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pH-Responsive ZIF-8@Polydopamine Nanocarrier for Co-Delivery of Letrozole and Cisplatin in Breast Cancer Therapy

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Research Article

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Abstract

A ZIF-8/polydopamine (PDA) nanocarrier was prepared for co-delivery of letrozole and cisplatin and evaluated by FTIR, SEM, TGA, XRD, buffer-release testing, and MCF-7 viability assays. The supplied release curves show enhanced release in acidic medium (pH 5.5) relative to the nominally neutral condition, with approximately 67% of letrozole and 85% of cisplatin released by 48 h in acidic medium. The neutral-buffer pH and some plotted release values require verification against experimental records. According to Table 1, the ZLDC IC_{50} values were 617.42 μg/mL after 24 h and 26.08 μg/mL after 48 h. However, the reported combination-index calculation and kinetic-fit parameters are not fully reconcilable with the available supporting figures. The findings provide preliminary evidence for pH-associated release and in vitro anticancer activity, while further data verification is needed before mechanistic, synergy, or in vivo safety claims can be established.

1. Introduction

Combination therapy is being investigated for breast cancer because agents with different mechanisms of action may offer complementary activity. Delivering letrozole and cisplatin together, however, introduces challenges related to their different physicochemical properties and release requirements [1, 2]. Metal–organic frameworks (MOFs), including zeolitic imidazolate framework-8 (ZIF-8), are of interest as drug carriers because of their porosity and susceptibility to destabilization under acidic conditions [3, 4].

Unmodified ZIF-8 may undergo premature cargo release under some conditions. Polydopamine (PDA), a mussel-inspired coating material, offers functional groups that can interact with drugs and metal-containing compounds [5, 6]. A PDA shell may therefore modify cargo retention and release; however, its protective effect at physiological pH and the precise mode of cisplatin binding require experimental confirmation [7, 8].

Here, we prepared ZIF-8@Letrozole@PDA@Cisplatin (ZLDC) by incorporating letrozole during ZIF-8 synthesis, applying a PDA coating, and exposing the coated particles to cisplatin. We examined physicochemical characteristics, pH-dependent release, release-model fits, and MCF-7 cell viability. We expected the two drugs to display different release profiles because of their different locations and interactions within the composite (Fig. 1).

2. Experimental Section

2.1. Materials

Letrozole, zinc nitrate hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$), 2-methylimidazole, dopamine hydrochloride, and methanol were obtained from Merck and used as received. The formulations are denoted Z (ZIF-8), ZL (ZIF-8@Letrozole), ZL@PDA (PDA-coated ZL), and ZLDC (ZIF-8@Letrozole@PDA@Cisplatin). The

sources and specifications of cisplatin and additional buffers and reagents should be added before submission.

2.2. Characterization

Fourier-transform infrared (FTIR) spectra were acquired using a Bruker Vector spectrometer. Thermogravimetric analysis (TGA; DuPont 951) was performed under nitrogen at a heating rate of 5°C/min. Morphology was examined by scanning electron microscopy (SEM; TESCAN VEGA/XMU) after gold coating. UV–Vis absorbance measurements were obtained using a Model 2100 spectrophotometer. Instrumental conditions, calibration procedures, and the X-ray diffraction (XRD) instrument details should be reported for reproducibility.

2.3. Synthesis of the ZLDC Delivery System

2.3.1. Preparation of ZL:

Letrozole (5, 10, or 15 mg) was dispersed in methanol with sonication. Zinc nitrate hexahydrate (50 mg) was dissolved in methanol and combined with the drug solution. Next, 2-methylimidazole (110 mg) in methanol was added dropwise under vigorous stirring. The mixture was stirred for 24 h at room temperature in the dark. The product was recovered by centrifugation (5000 rpm, 20 min), washed three times with methanol, and vacuum-dried at 40°C. The stated quantities correspond to a Zn^{2+}:2-methylimidazole molar ratio of approximately 1:8.

2.3.2. Surface Functionalization with Polydopamine (ZL@PDA):

50 mg of ZL nanoparticles were dispersed in 50 mL of Tris-HCl buffer (pH 8.5). Upon adding 10 mg of dopamine hydrochloride, the mixture was stirred for 12 h in an open-air environment to facilitate the oxidative self-polymerization of dopamine. The PDA-coated nanoparticles were collected, washed with deionized water, and dried under vacuum.

