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Research Article

Evaluating Polymeric-Based Nanoparticles of Oncolytic Measles Virus for the Treatment of Cervical Cancer

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Abstract

Globally, cervical cancer is a leading cause of mortality in women, highlighting the need for effective treatment strategies. Oncolytic viruses (OVs), including Oncolytic Measles Virus (OMV), not only preferentially infect the cancer cells but also enhance anti-tumor immunity and safety. This paper focuses on developing polymeric OMV nanoparticles (NPs) for cervical cancer therapy. NPs with hyaluronic acid (HA) were synthesized through a green synthesis process, in a preparation with biodegradable materials. Moreover, the ionic gelation method was used to encapsulate OMV. Chitosan was cross-linked with glacial acetic acid while OMV was incorporated under stirring for better encapsulation. OMV-loaded NPs had a size of 1236 nm with a PDI of 0.972, while blank NPs had a size of 522.5 nm, indicating stability with a negative charge of -14.5 mV. X-ray diffraction (XRD) and Scanning Electron Microscope (SEM) reveal the amorphous nature and proper encapsulation of OMV NPs for targeted drug delivery. The HA-coated NPs synthesized in this study had better stability, cellular penetration, and CD44-mediated cancer therapy applications. This approach seems to offer a viable strategy to promote the clinical efficacy of OMV-based therapeutics in cervical cancer therapy.

Keywords: Nanoparticles, cervical cancer, oncolytic Measles virus, targeted therapy, polymers

1. Introduction

Cervical cancer is an abnormal growth of cells in the cervix, primarily caused by the Human Papillomavirus (HPV), especially strains HPV-16 and HPV-18. The virus integrates its genome into host cells, leading to disorganized cell growth and spreading throughout the epithelium. Reactive Oxygen Species (ROS) and oxidative stress also play critical roles in cancer development by damaging proteins, lipids, and nucleic acids. Key biomarkers, including antioxidative enzymes, malondialdehyde (MDA), and 8-hydroxy-2-deoxyguanosine (8-OHdG), have been linked to elevated oxidative stress levels in cancer (Kousar et al., 2022). Studies show that cancer patients have lower antioxidant enzyme activity but higher MDA and 8-OHdG levels, suggesting these biomarkers may help with assessing cancer prognostication. Further research is needed to confirm their effectiveness. Much like the other biological processes, the female reproductive system relies on cell signaling for functions like implantation, oogenesis, and embryo formation. Exosomes, carrying genetic and proteomic information, are essential for cell signaling in reproduction and may serve as biomarkers in pregnancy. Cervical cancer treatments traditionally include radiotherapy, chemotherapy, and brachytherapy. However novel methods like novel methods like CAR-T cell therapy are emerging due to the debilitating effects of conventional therapies Nanotechnology offers

innovative drug delivery options for cervical cancer treatment, with polymeric NPs, and like PLGA, allowing targeted as well as controlled drug release. Nanomedicine, through therapies like immunotherapy and gene therapy, minimizes side effects by delivering drugs specifically and precisely to cancer cells, enhancing treatment efficacy. Furthermore, nanocarriers enable treatment monitoring and personalized therapy by incorporating imaging agents, optimizing therapeutic outcomes, and reducing side effects. OVs are a promising new cancer treatment, selectively targeting and destroying cancer cells while sparing healthy cells (Naseer et al., 2022a). Both naturally occurring and genetically engineered OVs are either in use or under trial. For example, Reolysin targets solid tumors, while T-Vec, approved by the FDA, treats melanoma. Other viruses like the measles virus are under study for their potential to target cancer cells selectively, demonstrating the therapeutic potential of oncolytic virotherapy in cancer treatment. OVs, including measles virus (MeV), are promising cancer treatments due to their selective targeting of cancer cells, and enhancing immune responses. MeV attaches to receptors like CD150/SLAMF1 on immune cells and Nectin-4 on airway cells, contributing to its natural oncotropism (Naseer et al., 2022b). This specificity allows MeV to target cancer cells effectively, particularly where interferon responses are weakened, as observed in various types of cancer. Vaccine strains of MeV, modified for safety and tumor-specific targeting, are commonly used in research due to their low toxicity and adaptability for genetic modifications. MeV-based virotherapy has shown potential in cancer treatment due to natural tumor selectivity, low toxicity, and the ability to genetically engineer tumor-specificity. However, a challenge in MeV therapy is that most of the Western patients are immune to MeV due to vaccination, which limits its clinical utility. To overcome this, strategies like pseudotyping, infected cell carriers, and surface modifications (e.g., coating polyethylene glycol) are used to evade neutralizing antibodies. Additionally, using immunosuppressants, and polymeric coatings are being explored to protect MeV from antibody neutralization, enhancing oncolytic efficacy (Naseer et al., 2021). OVs stimulate anti-tumor immunity, making them effective in combination with other treatments like immunotherapy, and radiotherapy. This study aims to explore the use of polymeric NPs carrying oncolytic measles virus (OMV) for cervical cancer treatment, utilizing nanotechnology for enhanced delivery and efficacy. OVs have shown potential as cancer therapeutics by selectively replicating in cancer cells, inducing immunogenic cell death, and supporting anti-tumor immunity, marking a significant advancement over traditional cancer treatments.

