue after assembly loss	Constituents are recovered elsewhere despite falling particle signal
Carrier reporter versus chemical mass	Fluorescence or afterglow; orthogonal molecular assay	Free-label control; excitation history; matched time points	Chemical and reporter signals agree with confirmed residence	Optical signal diverges because of reporter transformation or photophysics
Organ enrichment versus elimination	Organ amount; urinary and fecal constituent recovery	Whole-organ sampling; collection efficiency; background subtraction	Chemically assigned material remains despite limited excreted recovery	Organ-associated signal reflects transient uptake or assay background
First dose versus repeated dose	Pre-dose retained species; antibody/innate markers; second-dose delivery	Matched dose formulation and interval; immune perturbation if justified	Subsequent effect tracks retained constituent after immune adjustment	Effect tracks immune history despite similar measured residual mass
Intravenous versus pulmonary exposure	Route-specific deposition; chemical species; functional endpoints	Formulation-matched feasible controls; tissue-specific follow-up	Similar species persist after route-specific normalization	Different barrier and deposition processes explain divergent signals

Table note. All comparisons are proposed prospective tests, not completed measurements. "Favors" means discriminates between specified mechanisms only if dose, assay recovery, and relevant biological confounding are adequately controlled. No universal detection limit, acceptable residue threshold, or numerical follow-up schedule is assumed.

Discussion, Competing Mechanisms, and Future Research

The biological afterlife hypothesis is useful only insofar as it distinguishes experimentally different explanations for the same observation. An apparently long-lived carrier signal might indicate a retained intact assembly, stable fragments, re-associated labels, or an optical state with dynamics unrelated to material conservation. Conversely, rapid therapeutic action need not require deep penetration of intact particles. A vascularly accumulated nanocarrier can release a drug rapidly near its target, as demonstrated by a flash-release strategy [34]. The clinical or experimental benefit of that strategy supplies no direct estimate of the carrier's later chemical recovery. Separating the drug-release clock from constituent disappearance prevents the theory from confusing therapeutic timescale with material half-life.

Immune priming represents a serious rival for explaining second-dose failure. An antibody- or receptor-associated response may alter capture and delivery despite low retained material. Intracellular damage sensing can also change inflammation in ways that are not reducible to residue burden. A material-persistence hypothesis is strengthened only when specified retained species precede the relevant change in function and appropriately controlled manipulations of their abundance modify that effect. Repeated-dose designs should therefore distinguish prior dose, immune history, current delivered amount, and actual constituent species. Any single-marker association is underdetermined because these quantities often change together.

Conventional chemical-species pharmacokinetics provides another demanding comparator. If a sufficiently resolved compartmental model, supplemented by independently characterized immune pathways, predicts the longitudinal measurements without introducing a novel clock interaction, the added theory has no practical explanatory advantage. Its value lies in exposing otherwise hidden mismatches of observation and kinetics, and in specifying discriminating studies—not in adding terminology to well-established mass transport. Future work should compare models prospectively using held-out time points, chemically independent tracer channels, and designs in which formulation chemistry and dosing history can vary separately. Such validation remains to be performed; the cited experimental literature does not establish universal superiority of the proposed account.

Limitations and boundaries of long-term risk interpretation

Material conservation establishes an accounting obligation, not a toxicity mechanism. The same recovered amount can represent different molecular species, cellular locations, interactions, or clearance opportunities. Analytical extraction may fail for bound molecules; isotope labels may exchange; endogenous elements can complicate background subtraction; and serial destructive tissue sampling may require separate animals. Consequently, an unrecovered fraction is a limitation of observation and collection, never evidence of an occult organ deposit. Even chemically secure retention proves exposure, not injury. Long-term causal interpretation requires concurrent functional endpoints and appropriately timed comparisons that separate continued material exposure from earlier biological events.

