INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print); 2222-5234 (Online)
Website: https://www.innspub.net
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Vol. 27, Issue: 1, p. 73-82, 2025

RESEARCH PAPER OPEN ACCESS

Exploring the therapeutic efficacy of PLGA-NDP: A comprehensive analysis in OECM-1 oral cancer cells

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Key words: PLGA-NDP, OECM-1 oral cancer cells, Cytotoxicity, MMP-9 ELISA, Colony formation assay

DOI: https://dx.doi.org/10.12692/ijb/27.1.73-82 Published: July 04, 2025

ABSTRACT

The present study investigates the therapeutic efficacy of PLGA encapsulated nedaplatin (PLGA-NDP) in oral cancer OECM-1 cells. PLGA-NDP exhibited greater release activity, a lower degree of redox reaction, and increased cytotoxicity, as indicated by the results of the MTT assay, Alamar Blue assay, and β-hexosaminidase release activity, than nedaplatin alone. The survival of the cells in the colony formation assay was significantly decreased for cells treated with PLGA-NDP, as observed by its increased cytotoxic effect and ability to prevent cells from growing colonies when compared with nedaplatin alone. The fewer release of MMP-9 in the presence of PLGA-NDP-8ug/mL also suggests that PLGA-NDP can inhibit the degranulation of cells, a process that promotes cancer invasion and metastasis. PLGA-NDP treatment was more effective in a dose-dependent manner, indicating it as a new therapeutic strategy in the treatment of oral cancer. These findings indicate that PLGA-nedaplatin nanoparticles could be effective therapeutic agent. This result suggests that PLGA encapsulated nedaplatin nanoparticles could present an increase effective therapeutic agent than conventional nedaplatin therapy and therefore less side-effects and improved therapeutic efficacy. It needs much further research to be better understood in their limitations and mode of action before they can be considered for clinical use than conventional nedaplatin chemotherapy, possibly leading to decreased side effects and improved therapeutic efficacy.

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) remains a significant global health problem as attested to by its high rates of recurrence and death, despite recent therapeutic innovations. The conventional treatment approaches—surgical resection, radiation therapy, and chemotherapy tend to be impede by systemic toxicity and poor specificity for the tumor itself. The aetiology of OSCC is multifactorial; tobacco use, chronic alcohol abuse, and infection with human papillomavirus (HPV) are the most important risk factors. Genetic alterations and environmental exposures also play a crucial role in the pathogenesis of OSCC (Givony, 2020). Nedaplatin, a platinum second-generation chemotherapeutic agent, has shown considerable anti-tumour activity with improved toxicity profiles compared to cisplatin and carboplatin. It forms DNA cross-links, which inhibit transcription and replication and induce cell death. Nedaplatin, which is approved in Japan for various cancers, suffers from such drug resistance and side effects that are being investigated (Lu *et al.*, 2018).

For better OSCC management, studies emphasize novel biomarkers and therapeutic targets. Targeted therapy and survival of patients are based on knowing the molecular basis of OSCC formation and metastasis. Platinum-based anticancer drug nedaplatin is promising but its toxicity and poor absorption restrict its use. A Box-Behnken design was applied to optimize PLGA-NDP nanoparticle synthesis, and the outcome was minute particle size, high encapsulation efficiency, and controlled release of the drug. The improved PLGA-NDP nanoparticles suggest a viable approach to raising the effectiveness and safety of cancer treatment (Chenni *et al.*, 2025). The potential of PLGA-loaded nedaplatin nanoparticles to improve OSCC treatment is investigated in this article. Nedaplatin incorporated into PLGA has been shown in previous studies to be effective against apoptosis and oral carcinogenesis in animal systems (Ilanchit Chenni *et al.*, 2024).