2. Materials & Methods

In this study, a live attenuated OMV strain was used to design and formulate a ligand-based nanoformulation with polymeric surface coating. Analytical parameter characterization of OMV was done after trapping in chitosan and layering it with hyaluronic acid (HA) using the ionic gelation technique. The materials employed to formulate OMV-loaded NPs were chitosan, HA with the molecular weight of ~1.5- 1.8× 10E6 Dalton, and less than 1% of protein impurities, bacterial glycosaminoglycan polysaccharide manufactured by Sigma Aldrich, Lot number BCCF8620, and expiry date of April 2026, and measles vaccine (live attenuated freeze-dried BP) manufactured by National Institute of Health (NIH), Islamabad.

2.1. Preparation of Polymer and Cross-Linker Solution

The synthesis of virus-encapsulated NPs is based on green synthesis through an ionic gelation process, mediated by merely biodegradable materials. The choice of eliminating hazardous solvents as well as acids makes this approach more energy-efficient and environment-friendly than chemical synthesis. Chitosan solution (1% w/v)

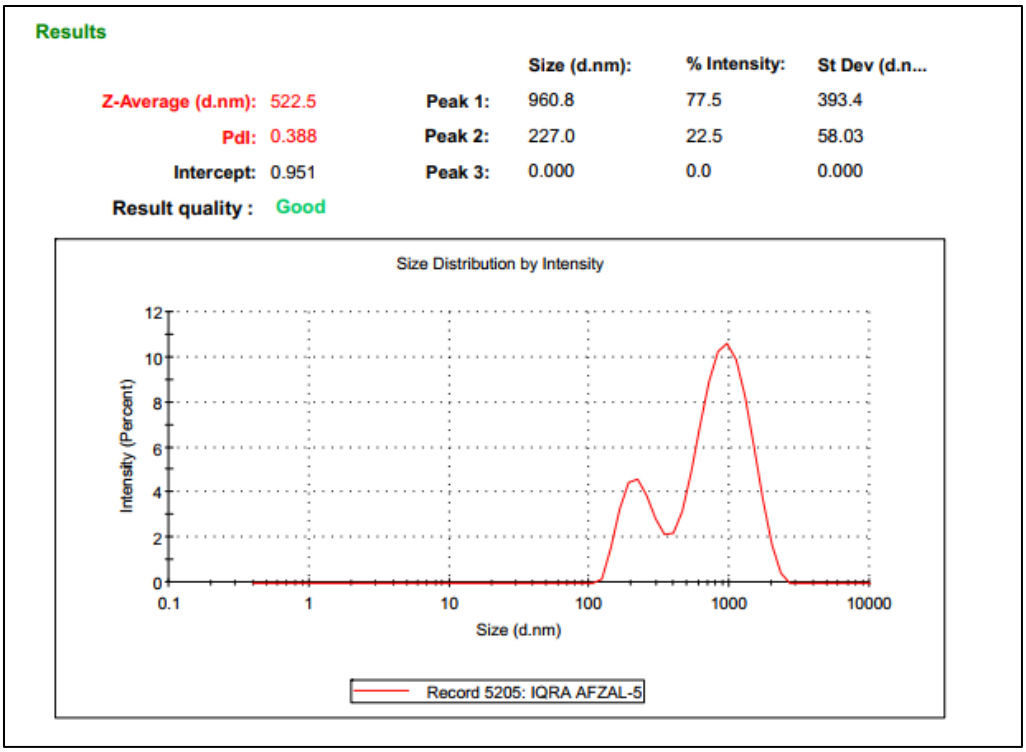

Figure 1. Zeta analysis of blank solution.