2.3.3. Cisplatin Conjugation (ZLDC):

30 mg of ZL@PDA were re-dispersed in PBS (pH 7.4). Cisplatin solution (0.5–1.5 mL, 1 mg/mL) was added dropwise in a light-protected environment. The mixture was stirred for 24 h to allow Pt(II) coordination with the catechol and amine moieties of the PDA shell. The final ZLDC nanocomposites were recovered by centrifugation and vacuum-dried.

2.4. In Vitro Drug Release

Release was evaluated in phosphate-buffered saline (PBS, pH 7.4) and acetate buffer (pH 5.5) at 37°C. ZLDC (0.3 g) was suspended in 10 mL of release medium under agitation. At predefined intervals, 1 mL of medium was withdrawn and replaced with fresh buffer. Letrozole and cisplatin were quantified using UV–Vis measurements at 240 and 215 nm, respectively; all assays were carried out in triplicate. Drug-specific calibration, possible absorbance overlap, correction for cumulative sampling, and verification of

sink conditions should be described. The reported cisplatin signal at 215 nm should be supported by a validated, selective assay.

2.5. Cytotoxicity Evaluation (MTT Assay)

MCF-7 human breast cancer cells (Pasteur Institute of Iran) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum at 37°C and 5% CO_2. Passage-18 cells were seeded in 96-well plates at 10,000 cells/well for the 24-h assay and 8,000 cells/well for the 48-h assay. After 24 h for attachment, cells were exposed to treatments at 100–500 µg/mL. MTT solution (40 µL, 5 mg/mL) was added after 24 or 48 h of treatment and incubated for 3 h; formazan was dissolved in 100 µL of DMSO. Absorbance was measured at 570 nm with a 630-nm reference (BioTek ELx800), and viability was normalized to untreated controls. The number of biological replicates, vehicle controls, and whether formulation concentrations refer to total nanocomposite mass or active-drug mass should be specified.

3. Results and Discussion

3.1. Structural and Chemical Characterization (FT-IR)

FTIR spectra were consistent with the presence of the imidazolate framework. Bands assigned to aromatic C–H stretching (approximately 3000–3100 cm^{-1}), ring vibrations (approximately 1350–1500 cm^{-1}), and Zn–N vibrations (near 421 cm^{-1}) were observed for ZIF-8. The persistence of framework-associated bands after letrozole incorporation suggests retention of the principal bonding environment. Broadening in the 3200–3500 cm^{-1} region after PDA coating is compatible with O–H/N–H groups. However, these overlapping FTIR bands alone do not conclusively establish cisplatin coordination or distinguish surface adsorption from incorporation within pores (Figs. 1S–3S, Supporting Information).

3.2. Morphological Analysis (SEM)

The SEM images (Fig. 4S, Supporting Information) showed particles with broadly polyhedral morphology. The reported apparent particle size increased from approximately 20 nm for ZIF-8 to approximately 45 nm after surface modification. This trend is consistent with coating and/or aggregation, but the images alone cannot establish a uniform shell thickness. Particle-size distributions from multiple particles should be provided to substantiate the reported averages.

3.3. Thermal Behavior (TGA/DTG)

TGA/DTG data for ZL and ZLDC are designated as Figs. 5S and 6S in the Supporting Information. However, the supplied panels appear to reproduce the same thermogram, including a residual-mass annotation of approximately 16.81%. Consequently, the previously reported ZLDC residue of 4.27% and comparative interpretations cannot be independently supported by the current supplementary figures. The correct ZLDC thermogram should be supplied before assigning distinct thermal events or reporting sample-to-sample differences.

The overlapping degradation of ZIF-8, letrozole, PDA, and cisplatin-associated species makes definitive interpretation of the TGA profile challenging. In particular, a lower final residue cannot by itself prove that the PDA shell functions as a thermal barrier or confirm the spatial arrangement of the different components. Additional controls (such as ZIF-8, PDA-coated ZIF-8, and free-drug reference curves) would strengthen these assignments.