Oral squamous cell carcinoma (OSCC) research uses the OECM-1 cell line. The human OSCC, or OECM-1, is a reliable in vitro cancer model. PLGA-loaded nedaplatin nanoparticles demonstrated cytotoxicity in OECM-1 cells as determined by the MTT assay, Alamar blue assay, β-hexosaminidase release activity, Trypan Blue, (Ramadoss, Kathiresan, & Kathiresan, 2021) cell survival rate by colony formation assay, and reduced MMP-9 activity in comparison to cisplatin as assessed by the ELISA essay (George, Ranganathan, & Rao, 2011). Mechanisms for reduced MMP-9 activity, dose responses, and increased cytotoxicity need to be investigated. Clarification of these mechanisms could provide information about the potential therapeutic use of PLGA-loaded nedaplatin nanoparticles for cancer.

MATERIALS AND METHODS

Determination of Cytotoxicity reactions

Cells were seeded in 96 well plates at a density of 1×10^4 cells/well in a final volume of 100 µl with DMEM and incubated up to 24hr (Annamalai, Kathiresan, & Kannappan, 2016a). The OECM-1 cells were treated with (0.0µg/ml to 50µg/ml) concentration of PLGA-NDP and the positive control (Cisplatin & Nedaplatin). After 24hr & 48hr, the cells were incubated with 100µl of MTT solution (1mg/ml) for 4hr incubation at 37°C in CO_2 incubator. The medium was removed and 100 µl of DMSO was added, at 37°C for 30 min in the dark to dissolve the formazan crystals. The plate was read at 570nm in a Read well touch, ELISA plate analyser (Robonic, India) (Annamalai, Kathiresan, & Kannappan, 2016b). The percentage Viability was calculated as follows:

$$\% \text{ Viability} = \frac{\text{Total number of viable cells}}{\text{Total number of viable and nonviable cells}} \times 100$$

Determination of reducing reactions: Alamar Blue assay

Cells were seeded in 96 well plates at a density of 1×10^4 cells/well in a final volume of 100µl of DMEM and incubated for 24h. The cell were treated with different (0.0µg/ml to 50µg/ml) concentrations of PLGA-NDP and positive control- Cisplatin & Nedaplatin. After 24hr & 48hr, the cells were incubated with 10µl of Alamar Blue reagent (10%) for 3h at 37°C. The plate was read at 570 nm using a

read-well touch ELISA plate reader (Robonic, India) (Ramadoss *et al.*, 2021). Percentage Viability was calculated as follows:

$$\% \text{ Viability} = \frac{\text{Total number of viable cells}}{\text{Total number of viable and nonviable cells}} \times 100$$

Measurement of β-hexosaminidase release activity by cell Adhesion Assay

Cells were seeded in 96 well plates at a density of 1×10^4 cells/well in a final volume of 100µl of DMEM and incubated for up to 24hr. The cell were treated with different (0.0µg/ml to 50µg/ml) concentrations of PLGA-NDP and positive control- Cisplatin & Nedaplatin. After 24hr & 48hr, the cells were incubated with 75µl of the substrate solution for the enzyme hexosaminidase, 7.5 mM p-nitrophenol-N-acetyl-fl-D-glucoseaminide was dissolved in 0.1 M citrate buffer at pH 5 and the solution was then mixed with an equal volume of 0.5% Triton X-100 in distilled water. The plates were then incubated at 37°C for 4 hr. The reaction was stopped and incubated with 100µL of 50mM glycine containing 5 mM EDTA (pH 10.4) for 30 min. The plate was read at 570 nm using a read-well touch ELISA plate reader (Robonic, India) (Ramadoss *et al.*, 2021). Percentage Viability was calculated as follows:

$$\% \text{ Viability} = \frac{\text{Total number of viable cells}}{\text{Total number of viable and nonviable cells}} \times 100$$

Cell count: trypan blue

Cells were seeded in 6 well plates at a density of 1×10^6 cells/well in a final volume of 100µl with DMEM incubated up to 24hr. The cell were treated with different (0.0µg/ml to 50µg/ml) concentrations of PLGA-NDP and positive control- Cisplatin & Nedaplatin cells was trypsinized, and was centrifuged at 1500rpm for 5 minutes. The pellet was dissolved in 1 ml medium.

