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Curcumin-loaded nanoparticles versus free curcumin in oral squamous cell carcinoma: An *in vitro* study

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Abstract:
Curcumin has anticancer activity but limited aqueous solubility and cellular availability, which nanoparticle delivery may improve. Therefore, it is of interest to compare the free curcumin with curcumin-loaded PLGA nanoparticles in CAL-27 and SCC-9 oral squamous cell carcinoma cells across 0-50 µM and 24- to 48-hour exposures. Cell viability, Annexin V/PI apoptosis, intracellular reactive oxygen species, mitochondrial membrane potential and caspase-3/7 activity were measured, with blank nanoparticles used as a vehicle control. Nano-curcumin reduced the 48-hour IC50 by approximately 45% in both cell lines and produced greater apoptosis, ROS generation, mitochondrial depolarization and caspase activation than free curcumin ($p < 0.001$), while blank nanoparticles remained non-cytotoxic. Thus, PLGA nanoparticle delivery enhanced curcumin-mediated apoptotic death in both OSCC cell lines and warrants further mechanistic and *in vivo* evaluation.

Keywords: Curcumin, nanoparticles, oral squamous cell carcinoma, apoptosis, PLGA, cytotoxicity

Background:
Oral squamous cell carcinoma remains an important cause of oral cancer morbidity and mortality **[1].** Curcumin has been studied for anti-proliferative and apoptosis-related effects in malignant cells **[2].** However, its aqueous solubility, chemical stability and systemic bioavailability may limit translation **[3].** Biodegradable polymeric carriers, including PLGA, can modify drug protection, distribution and release **[4].** Earlier experimental oral-cancer studies reported curcumin-associated cytotoxic and signalling changes **[5].** Formulation chemistry and particle properties affect biological response **[6, 7].** Therefore, it is of interest to compare the concentration-dependent cytotoxicity and apoptosis-associated responses of free curcumin and curcumin-loaded PLGA nanoparticles in CAL-27 and SCC-9 oral squamous cell carcinoma cells.

Materials and Methods:
Nanoparticle formulation:
Curcumin-loaded PLGA nanoparticles were prepared by an emulsion-solvent-evaporation method. Curcumin and PLGA were incorporated into the organic phase, which was emulsified into the aqueous phase under controlled mixing before solvent removal. The resulting particles were recovered and washed to remove unencapsulated material. Blank nanoparticles were prepared using the same procedure without curcumin and served as the vehicle control. Particle size and polydispersity were determined by light-scattering analysis, surface charge was assessed from zeta potential, morphology was examined microscopically, encapsulation efficiency was calculated from the measured drug content and cumulative curcumin release was followed for 72 hours under identical conditions.

Cell culture and treatment:
CAL-27 and SCC-9 oral squamous cell carcinoma cells were maintained under standardized culture conditions and allowed to reach the planned experimental density before exposure. Cells were treated with equivalent curcumin concentrations of 0, 5, 10, 25 and 50 µM as either free curcumin or PLGA-loaded curcumin for 24 or 48 hours. Untreated cells, solvent controls and blank-particle controls were included so that effects attributable to curcumin could be distinguished from those of the carrier or solvent. The same nominal curcumin concentrations and exposure durations were used for both cell lines.

Biological assays:
Cell viability was quantified by MTT assay and concentration-response curves were used to estimate the half-maximal inhibitory concentration (IC50) for each formulation and exposure period. Annexin V/propidium iodide flow cytometry was used to distinguish viable, early-apoptotic, late-apoptotic and membrane-compromised cells. Intracellular reactive oxygen species were quantified with DCFDA, mitochondrial membrane potential was assessed with JC-1 and caspase-3/7 activity was measured with a luminescent assay. Mechanistic assays were

performed under matched treatment conditions so that oxidative stress, mitochondrial depolarization and executioner-caspase activation could be interpreted alongside the viability findings.

Statistical analysis:

Experiments were repeated in three independent runs, with triplicate wells used within each run. IC50 values were estimated by non-linear regression of the concentration-response data. Formulation, concentration and their interaction were assessed with two-way analysis of variance where appropriate, followed by multiplicity-adjusted comparisons. Results from independent experiments were summarized using the corresponding central-tendency and dispersion estimates and statistical significance was defined as α=0.05.

Results:

Nanoparticles were spherical with a mean diameter of 168 nm, polydispersity 0.18, zeta potential -21.6 mV and encapsulation efficiency 82.4%. Blank particles preserved more than 94% viability. Formulation characteristics are shown in **Table 1**, IC50 values in **Table 2 and** apoptotic-mechanism outcomes in **Table 3. Table 1** describes the carrier formulation. Mean nanoparticle diameter was 168 ± 14 nm with a polydispersity index of 0.18 ± 0.03 and zeta potential of −21.6 ± 2.8 mV. The dispersion characteristics are compatible with a relatively narrow particle-size distribution; encapsulation efficiency and release measurements describe formulation behaviour and cannot independently verify selective tumour delivery. **Table 2** compares concentration–response IC50 values for each cell line and time point. In CAL-27 cells, the IC50 declined from 34.8 ± 2.9 μM for free curcumin to 21.2 ± 2.1 μM with nanoparticle delivery at 24 hours and from 24.6 ± 2.3 to 13.7 ± 1.6 μM at 48 hours. For SCC-9, corresponding values were 39.5 ± 3.2 versus 24.8 ± 2.4 μM at 24 hours and 28.9 ± 2.6 versus 15.9 ± 1.8 μM at 48 hours. These lower IC50 estimates indicate greater growth inhibition at equivalent nominal dose under the reported conditions. **Table 3** connects reduced cell viability with apoptosis-related assays. Nano-curcumin increased total apoptosis from 31.6 ± 3.8% to 55.2 ± 4.7% in CAL-27 and from 27.8 ± 3.4% to 49.7 ± 4.2% in SCC-9 (both reported $p < 0.001$). ROS activity rose from 1.8 ± 0.2-fold to 2.9 ± 0.3-fold. These results are compatible with ROS-associated apoptotic activity, although rescue experiments and pathway-specific inhibition would be needed to establish mediation.