The previously stated 12.54-percentage-point difference in final residue and an estimated drug/polymer loading of 20–25 wt.% cannot be verified from the supplied figures. TGA alone cannot reliably separate individual drug loading from PDA and framework degradation without suitable controls and mass-balance analysis. These quantities have therefore not been used as validated loading estimates.

3.4. Crystallinity and Phase Purity (XRD)

The supplied XRD panels (Fig. 7S) show markedly different patterns for the starting material and the final composite. The displayed axis ranges and broad scattering in the latter panel do not provide unambiguous evidence for retention of characteristic ZIF-8 diffraction peaks. Phase retention and the peak assignment previously made near 2θ = 10.66° should be re-evaluated using correctly calibrated diffraction data and a common, clearly labeled 2θ range.

3.5. In Vitro Drug Release Behavior

Figure 8S suggests greater cumulative release at pH 5.5 than at the nominally neutral condition for both drugs. At 48 h, the acidic curves reach approximately 67% for letrozole and 85% for cisplatin. The neutral-condition curves in the supplied figure appear closer to approximately 26% and 33%, respectively, rather than the 42% and 40% previously described in the manuscript. The figure legend also labels the neutral condition as pH 7.0, whereas the methods specify pH 7.4. The exact neutral-buffer values and pH must be checked against the original measurements before final numerical comparisons are reported.

Acidic conditions may promote protonation of imidazolate groups and destabilization of the ZIF-8 framework, thereby increasing drug release. Altered drug–carrier interactions at the PDA-coated surface may also contribute. The relative contributions of framework degradation, diffusion, and drug desorption were not directly resolved in this study; mechanistic assignments should therefore be regarded as hypotheses consistent with the measured release trends.

At 48 h under acidic conditions, the supplied curves indicate a larger released fraction for cisplatin than for letrozole. Distinct molecular properties and drug–carrier association could contribute to the difference, but the experiment does not independently establish which mechanism dominates. Furthermore, the profiles do not demonstrate tumor selectivity or reduced systemic toxicity.

3.6. In Vitro Cytotoxicity and IC_{50} Analysis

The viability of MCF-7 breast cancer cells was assessed after 24 and 48 h of exposure to the tested formulations over the reported concentration range of 100–500 µg/mL (Fig. 2). Table 1 reports the IC_{50} values for cisplatin (PT), ZLDC, letrozole (L), ZL, and the unloaded carrier (Z). Comparisons of equal

mass concentrations between free drugs and nanocomposites should be interpreted cautiously because the administered amounts of active drug may differ.

Figure 2 shows concentration-dependent changes in MCF-7 viability after treatment. At 48 h, the plotted PT response falls sharply with increasing concentration, while the other groups show distinct dose-response patterns. The plots support a description of in vitro cytotoxicity but do not by themselves establish reduced systemic toxicity, improved patient compliance, or a sustained therapeutic concentration in vivo. The marked differences between 24- and 48-h responses should be considered alongside the fitted IC_{50} estimates and their uncertainty.

According to Table 1, the IC_{50} values at 24 and 48 h were 635.55 and 40.5 μg/mL for PT; 617.42 and 26.08 μg/mL for ZLDC; 125.71 and 166.37 μg/mL for free letrozole (L); 590.12 and 57.2 μg/mL for ZL; and 1046.33 and 947.34 μg/mL for unloaded ZIF-8 (Z). The decrease in the reported ZLDC IC_{50} over time is compatible with greater apparent cytotoxic potency at 48 h than at 24 h. However, the reported 48-h ZLDC IC_{50} of 26.08 μg/mL is inconsistent with the 556.08 μg/mL listed in Table 2; this discrepancy must be reconciled using the original data. Because several reported IC_{50} values lie outside the tested 100–500 μg/mL interval, these should be identified as extrapolated model estimates if they were derived from curve fitting. High viability of unloaded ZIF-8 in this cancer cell line cannot establish biocompatibility in normal tissues.

Table 1
IC_{50} values (μg mL^{-1}) of different formulations against MCF-7 cells after 24 and 48 h of treatment.