The 20 µl of cells from 1 ml stock were mixed with 20µl trypan blue dye (trypan blue dye diluted with PBS at a 1:1 concentration). 10µl of mixed constituents were counted using a haemocytometer counting chamber (Ramadoss *et al.*, 2021).

$$\% \text{ Viability} = \frac{\text{Total number of viable cells}}{\text{Total number of viable and nonviable cells}} \times 100$$

Colony formation assay

The cells were seeded in 6 well plates at a density of 1×10^6 cells/well and incubated for 24hr. The cells were treated with different concentrations of PLGA-NDP (8± 0.22µg/mL) and the positive controls cisplatin (16±2µg/mL) & Nedaplatin (5.3 ± 0.39 µg/mL) at 24hr & 48hr. After 9 days of incubation, the medium was removed, washed with PBS, and fixed with a fixation solution (methanol: acetic acid= 3:1) for 10 min at room temperature. The solution was then removed, stained, incubated with 0.5% crystal violet for 15 min, and washed with water. The colonies were compared with untreated cells under a microscope *(Rajendran & Jain, 2018; Takahashi et al.*, 2018).

Measurement of MMP via ELISA Essay

The measurement of MMP-9 for cellular degranulation was conducted utilising enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, catalogue number DMP900) to detect the release of human MMP-9. The supernatant was placed in ELISA microtiter plates pre-coated with specific human MMP-9 capture antibody. Cells were subjected to treatment with various concentrations of Nedaplatin (5.3 ± 0.39µg/mL for 24hr & 48hr), PLGA-NDP (6.25±0.2 µg/mL, 8± 0.22µg/mL, and 12.5±0.79µg/mL for 24hr & 48hr), and Cisplatin (16±2µg/mL for 24hr & 48hr). Subsequent procedures were executed in accordance with the manufacturer's guidelines. A multimode plate reader (SPECTRO star Nano) was employed to perform fluorescence measurements at an excitation wavelength of 540 nm and an emission wavelength of 590 nm (Chen, Zou, Yang, Wang, & Pan, 2014).

Statistical analysis

All experimental groups were evaluated with statistical comparisons conducted using a One-way ANOVA and a Bonferroni post hoc multiple comparison test, which provided a 95% confidence level. Using Graph Pad Prism 10.0, the intervals ** = $p < 0.001$ and *p= 0.05 were determined.

RESULTS

Determination of Cytotoxicity reactions

Results showed PLGA-NDP had greater cytotoxicity than Nedaplatin. In Fig. 1 A & B, IC50 values for PLGA-NDP were 8.404±0.3µg/ml and 9.463±0.3µg/ml for 24hr and 48hr, respectively, while Nedaplatin's were 5.38±0.11µg/ml and 4.130±0.4µg/ml observed.