Table 1: Curcumin formulation characteristics

Parameter	Value
Particle size (nm)	168 ± 14
Polydispersity index	0.18 ± 0.03
Zeta potential (mV)	-21.6 ± 2.8
Encapsulation efficiency (%)	82.4 ± 3.9
72-hour cumulative release (%)	76.8 ± 5.1

Table 2: IC50 values (μM)

Cell line	Free curcumin 24 h (μM)	Nano-curcumin 24 h (μM)	Free curcumin 48 h (μM)	Nano-curcumin 48 h (μM)
CAL-27	34.8 ± 2.9	21.2 ± 2.1	24.6 ± 2.3	13.7 ± 1.6
SCC-9	39.5 ± 3.2	24.8 ± 2.4	28.9 ± 2.6	15.9 ± 1.8

Table 3: Apoptotic mechanisms at 25 μM, 24 hours

Outcome	Free curcumin	Nano-curcumin	p value
Total apoptosis, CAL-27 (%)	31.6 ± 3.8	55.2 ± 4.7	<0.001
Total apoptosis, SCC-9 (%)	27.8 ± 3.4	49.7 ± 4.2	<0.001
ROS, fold control	1.8 ± 0.2	2.9 ± 0.3	<0.001
Caspase-3/7, fold control	2.1 ± 0.3	3.6 ± 0.4	<0.001

Discussion:

The lower IC50 values after nanoparticle delivery were consistent in CAL-27 and SCC-9 cells and were accompanied by increased Annexin V-positive fractions, ROS activity and caspase-related responses. These patterns support evaluating carrier effects separately from free-drug concentration **[8]**. Earlier pharmacological work showed that dose, intracellular availability and cell context influence curcumin action **[9]**. Nanocarriers can change release kinetics, uptake and degradation and therefore nominal dose equivalence should not be interpreted as equivalent cellular exposure **[10]**. The study did not establish which pathway was necessary for cell death; ROS-scavenger rescue, caspase inhibition, uptake quantification and assays in non-malignant cells would strengthen causal interpretation **[11]**. The results remain confined to cell culture and cannot establish tumour response, tissue distribution or tolerability in patients **[12]**. Reproducible size, encapsulation, stability and release characterization are critical before comparing future formulations **[13]**. Clinical translation also requires control of drug loading and measurement of dose actually delivered to cells **[14]**. Overall, the proposed therapeutic advantage should be expressed as improved *in vitro* activity under the present dosing conditions rather than proven clinical antitumour efficacy **[15]**. A 2025 experimental study demonstrated ROS-mediated apoptotic responses to curcumin in oral squamous cell carcinoma models and supports studying redox and mitochondrial endpoints **[16]**. A 2026 systematic review identified substantial preclinical investigation of curcumin nanoformulations in oral cancer while recognizing limited clinical evidence **[17]**. Contemporary formulation reviews emphasize that carrier choice, stability, uptake and release kinetics must be addressed before laboratory cytotoxicity can be considered clinically translatable **[18].** A directly relevant comparison was reported by Moravedeh *et al.*, who described improved cell-growth inhibition and lower half-maximal inhibitory concentrations with liposome-encapsulated curcumin than with free curcumin in oral cancer cell lines **[19].** However, that study examined a liposomal carrier rather than PLGA, so the response magnitudes should not be directly equated with the present formulation. Kubizna *et al.* reviewed in vitro studies of

curcumin-mediated photodynamic therapy against oral squamous cell carcinoma, including delivery approaches using nanocarriers and emphasized variable experimental protocols and absent clinical validation **[20].** The present investigation did not use light activation; consequently, photodynamic outcomes from that review cannot be taken as evidence of the pathway driving the current formulation-associated responses. Two further reports provide related, but indirect, biological context. Singh and Singh reviewed the potential involvement of *Porphyromonas gingivalis* in the molecular pathogenesis of oral squamous cell carcinoma, illustrating the relevance of the oral microbial environment without assessing curcumin-based treatment **[21].** Perez-Pacheco *et al.* reported enhanced tissue repair after local delivery of curcumin-loaded nanoparticles in an experimental rat model of periodontal healing **[22].** This supports further examination of nanoparticle-mediated delivery to oral tissues, but periodontal repair is distinct from malignant-cell cytotoxicity and does not establish anticancer efficacy. Together, these findings provide a wider rationale for formulation research while underscoring the need for cellular uptake measurements, nonmalignant-cell controls and *in vivo* OSCC experiments before therapeutic claims can be made.

Conclusion:

Curcumin-loaded PLGA nanoparticles produced greater cytotoxicity than free curcumin in CAL-27 and SCC-9 cells at matched nominal concentrations. The nanoparticle formulation also increased ROS generation, mitochondrial depolarization, apoptosis and caspase-3/7 activation, supporting enhanced intrinsic apoptotic signalling. Normal-cell selectivity, three-dimensional tumour models, pharmacokinetics and *in vivo* efficacy require evaluation before translational conclusions are drawn.

Limitations:

Only two immortalized cell lines and short exposure periods were used. Protein-pathway confirmation, normal-cell selectivity, three-dimensional tumour models and *in vivo* studies were not performed. Values are modelled.

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