was prepared by dissolving chitosan in distilled water with 1 mL of glacial acetic acid, owing to its ability to control the pH and solubility of chitosan. HA was dissolved as a 0.5% w/v solution in another container. HA has functional groups that allow the conjugation of ligands and cross-linking of bioactive species, especially in cases of binding of CD44 overexpressing tumor cells. The HA solution was slowly incorporated into the chitosan solution under stirring. An hour later, NPs were formed on the walls of the beaker.

2.2. Synthesis of OMV-Loaded Nanoparticles

The loaded NPs were synthesized employing a green synthesis procedure through the ion gelation crosslinking method. The overall concentration of the solution that was prepared at the onset was 21 mL, including a polymer or cross-linker solution. From this solution, 10 mL was set aside as a blank, and 11 mL was used in OMV loading. . To prepare NPs with different concentrations of OMV, two preparations of the polymer and cross-linker solution were prepared. For the 0.1% OMV-loaded NPs, 10 mL of the prepared solution was put on a magnetic stirrer at 550 rpm. 1 ml of OMV was withdrawn from the vial using a syringe and added into the solution dropwise. The solution was stirred for 1 hour. Likewise, for 0.2% OMV-loaded NPs, 10 mL of the prepared solution was stirred on a magnetic stirrer at 550 rpm. 2 ml of OMV was taken from the vial containing the OMV using a syringe and added to the solution drop by drop. The solution was stirred for 1 hour. Thorough stirring for an hour ensures proper distribution and encapsulation of the OMVs within the NPs. Following this, OMV-encapsulated NPs with HA functioning as a targeting moiety were formulated. These NPs were named HA-coated OMV-loaded NPs.

2.3. Characterization of OMV-Loaded NPs

2.3.1. X-ray Diffraction

XRD technique is employed to investigate the crystalline framework of NPs with details of atomic packing, crystal structure, & crystallite size. It operates by analyzing some form of XRD patterns emitted by the crystal lattice. The Scherrer equation is widely used to determine the size of

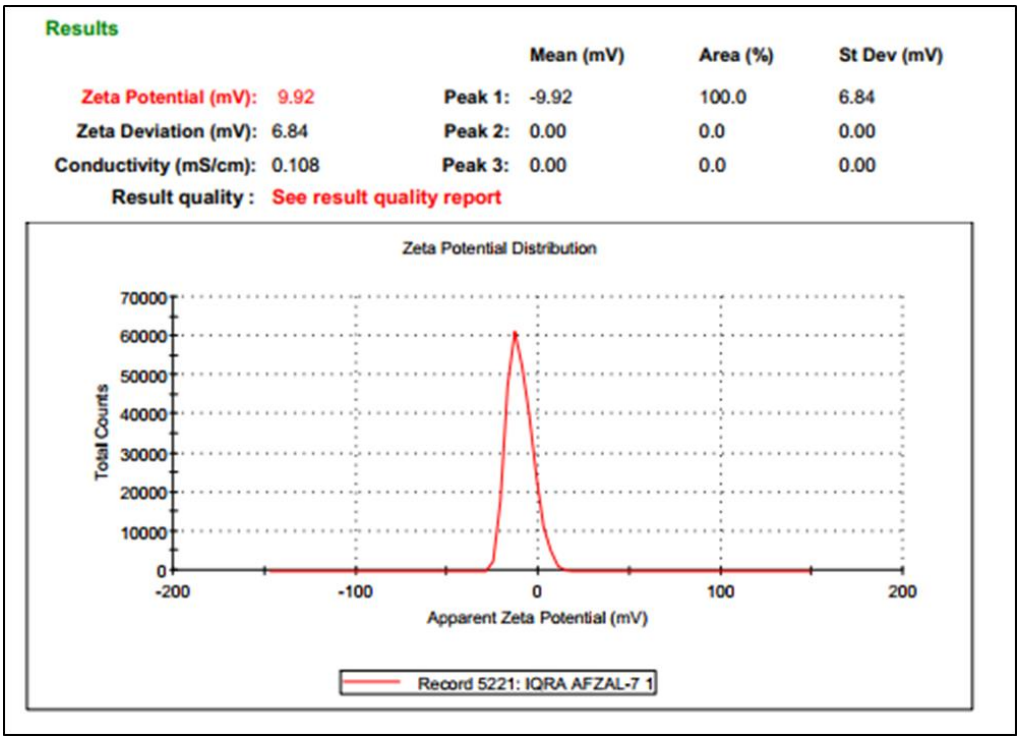

Figure 2. Zeta potential of the blank solution.