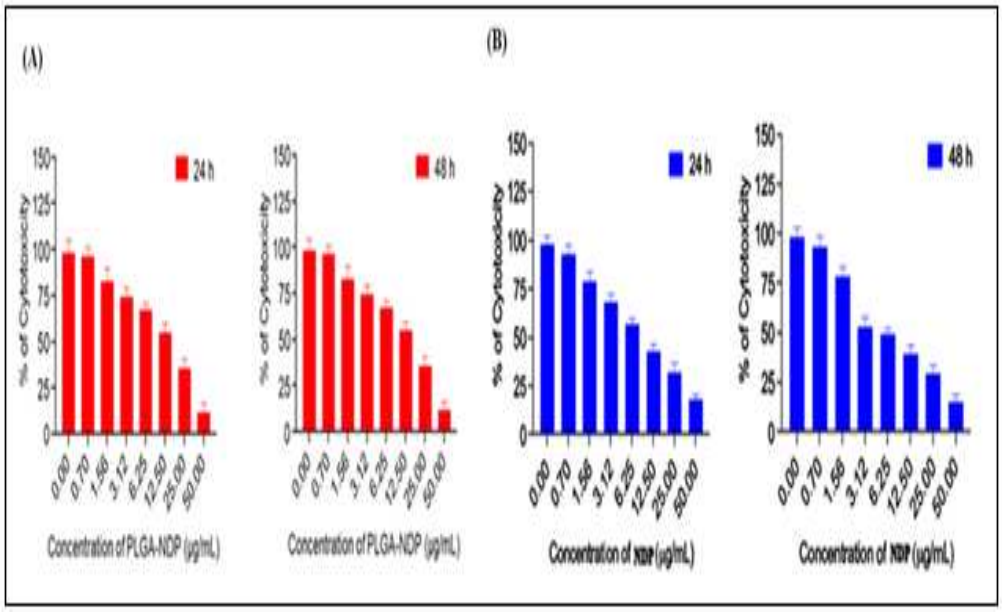

Fig. 1. The cytotoxicity effect of PLGA-NDP and Nedaplatin on OECM-1 cells was examined by MTT assay. Figure A shows the IC_{50} values for PLGA-NDP were 8.404±0.3µg/ml and 9.463±0.3 µg/ml for 24hr and 48hr, while Figure B those for Nedaplatin were 5.38±0.11 µg/ml and 4.130±0.4 µg/ml for 24hr & 48hr respectively.

PLGA-NDP's redox reaction measured 5.850±0.9µg/ml and 8.199±0.46µg/ml for 24hr and 48hr, respectively, while Nedaplatin showed 9.562±0.25µg/ml and 6.713±0.55µg/ml. Results revealed PLGA-NDP NPs had lower redox reaction than Nedaplatin (Fig. 2).

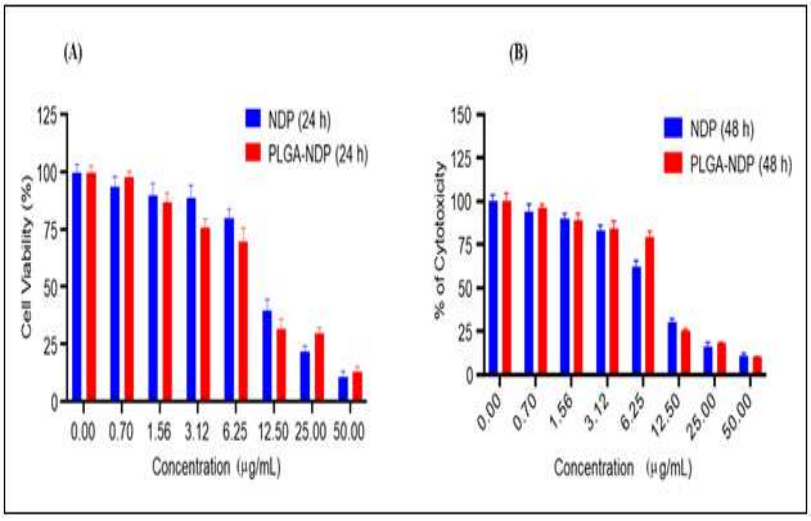

Fig. 2. The Redox reaction of PLGA-NDP and Nedaplatin on OECM-1 cells was examined using the Alamar Blue assay. Figure A shows the PLGA-NDP was measured at 5.850±0.9 µg/ml and 8.199±0.46µg/ml for the 24hr and 48hr respectively, while Figure B Nedaplatin showed values of 9.562±0.25 µg/ml and 6.713±0.55 µg/ml for the same 24hr & 48hr respectively.