Organ targeting illustrates why universal predictions are inappropriate. Barcoded polymeric nanocapsules can exhibit kidney-enriched distribution in mouse experiments [35], but renal tissue association does not establish urinary passage or degradation. Size-controlled mesoporous silica carriers cross dystrophic-muscle vasculature through experimentally studied transcytotic mechanisms and improve selected outcomes in a mouse disease model [36]. Neither finding transfers directly to healthy tissue, humans, or a chemically unrelated lipid formulation. Follow-up limited to delivery or short-term efficacy similarly cannot resolve chronic accumulation or delayed clinical toxicity. The theory applies only when the relevant constituent identity, population, administration route, sampling interval, and detection properties are stated; it does not forecast patient-level risk from incomplete preclinical observations.

CONCLUSION

Nanocarrier degradation, tissue retention, repeat-dose response, and biological recovery can proceed on different clocks because they concern distinct chemical states and biological mechanisms. A defensible account conserves appropriately traced administered constituents across transformation, redistribution, and recovered elimination while keeping unrecovered analytical material explicit. That approach explains why losing an intact-particle, optical, or payload signal cannot alone establish elimination, and why a changed second-dose response need not imply accumulated fragments. Its scientific value will depend on prospectively separating chemically identified residues from immune and transport alternatives. Long-term pharmaceutical safety ultimately requires linked evidence of material identity, anatomical exposure, biological consequence, and observation over the time intervals relevant to the formulation; no universal toxicity or clearance prediction follows from the theory alone.

ACKNOWLEDGMENTS: None

CONFLICT OF INTEREST: None

FINANCIAL SUPPORT: None

ETHICS STATEMENT: None

REFERENCES

1. Cabral H, Li J, Miyata K, Kataoka K. Controlling the biodistribution and clearance of nanomedicines. Nat Rev Bioeng. 2024;2(3):214-32. doi:10.1038/s44222-023-00138-1
2. Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. Nat Rev Drug Discov. 2021;20(2):101-24. doi:10.1038/s41573-020-0090-8
3. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater. 2021;6(12):1078-94. doi:10.1038/s41578-021-00358-0
4. Lammers T. Nanomedicine tumor targeting. Adv Mater. 2024;36(26):e2312169. doi:10.1002/adma.202312169
5. Li X, Hu Y, Zhang X, Shi X, Parak WJ, Pich A. Transvascular transport of nanocarriers for tumor delivery. Nat Commun. 2024;15(1):8172. doi:10.1038/s41467-024-52416-0
6. Sindhwani S, Syed AM, Ngai J, Kingston BR, Maiorino L, Rothschild J, et al. The entry of nanoparticles into solid tumours. Nat Mater. 2020;19(5):566-75. doi:10.1038/s41563-019-0566-2
7. Wu JLY, Ji Q, Blackadar C, Nguyen LNM, Lin ZP, Sepahi Z, et al. The pathways for nanoparticle transport across tumour endothelium. Nat Nanotechnol. 2025;20(5):672-82. doi:10.1038/s41565-025-01877-5