the NPs and the width of the peaks (lower peak width implies larger particle size). XRD is particularly useful during phase transformations and in measuring crucial aspects of catalysis or drug delivery. Furthermore, in cases where samples are amorphous, the XRD produce broad, structureless patterns (Bunaciu, Udriştioiu, and Aboul-Enein 2015).

2.3.2. Scanning Electron Microscope (SEM)

SEM is used widely for the observation of NPs surface morphology and surface relief. In SEM, a high-resolution, 3D surface map is produced by scanning a beam of electrons across a target point on the sample and detecting the escaping low-energy electrons. This method offers characteristic features of surface texture, roughness, and aggregation of material without subsequent thinning of the sample as in Transmission Electron Microscopy (TEM). SEM can also be coordinated with energy-dispersive X-ray spectroscopy (EDX) for element analysis to describe the chemical composition of a material. Although SEM is better at surface imaging than TEM, it has lower resolution and lacks the internal imaging properties of TEM (Mohammed and Abdullah 2018).

2.3.3. Zeta Sizer

The stability of the NPs can be determined through zeta potential with values near ±30 mV deemed stable. Traditional approaches, such as phase analysis light scattering, are not compatible enough with polydisperse samples. TRPS increases accuracy by reducing variability arising from zeta potential and size for the particles in dilute suspension (Clogston and Patri 2011, Li et al. 2017).

3. Results

3.1 Physical Characterization of OMV-Loaded Nanoparticles

3.1.1. Zeta Analysis

The solution containing blank particles had a size of approximately 522.5 nm with a moderate polydispersity index (PDI) of 0.388. A PDI below 0.5 is suggestive of a relatively even distribution of