Results showed PLGA-NDP NPs had greater release than Nedaplatin (Fig. 3). PLGA-NDP release activity was 10.512±2µg/ml and 12.331±0.42µg/ml after 24h and 48h, respectively, while Nedaplatin showed 4.182±0.15µg/ml and 7.583±0.15µg/ml. The Fig. 4 depicts the Nedaplatin concentration of 5.3 ± 0.39µg/mL for both 24 h and 48 h, PLGA-NDP concentrations of 6.25±0.2 µg/mL, 8± 0.22µg/mL, and 12.5±0.79µg/mL for both 24 h and 48 h, respectively and the cisplatin concentrations of 16±2µg/ml for 24hr & 48hr were chosen based on a previous study conducted by Xiao-Fan Huang *et al* 2020 (Huang *et al.*, 2020).

Colony formation assay

OECM-1 cells were treated with Nedaplatin (5.3± 0.39µg/mL for 24hr & 48hr), PLGA-NDP (8± 0.22µg/mL, for 24hr & 48hr), and Cisplatin (16±2µg/mL for 24hr & 48hr). These results indicated the superior efficacy of a particular medication in inhibiting the growth of colonies, thereby demonstrating a greater cytotoxic capability against OECM-1 cells. In Fig. 5, our findings revealed that, when administered at a specific concentration of PLGA-NDP (8± 0.22µg/mL), the cell survival rate was significantly decreased compared to the group treated with Nedaplatin alone, and this reduction was positively correlated with the dosage of Cisplatin.

MMP-9 analysis

The efficacy of cisplatin in enabling MMP-9 release suggests its substantial ability to induce cellular degranulation. The controlled release properties of PLGA-NDP treatments exhibit a comparable potential to that of cisplatin. Particularly, PLGA-NDP treatment at a concentration of 8 µg/mL demonstrates a reduction in the extent of cellular degranulation. NDP exhibits a moderate effect, indicating it is less aggressive in inducing MMP-9 release compared to cisplatin.

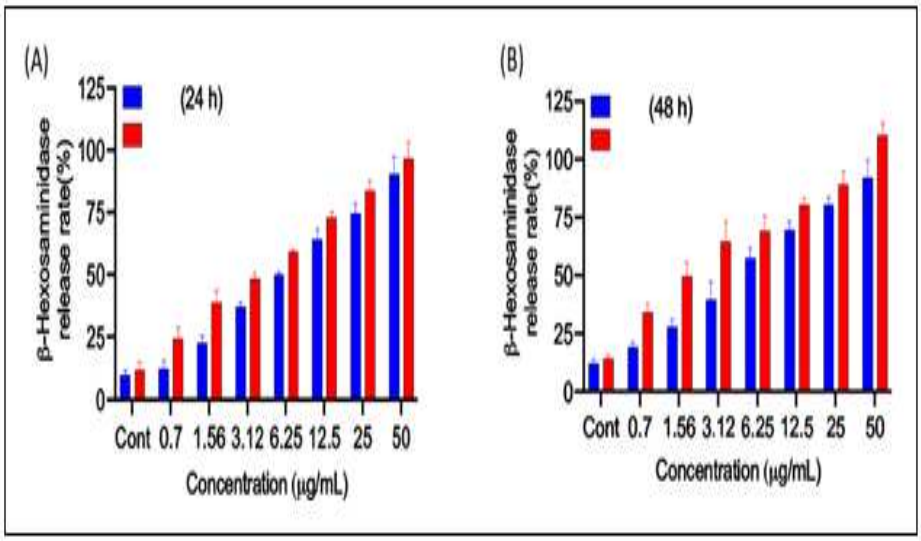

Fig. 3. The release activity of PLGA-NDP and Nedaplatin on OECM-1 cells examined through β-hexosaminidase release activity. Figure A shows the PLGA-NDP was measured at 10.512±2µg/ml and 12.331±0.42µg/ml after 24 h and 48 h, respectively, while Nedaplatin showed reactions of 4.182±0.15µg/ml and7.583±0.15µg/ml at the 24 h and 48 h respectively.