8. Bertrand N, Grenier P, Mahmoudi M, Lima EM, Appel EA, Dormont F, et al. Mechanistic understanding of in vivo protein corona formation on polymeric nanoparticles and impact on pharmacokinetics. Nat Commun. 2017;8(1):777. doi:10.1038/s41467-017-00600-w
9. Bussin B, MacDuff MGG, Ngo W, Wu JLY, Lin ZP, Granda Farias A, et al. Discovering nanoparticle corona ligands for liver macrophage capture. Nat Nanotechnol. 2025;20(7):914-25. doi:10.1038/s41565-025-01903-6
10. Jiang K, Tian K, Yu Y, Wu E, Yang M, Pan F, et al. Kupffer cells determine intrahepatic traffic of PEGylated liposomal doxorubicin. Nat Commun. 2024;15(1):6136. doi:10.1038/s41467-024-50568-7
11. Su K, Shi L, Sheng T, Yan X, Lin L, Meng C, et al. Reformulating lipid nanoparticles for organ-targeted mRNA accumulation and translation. Nat Commun. 2024;15(1):5659. doi:10.1038/s41467-024-50093-7
12. Han X, Xu J, Xu Y, Alameh MG, Xue L, Gong N, et al. In situ combinatorial synthesis of degradable branched lipidoids for systemic delivery of mRNA therapeutics and gene editors. Nat Commun. 2024;15(1):1762. doi:10.1038/s41467-024-45537-z
13. Dikpati A, Maio VDP, Ates E, Greffard K, Bertrand N. Studying the stability of polymer nanoparticles by size exclusion chromatography of radioactive polymers. J Control Release. 2024;369:394-403. doi:10.1016/j.jconrel.2024.03.053
14. Roussel S, Grenier P, Chénard V, Bertrand N. Dual-labelled nanoparticles inform on the stability of fluorescent labels in vivo. Pharmaceutics. 2023;15(3):769. doi:10.3390/pharmaceutics15030769
15. Piotrowski-Daspit AS, Bracaglia LG, Eaton DA, Richfield O, Binns TC, Albert C, et al. Enhancing in vivo cell and tissue targeting by modulation of polymer nanoparticles and macrophage decoys. Nat Commun. 2024;15(1):4247. doi:10.1038/s41467-024-48442-7
16. Zhou Z, Feng Y, Jiang M, Yao Z, Wang J, Pan F, et al. Ionizable polymeric micelles (IPMs) for efficient siRNA delivery. Nat Commun. 2025;16(1):360. doi:10.1038/s41467-024-55721-w
17. Dogra P, Adolphi NL, Wang Z, Lin YS, Butler KS, Durfee PN, et al. Establishing the effects of mesoporous silica nanoparticle properties on in vivo disposition using imaging-based pharmacokinetics. Nat Commun. 2018;9(1):4551. doi:10.1038/s41467-018-06730-z
18. Luo J, Molbay M, Chen Y, Horvath I, Kadletz K, Kick B, et al. Nanocarrier imaging at single-cell resolution across entire mouse bodies with deep learning. Nat Biotechnol. 2025;43(12):2009-22. doi:10.1038/s41587-024-02528-1
19. Moore ST, Lian X, Vaidya A, Chatterjee S, Santelli J, Sun Y, et al. Multiplexed lipid nanoparticle barcoding reveals tissue-dynamic kinetic insights and enriched cellular tropism in hepatic zones. Nat Commun. 2026;17(1):1345. doi:10.1038/s41467-025-68103-7
20. Johansson JM, Du Rietz H, Hedlund H, Eriksson HC, Oude Blenke E, Pote A, et al. Cellular and biophysical barriers to lipid nanoparticle mediated delivery of RNA to the cytosol. Nat Commun. 2025;16(1):5354. doi:10.1038/s41467-025-60959-z
21. Lee Y, Jeong M, Park J, Jung H, Lee H. Immunogenicity of lipid nanoparticles and its impact on the efficacy of mRNA vaccines and therapeutics. Exp Mol Med. 2023;55(10):2085-96. doi:10.1038/s12276-023-01086-x
22. Chaudhary N, Kasiewicz LN, Newby AN, Arral ML, Yerneni SS, Melamed JR, et al. Amine headgroups in ionizable lipids drive immune responses to lipid nanoparticles by binding to the receptors TLR4 and CD1d. Nat Biomed Eng. 2024;8(11):1483-98. doi:10.1038/s41551-024-01256-w
23. Sanchez AJDS, Loughrey D, Echeverri ES, Huayamares SG, Radmand A, Paunovska K, et al. Substituting poly(ethylene glycol) lipids with poly(2-ethyl-2-oxazoline) lipids improves lipid nanoparticle repeat dosing. Adv Healthc Mater. 2024;13(17):e2304033. doi:10.1002/adhm.202304033
24. Xiao Y, Lian X, Sun Y, Sung YC, Vaidya A, Chen Z, et al. High-density brush-shaped polymer lipids reduce anti-PEG antibody binding for repeated administration of mRNA therapeutics. Nat Mater. 2025;24(11):1840-51. doi:10.1038/s41563-024-02116-3
25. Luozhong S, Liu P, Li R, Yuan Z, Debley E, Chen Y, et al. Poly(carboxybetaine) lipids enhance mRNA therapeutics efficacy and reduce their immunogenicity. Nat Mater. 2025;24(11):1852-61. doi:10.1038/s41563-025-02240-8
26. Zeng JY, Zhang Y, Zhao G, Seow BYL, Krishnan NB, Goh JH, et al. Polypeptide-engineered lipid nanoparticles for mRNA delivery with limited immunogenicity. Nat Commun. 2026;17(1):6986. doi:10.1038/s41467-026-73698-6