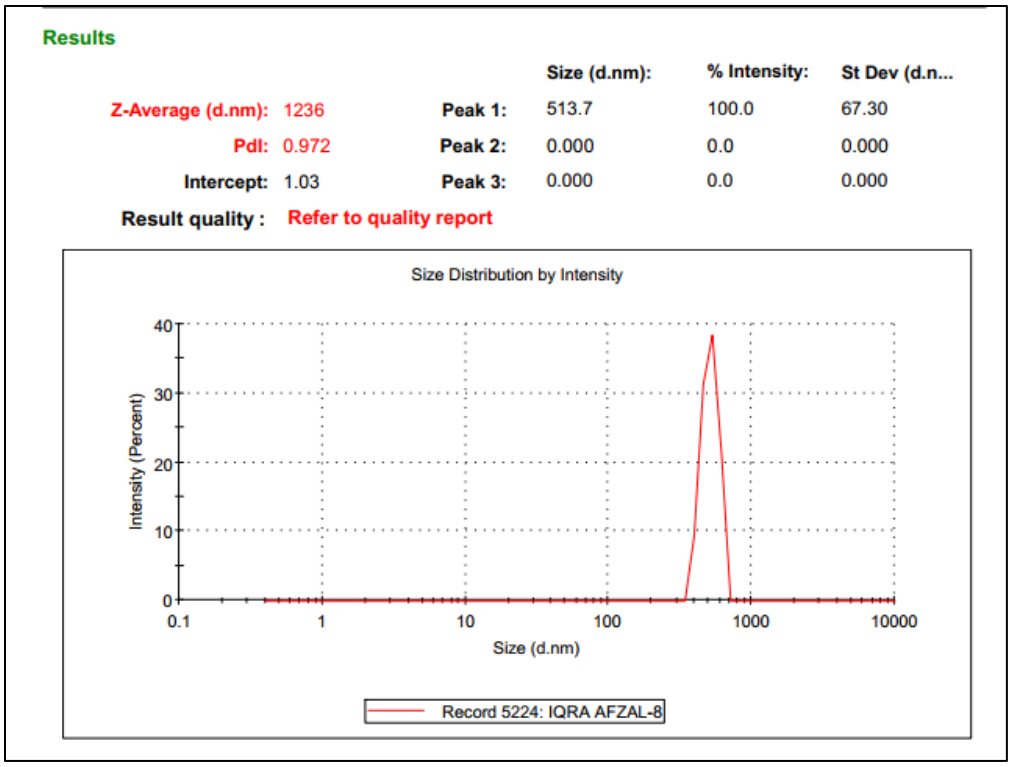

Figure 3. Zeta analysis of 2% OMV loaded NPs

.particle size, suitable for a blank solution. The particle size greater than 500 nm suggests some degree of aggregation, which would be appropriate depending on the desired controlled release pattern of the drug.

A high zeta potential of +9.92 mV is relatively low in the context of colloidal stability. Normally, NPs with zeta potential greater than ±30mV are acceptable as they thus have great stability due to interparticle repulsion. In this case, the blank NPs may be vulnerable to slight aggregation with time although they remain stable.

The size of the OMV-loaded NPs is much larger, approximately 1236 nm, which may lead to considerable aggregation or NPs growth after OMV loading. The PDI is also high (0.972) which indicates larger distribution and heterogeneity in the particle sizes present in the suspension. This indicates poor uniformity and is likely to compromise the rate of drug delivery and release. The zeta potential value, which is -14.5 mV is slightly better than the value of blank NPs. However, it is not high enough to assure good colloidal stability.

The larger size and widened biodistribution of the OMV-loaded NPs may decrease their extravasation ability or cellular uptake into the tumor, potentially reducing their therapeutic potential. In free drug delivery NPs usually have a particle size below 500nm for improving its cellular uptake and penetration across tumor tissue. From the above-mentioned zeta potential values one can see that the blank and OMV-loaded NPs have relatively low surface charge and thus, they could potentially aggregate over time, which will be detrimental to the NPs' circulation within the bloodstream. Enhancing the zeta potential, either by altering the formulation or altering the surface properties, could enhance the stability and performance of the NPs.

3.1.2. X-ray Diffraction

XRD peak profile analysis is a very effective technique to quantify the broadening of the peaks with crystallite size and lattice strain. The result

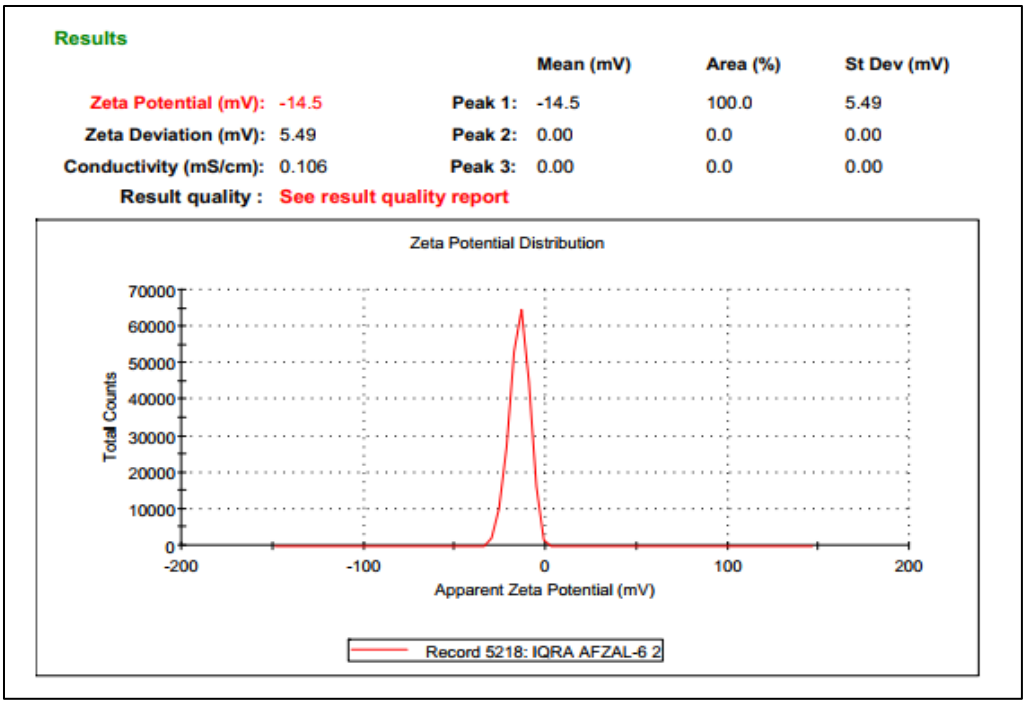

Figure 4. Zeta potential of 2%OMV loaded NPs.