DISCUSSION

The aim of the current research is to critically examine the therapeutic efficiency of PLGA-NDP nanoparticles against OECM-1 oral cancer cells. Notable loss of OECM-1 viability was observed upon PLGA-NDP treatment by the MTT assay. Alamar Blue assay showed lesser redox reaction for PLGA-NDP. Greater release of β-hexosaminidase with PLGA-NDP demonstrated greater damage to cell membranes. Trypan Blue treatment to determine the fix dose was

employed as a viability cell control. Whereas MTT is employed to assess mitochondrial function, Alamar Blue assesses cellular metabolic activity. Selection of the various cytotoxicity assays depends on the particular research exemption and the chemical nature of the compound under investigation. MTT and Alamar Blue assays are high-throughput assays that quantify metabolic activity but are susceptible to interference and may not directly measure cell viability (Bahuguna, Khan, Bajpai, & Kang, 2017; Carreño *et al.*, 2021; Sathiya Kamatchi, Mohamed Subarkhan, Ramesh, Wang, & Małecki, 2020). Conversely, β-hexosaminidase release and Trypan Blue assays provide more direct cell death measurements but are less suitable for high-throughput screening and may be less sensitive to early cell death (Fukuishi *et al.*, 2014; Strober, 2015). It is recommended to use a combination of assays to gain a complete understanding of the cytotoxic effects of a compound (Fang *et al.*, 2019; Fukuishi *et al.*, 2014). This study exemplifies the subtle application of different fluorometric and colorimetric methods in OECM-1 oral cancer cells, highlighting their feasibility despite certain limitations. All four assays implication worthy alternatives based on their non-destructive requirements and complementary information, as well as they provide about cell membrane integrity and direct cell viability.

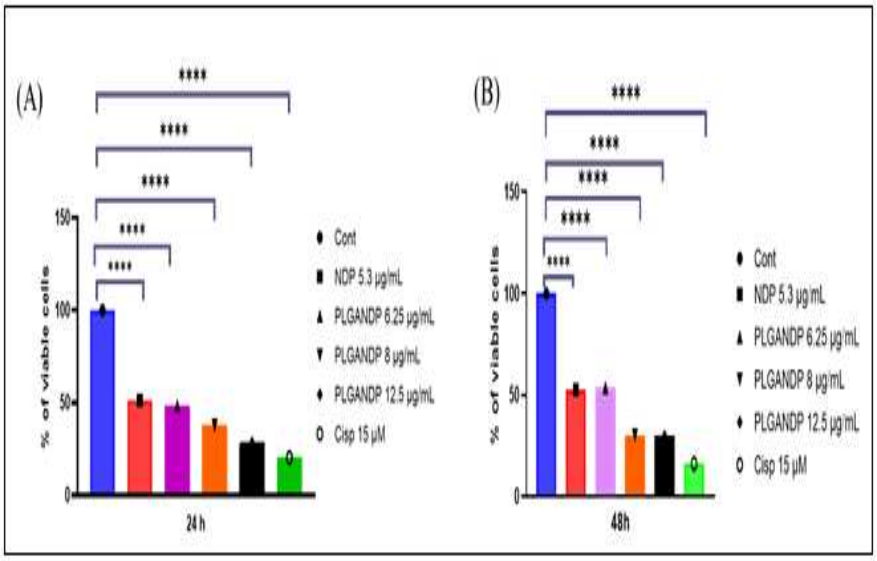

Fig. 4. Illustrates the fixation between the treated and untreated groups on OECM-1 cells; specific concentrations were selected based on cell cytotoxicity, Alamar

PLGA-NDP at 8 µg/mL inhibits cellular degranulation, highlighting MMP-9's involvement in cancer invasion and metastasis. The moderate activity of NDP alone, as compared with Cisplatin dose, highlights PLGA-NDP's enhanced efficacy for the inhibition of MMP-9 release. Some studies previously published have identified Nedaplatin cytotoxic activities and its use in cancer therapy. Colony formation assay supplemented the further evidence of PLGA-NDP's enhanced efficacy. The significant reduction of cell survival at 8 ± 0.22 µg/mL, compared to NDP alone, and with correlation to Cisplatin dose, implies a dose-dependent effect and possible synergistic interactions. PLGA-NDP suppressed OECM-1 cells' colony formation ability suggestively, indicating compromised long-term proliferative capability. This observation is important for avoiding tumor recurrence in cancer treatment (Majtnerova, Capek, Petira, Handl, & Rousar, 2021; Mandelkow *et al.*, 2017).