48 hr	24 hr	
40.5	635.55	PT
26.08	617.42	ZLDC
166.37	125.71	L
57.2	590.12	ZL
947.34	1046.33	Z

3.7. Quantitative Synergistic Evaluation (Combination Index)

The combination index (CI) was reported to examine the interaction of letrozole and cisplatin in the ZLDC formulation (Table 2). A conventional Chou–Talalay analysis requires dose–effect relationships for each single agent and the combination at defined effect levels, together with the corresponding drug-equivalent doses. The available summary data do not provide enough information to independently reproduce the reported CI value. IC_{50}

Table 2 lists free-drug 48-h IC_{50} values and a reported effective dose for ZLDC. A cumulative release percentage measured in buffer is not, by itself, an experimentally determined intracellular bioavailable dose. In addition, Table 2 reports a 48-h ZLDC IC_{50} of 556.08 μg/mL, whereas Table 1 reports 26.08 μg/mL for the same endpoint. The original dose-response curves, individual drug loadings, and detailed CI calculation should be checked before interpreting this analysis. IC_{50}

The CI value of 0.72 is retained in Table 2 as originally reported, but its validity cannot be confirmed from the presented information. Accordingly, these data are insufficient to make a definitive quantitative claim of synergy. The combined formulation remains of interest because it incorporates agents with distinct pharmacological mechanisms, although this study does not directly demonstrate aromatase inhibition, DNA damage, or intracellular co-delivery in MCF-7 cells.

Table 2
Quantitative synergy analysis and Combination Index (CI) for the ZLDC system compared to free drug formulations.

Formulation	IC_{50} at 48 h (μg/mL)	Effective Dose (μg/mL)	CI Value	Interaction Type
Free Letrozole	166.37	166.37	—	—
Free Cisplatin	40.50	40.50	—	—
ZLDC Nanoconstruct	556.08	~ 111.21*	0.72	Synergistic

**Note: The reported effective dose and CI have not been independently validated here. The method used to convert cumulative release fractions to a combined active-drug dose, including individual drug-loading fractions and the original dose–response data, should be documented. The 48-h ZLDC IC_{50} in this table also differs from* Table 1 *and requires source-data verification.*

4. In Vitro Release Kinetics and Mechanistic Interpretation

4.1. Mathematical Modeling and Kinetic Study

Release data at pH 5.5 were reportedly fitted to zero-order, first-order, Higuchi and Korsmeyer–Peppas expressions (Table 3). The supplementary fitted plots (Figs. 11S and 12S) display fit statistics and exponents different from those in Table 3, and some plotted time ranges extend beyond the 48-h protocol stated in the methods. Until the raw time–release dataset, fitted intervals, normalization and fitting procedure are confirmed, Table 3 must be regarded as the originally reported numerical summary rather than an independently verified kinetic analysis.

Table 3
Kinetic release parameters of Letrozole and Cisplatin from the nanocarrier at pH 5.5.

Drug	Model	Equation	k	R^2	n	Mechanism
Letrozole	Zero-order	$Q_t=kt+Q_0$	1.060	0.944	--	--
	First-order	$\ln(1-Q_t) = -kt$	0.017	0.991	--	Sustained
	Higuchi	$Q_t=kt^{0.5}$	7.162	0.887	--	--
	Korsmeyer-Peppas	$Q_t=kt^n$	2.610	0.976	0.775	Anomalous
Cisplatin	Zero-order	$Q_t=kt+Q_0$	1.532	-0.576	--	--
	First-order	$\ln(1-Q_t) = -kt$	0.073	0.674	--	--
	Higuchi	$Q_t=kt^{0.5}$	11.953	0.692	--	--
	Korsmeyer-Peppas	$Q_t=kt^n$	23.241	0.925	0.311	Quasi-Fickian

4.2.1. Letrozole Release: Model Fit and Interpretation

Table 3 reports the highest R^2 for the first-order description of letrozole release (0.991) and a Korsmeyer–Peppas exponent of 0.775. However, Fig. 11S displays different goodness-of-fit values and an exponent of about 1.007. Therefore, the mechanism cannot be assigned consistently from the supplied materials. The kinetic parameters and any inference about anomalous transport should be treated as provisional until the raw release series are refitted using a specified range and model definition.