displays a spectrum of XRD to compare the blank solution (Fig 4.2.a) and the OMV-loaded NPs (Fig. 4.2.b). There is intensity in arbitrary unit (a.u) versus 2θ (degree) plots, which indicates the crystalline structure and amorphous nature of the samples. The blank solution has XRD broader peaks with an absence of sharp diffraction peaks, reflecting amorphous nature of the material. The results also demonstrate that there is no clear indication of a crystalline structure. The broad pattern of the OMV-loaded NPs shows that they also have amorphous characteristics. However, the steepness of pattern in the OMV-loaded NPs demonstrated a slightly different slope and a little shift in peak location, which might imply that the drug and the material involved interact and can change its structural properties, but not enough to form crystals. In general, for both samples, the diffraction patterns share an amorphous phase, and no sharp crystalline peaks are observed. However, there are slight variations between the OMV-loaded polymer sample and the blank solution. These differences could be a result of the integration of the OMV in this formulation which might alter the arrangement of molecules in the matrix. The absence of sharp peaks in both cases suggests that OMV loading did not alter the crystal form of the drug as well as the matrix material. The results indicate that OMV loading did not transform the amorphous material into a crystalline form. It only changed the order of the molecules slightly, suggesting possible useful interactions without affecting the amorphous structure.

3.2. Morphological Analysis of OMV-Loaded Nanoparticles

3.2.1. Scanning Electron Microscopy

The Ultrastructure of both samples was photographed and published under X10, 000 Magnification. The scale bar in both the photomicrographs measures 1.0μm.

Blank Solution (right image): The particles in the blank solution appear rather fine, judging by the scale and the magnification the size of the particles could probably range between 100nm- 500nm. The particles are less dense and more uniformly distributed with a higher interconnection porosity.

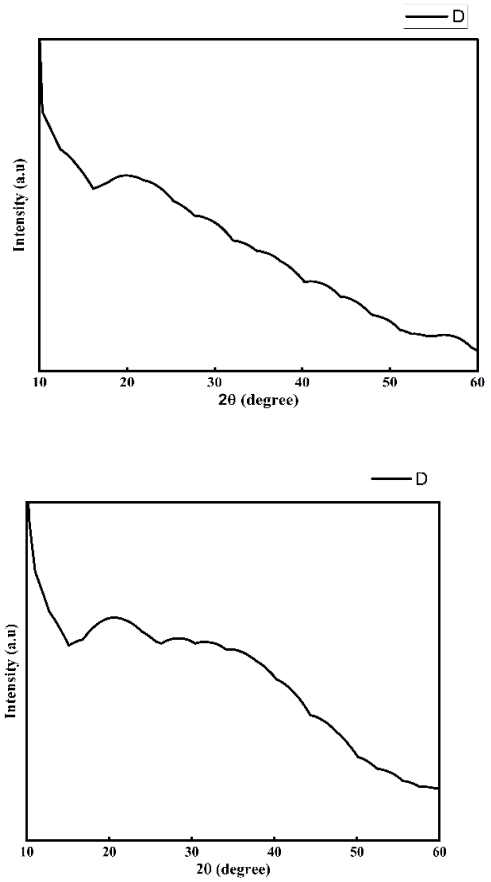

Figure 5. XRD Pattern of nanoparticles an XRD Pattern of Blank Solution. b XRD Pattern of OMV-loaded nanoparticles. (please confirm the labeling of the diagram)

OMV-Loaded NPs (left image): The OMV-loaded NPs look more aggregated; therefore, larger structures can be observed. The particle size is estimated to vary from 500 nm to greater than 1μm, depending on the extent of aggregation present.

These size ranges are typical for polymeric NPs and indicate that both – blank and OMV-loaded – formulations are within the optimal NP drug delivery system's range, according to which smaller particles are better for cellular uptake and biodistribution. However, the larger OMV-loaded structures could contribute to the controlled drug release due to their size and aggregation.