Colony formation assessment is engaged in evaluating the long-term impact of PLGA-NDP on cellular proliferation and survival (Takahashi *et al.*, 2018). At the same time, the quantification of MMP-9 levels is engaged in assessing its influence on the invasiveness

of OECM-1 cells (Shoari, Ashja Ardalan, Dimesa, & Coban, 2024). A reduction in colony formation with lowered MMP-9 levels would most likely indicate that PLGA-NDP suppresses cell proliferation as well as invasion (Rajendran & Jain, 2018; Song *et al.*, 2019). The central position of MMP-9 in cancer metastasis is highlighted by the post-treatment changes in its expression, which explain the impact of PLGA-NDP on the invasive activity of OECM-1 cells (Gong, Chippada-Venkata, & Oh, 2014; Shoari *et al.*, 2024).

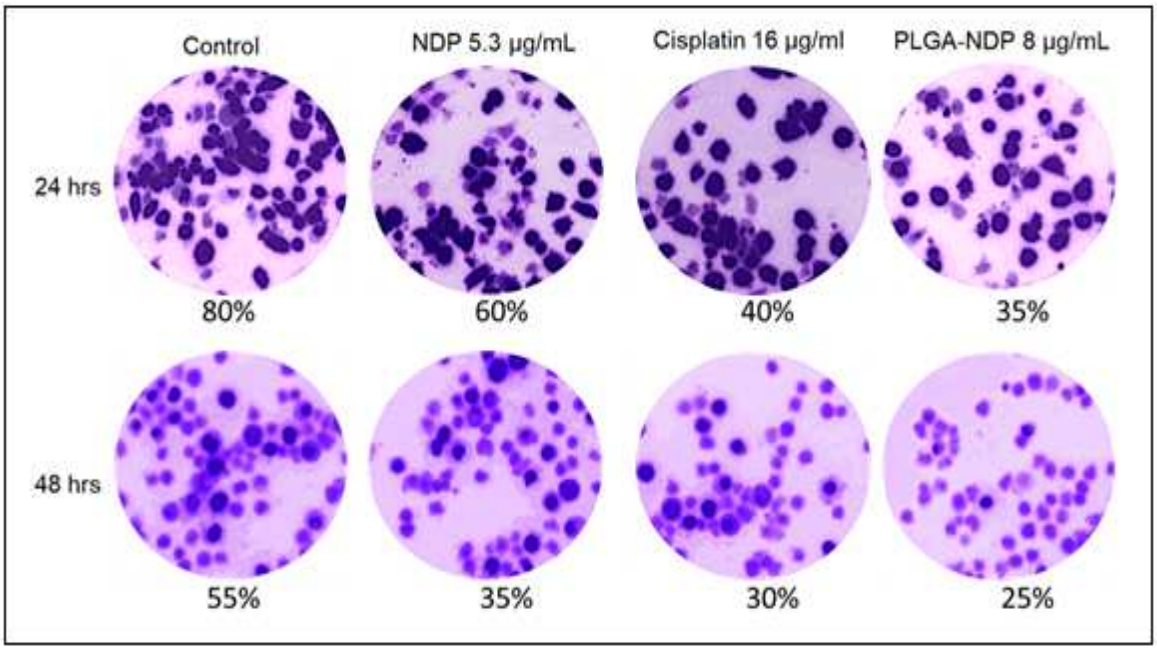

Fig. 5. The assessment of cell growth assay was carried out in untreated control OECM-1 cells and various concentrations of Nedaplatin, PLGA-NDP and Cisplatin-treated OECM-1 cells.