4.2.2. Cisplatin Release: Model Fit and Interpretation

Table 3 reports a Korsmeyer–Peppas exponent of 0.311 and R^2 of 0.925 for cisplatin, whereas the corresponding supplementary plot (Fig. 12S) displays an exponent of approximately 0.426 and R^2 of approximately 0.872. These estimates cannot both describe the same fitting procedure and dataset without further explanation. Hence, the classification as quasi-Fickian diffusion and mechanistic claims about cleavage of cisplatin–PDA coordination bonds remain tentative.

5. Conclusion

A ZIF-8/PDA-based formulation was prepared for combined delivery of letrozole and cisplatin, and the available characterization and in vitro experiments provide preliminary evidence for pH-associated release and concentration-dependent effects on MCF-7 cell viability. According to the verified Table 1, the ZLDC IC_{50} decreased from 617.42 µg/mL at 24 h to 26.08 µg/mL at 48 h. This is an observation at the formulation level and does not, on its own, establish pharmacological synergy or safety in vivo.

Differences between the principal tables and supplementary thermal, release and kinetic plots must be resolved using original records before quantitative mechanistic or therapeutic claims can be confirmed.

Declarations

Author Contribution

Fatemeh Khodabandeh: Investigation, Methodology, Data Curation. Seyed Meysam Baghbanian: Conceptualization, Methodology, Supervision, Project Administration, Writing-Original Draft, Writing-Review & Editing. Milad Arab-Nozari: Supervision, Validation. Maryam Khavarpoor: Supervision, Validation. All authors reviewed and approved the final version of the manuscript.

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Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures

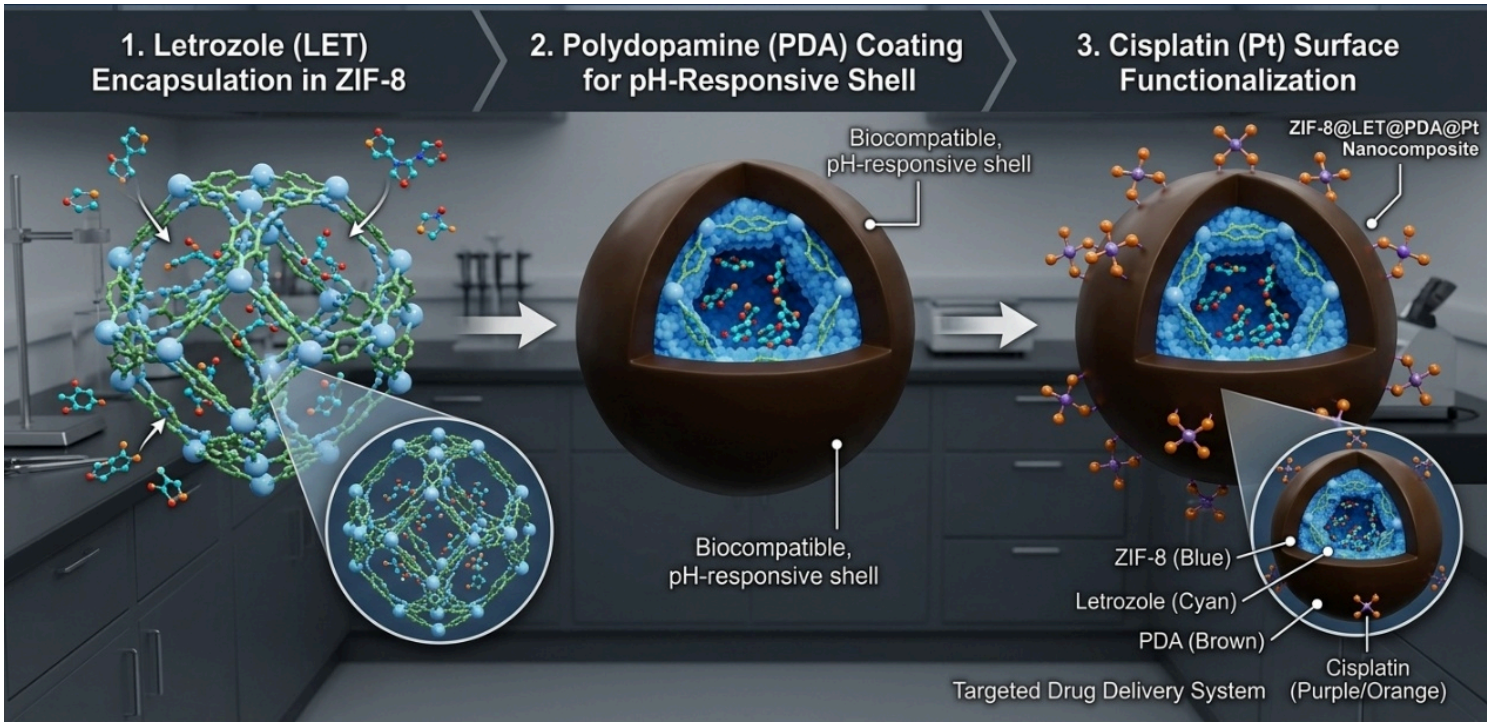


Figure 1

Schematic diagram of hierarchical and pH-responsive ZIF-8@Letrozole@Polydopamine@Cisplatin

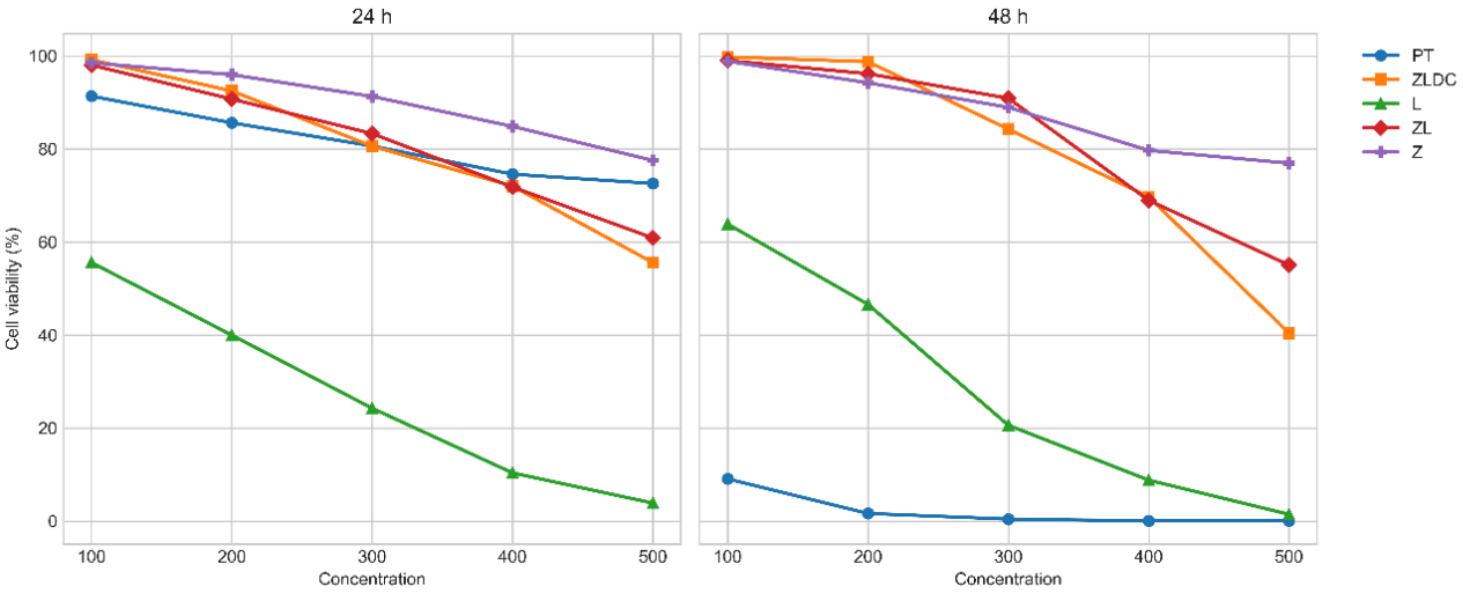


Figure 2

MCF-7 cell viability as a function of nominal treatment concentration after 24 and 48 h for PT, ZLDC, L, ZL, and Z. Concentration units and error bars/replicate information should be verified against the underlying dataset.

Supplementary Files

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