4. Discussion

SEM analysis differentiates blank NPs from the OMV-loaded ones. Blank NPs are smaller with a porous, irregular structure, which facilitates rapid drug release (Choi et al., 2021). On the other hand, OMV-loaded NPs are denser and more compact, indicating better encapsulation. This structure supports sustained release, which is better for challenging treatments like cervical cancer therapy, where a steady release of drug is important. The zeta potential analysis shows moderate stability in both types of NPs. Blank NPs have a zeta potential of 9.92 mV, while OMV-loaded particles have 14.5 mV. Both values fall short of the ±30 mV (required for high stability), indicating that aggregation could happen over time due to insufficient repulsive forces (Rojas et al., 2020). To improve stability, further modifications may be essential. Raman spectroscopy, a non-destructive technique for analyzing molecular structures by observing vibrational modes, is highlighted. Its potential to identify chemical bonds and molecular structures makes it widely applicable in scientific fields, including medicine and materials science (Kousar et al., 2022). In a study on ZnO NPs, the introduction of copper led to morphological changes and particle size reduction, further demonstrating how chemical composition influences NPs structure and performance in drug delivery applications (Sajjad et al. 2018).

The future of NPs is promising, especially for cancer treatment. Their potential for targeted delivery and low toxicity makes them appropriate for precision medicine (Anjum et al., 2023). By utilizing surface modification, NPs can be engineered and modified to bind specifically to tumor cell receptors, improving their targeting efficiency and lowering the side effects (Gavas, Quazi, and Karpiński 2021). NPs are also significant in diagnostic imaging, enabling real-time monitoring of drug delivery, a concept known as theranostics, which merges therapeutic and diagnostic functions for personalized

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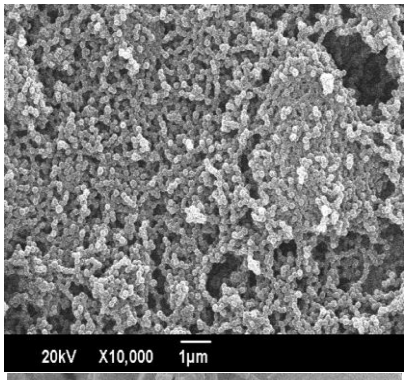

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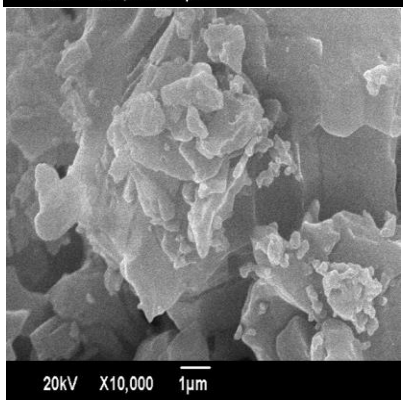

Figure 6. SEM results, a. SEM results of 2% OMV loaded NPs b. SEM results of the blank solution.

treatment. Future research should be focused on improving NPs' stability, biocompatibility, and efficacy by using biodegradable materials (Swain et al. 2016). Developments in nanoparticle technology may also lead to multifunctional systems that combine drug delivery with immunotherapy, gene therapy, or virotherapy, enabling simultaneous cancer cell targeting, immune modulation, and controlled therapeutic release. This emerging technology holds immense potential for improving the effectiveness of cancer treatment, especially for complex diseases like cervical cancer.

5. Conclusion

The conclusion of this study underscores the innovative potential of OMV as a therapeutic strategy for treating cervical cancer, particularly when encapsulated in polymeric-based NPs. The study evaluated the cytotoxic effects, selectivity, and safety of OMV-loaded NPs, revealing encouraging results in targeting cancer cells while sparing healthy tissues. The NPs' enhanced stability and selective targeting, especially through HA-coated delivery systems, demonstrated significant potential for improved clinical outcomes. However, further optimization is required, especially in terms of particle size and stability, to fully harness the efficacy of this novel approach in treating cervical cancer.

Conflict of Interest

The authors declare that they have no conflicts of interest to disclose.

Funding

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Study Approval

There are no animal/human subjects involved so, this study requires no institutional or ethical review board approval.

Consent Forms

NA.

Authors Contributions

FN conceptualized the study and wrote the final manuscript; HA, LK, and TF performed experimental work and wrote the first draft, AM & LB did the literature search and review of the studies, and FN supervised the whole project.

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