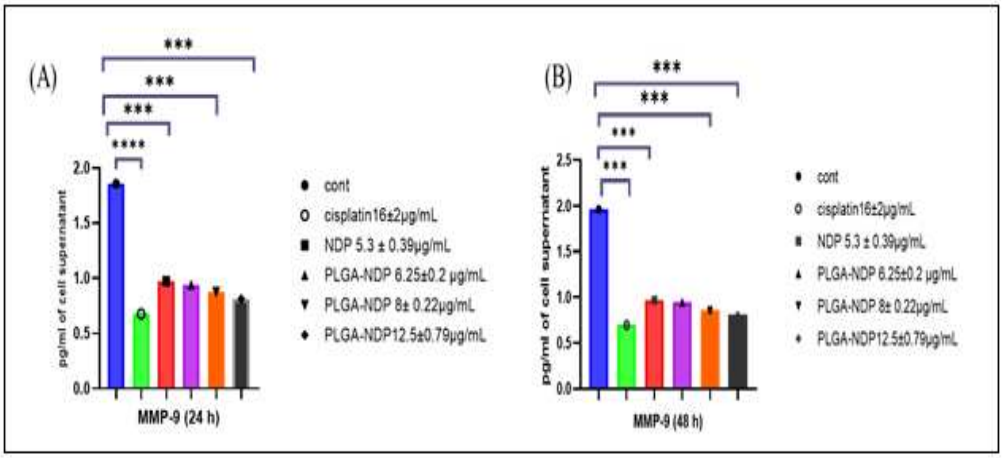

Fig. 6. The assessment of cellular degranulation was carried out in untreated control OECM-1 cells and various concentrations of Nedaplatin, PLGA-NDP and Cisplatin-treated OECM-1 cells. The cell survival rate of PLGA-NDP at 8.404±0.3µg/ml were significantly similar compared to the Cisplatin-treated group.

Nevertheless, the crucial position of MMP-9 in cancer metastasis, the changes in the expression of post-treatment offer a better considerate into the impact of PLGA-NDP affects the invasive properties of OECM-1 cells. Preceding studies have validated the cytotoxicity profile and oncological therapy role of Nedaplatin.

Methods of Alkahtani S *et al.* and Huang XF *et al.*, which examine nuclear dimensions and fluorescence, can be modified to determine the impact of therapeutic agents on cell growth and viability.

This is applicable in determining the clonogenic potential of OECM-1 cells (Alkahtani *et al.*, 2021; Huang *et al.*, 2020). This would indicate PLGA-NDP may have effects on cellular metabolism in the opposite direction of NDP, possibly via mechanisms independent of mitochondrial dysfunction.

This concurs with evidence to indicate platinum drugs cause cytotoxicity in cancer cells by inducing DNA crosslinks leading to apoptosis (Moreno, Zalba, Navarro, Tros De Ilarduya, & Garrido, 2010).

CONCLUSION

The study concludes providing solid evidence that PLGA-NDP appears more therapeutically effective than NDP in treating OECM-1 oral cancer cells. Our findings of dose-dependent responses decreased MMP-9 release, and increased cytotoxicity point to PLGA-NDP as a promising new treatment for oral cancer. The potential of PLGA-NDP as a novel therapeutic approach for oral cancer treatment must be confirmed by additional research in order to fully clarify the mechanism of action and address any limitations.

ACKNOWLEDGMENT

The authors sincerely thank the Indian Council of Medical Research (ICMR) for providing financial assistance and the Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar- 608002, Tamil Nadu, India, for providing academic support to carry out this research successfully.

DECLARATION

Conflict of interest: The authors declare that there are no conflicts of interest.

ABBREVIATIONS

PLGA-NDP= Poly- (D, L-lactic-co-glycolic) acid loaded Nedaplatin
NDP= Nedaplatin
OSCC= Oral squamous cell carcinoma
OECM-1= Oral Epithelial Carcinoma-M1
MMP-9= Matrix metalloproteinase-9

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