27. Lee Y, Jeong M, Lee G, Park J, Jung H, Im S, et al. Development of lipid nanoparticle formulation for the repeated administration of mRNA therapeutics. Biomater Res. 2024;28:0017. doi:10.34133/bmr.0017
28. Omo-Lamai S, Wang Y, Patel MN, Milosavljevic A, Zuschlag D, Poddar S, et al. Limiting endosomal damage sensing reduces inflammation triggered by lipid nanoparticle endosomal escape. Nat Nanotechnol. 2025;20(9):1285-97. doi:10.1038/s41565-025-01974-5
29. Zhao Z, Shan X, Ding J, Ma B, Li B, Huang W, et al. Boosting RNA nanotherapeutics with V-ATPase activating non-inflammatory lipid nanoparticles to treat chronic lung injury. Nat Commun. 2025;16(1):6477. doi:10.1038/s41467-025-61688-z
30. Gomerdinger VF, Nabar N, Hammond PT. Advancing engineering design strategies for targeted cancer nanomedicine. Nat Rev Cancer. 2025;25(9):657-83. doi:10.1038/s41568-025-00847-2
31. Ngo W, Wu JLY, Lin ZP, Zhang Y, Bussin B, Granda Farias A, et al. Identifying cell receptors for the nanoparticle protein corona using genome screens. Nat Chem Biol. 2022;18(9):1023-31. doi:10.1038/s41589-022-01093-5
32. Jiang Y, Huang J, Zhen X, Zeng Z, Li J, Xie C, et al. A generic approach towards afterglow luminescent nanoparticles for ultrasensitive in vivo imaging. Nat Commun. 2019;10(1):2064. doi:10.1038/s41467-019-10119-x
33. Lokugamage MP, Vanover D, Beyersdorf J, Hatit MZC, Rotolo L, Echeverri ES, et al. Optimization of lipid nanoparticles for the delivery of nebulized therapeutic mRNA to the lungs. Nat Biomed Eng. 2021;5(9):1059-68. doi:10.1038/s41551-021-00786-x
34. Zelepukin IV, Griaznova OY, Shevchenko KG, Ivanov AV, Baidyuk EV, Serejnikova NB, et al. Flash drug release from nanoparticles accumulated in the targeted blood vessels facilitates the tumour treatment. Nat Commun. 2022;13(1):6910. doi:10.1038/s41467-022-34718-3
35. Wang L, Zhou W, Chen H, Jia X, Zheng P, Jiang H, et al. Barcoded screening identifies nanocarriers for protein delivery to kidney. Nat Commun. 2025;16(1):899. doi:10.1038/s41467-025-56257-3
36. Liu X, Hicks MR, Feng Z, Jiang J, Li Y, Young CS, et al. Transcytotic transportation of size-controlled nanocarriers into dystrophic skeletal muscle leads to therapeutic outcome in mice. Nat Commun. 2025;16(1):11217. doi:10.1038/s41467-025-66061-8