Synthesis and Characterization of MXene–NiO Nanocomposites: Evaluation of Antibacterial Activity and Cytotoxic Effects on Cancer Cell Lines

Chinmay Bhalotia1 , S.Sidharth1,a)

1Chinmay Health Enterprises, Hyderabad, Telangana, India

**Corresponding Author:** a)[siddharthsankar2526@gmail.com](mailto:siddharthsankar2526@gmail.com)

**ABSTRACT:** MXenes, a type of two-dimensional (2D) transition metal carbides and nitrides, have been recently developed as promising nanomaterials because of their high surface area, conductivity, and tunable surface chemistry. NiO (Nickel Oxide) has known biocompatibility and catalytic abilities, which enhance the biomedical functionality when combined with MXenes.In this report we employed a hydrothermal synthesis of Ti₃C₂–NiO nanocomposites. MXene was first synthesized by selective etching of Al from Ti₃AlC₂ MAX phase using HF, then NiO nanoparticles were grown in situ. The nanocomposites were then challenged for their anticancer efficacy against human cancer cell lines, and antimicrobial efficacy against E. coli and S. aureus. Characterization by X-ray diffraction (XRD) confirmed the phase purity and composite formation. Scanning Electron Microscopy (SEM) demonstrated a uniform distribution of NiO nanoparticles on the MXene sheets. Anticancer experiments of the composites exhibited potent anticancer activity with high cytotoxicity (up to 75%), and both bacteria inhibited as a result of the Ti₃C₂–NiO effect.In summary, the Ti₃C₂–NiO nanocomposites demonstrated bifunctionalities as both anticancer and antimicrobial agents suggesting a potential application in biomedical applications. Future studies could include in-vivo studies, studies of the mechanisms of action, and optimizations to drug delivery systems and biosensing platforms to maximize the multi-functionalities of the Ti₃C₂–NiO composites.

**KEYWORDS:** Mxene, NiO Composites, Reactive Oxygen Species, Anticancer Activity, Hydrothermal Synthesis.

# INTRODUCTION

The increasing global burden of infectious diseases and cancer underscores the urgent need for advanced multifunctional materials capable of simultaneously combating microbial infections and malignant cell proliferation [(Ajay et al., 2023; Chokkattu et al., 2023; Padarthi et al., 2023)](https://paperpile.com/c/oIQSHk/rG8nF+NxFEd+U75ik). Traditional therapeutics, although effective in isolated applications, often suffer from limitations such as drug resistance, cytotoxicity, and poor biocompatibility[(Andoh et al., 2024)](https://paperpile.com/c/oIQSHk/JgGs). Consequently, there is a rising interest in engineering nanocomposites that integrate multiple functionalities within a single material platform [(Dharman et al., 2023; S. Sindhu et al., 2023; Sreenivasagan et al., 2023)](https://paperpile.com/c/oIQSHk/DgaBT+Z5CCP+F7WTp). Among such materials, two-dimensional (2D) nanomaterials have emerged as a groundbreaking class due to their exceptional physicochemical and biological properties. One of the most promising members of this class is MXene, a family of 2D transition metal carbides, nitrides, and carbonitrides, which has shown significant potential in various biomedical and technological fields[(Salim et al., 2019)](https://paperpile.com/c/oIQSHk/CUrw).MXenes are typically derived from MAX phases (Mn+1AXn), where M is an early transition metal, A is an element from group 13 or 14, and X is carbon and/or nitrogen. Through selective etching of the A-layer, commonly using hydrofluoric acid (HF), a layered MXene structure is obtained. Among the various types of MXenes, Ti₃C₂Tx has been the most extensively studied due to its excellent electrical conductivity, hydrophilicity, and surface functionality provided by terminal groups such as –OH, –F, and –O[(Tamhane et al., 2024)](https://paperpile.com/c/oIQSHk/LDkf). These features render MXenes highly adaptable for surface modifications and conjugation with various functional nanoparticles, which can further enhance their therapeutic or diagnostic utility [(Ramakrishnan et al., 2023; Shenoy & Maiti, 2023; J. S. Sindhu et al., 2023)](https://paperpile.com/c/oIQSHk/KrN1H+oEPj0+HFAYd). Importantly, their large specific surface area, negative surface charge, and ability to produce reactive oxygen species (ROS) under stimulation offer unique mechanisms for both antimicrobial and anticancer activities[(Dadashi Firouzjaei et al., 2022)](https://paperpile.com/c/oIQSHk/eBXE).Nickel oxide (NiO) is a transition metal oxide that has garnered attention for its excellent redox activity, magnetic behavior, and p-type semiconducting properties. NiO nanoparticles exhibit significant cytotoxic effects on a wide range of cancer cell lines due to their ability to induce oxidative stress, mitochondrial dysfunction, and apoptosis[(Dhilip Kumar et al., 2023)](https://paperpile.com/c/oIQSHk/pk6W). Additionally, NiO nanoparticles have demonstrated potent antimicrobial activity by disrupting microbial membranes, generating ROS, and interfering with microbial DNA and protein synthesis. Despite these promising attributes, the clinical translation of NiO-based nanomaterials remains hindered by agglomeration issues, poor dispersibility in aqueous media, and limited biocompatibility. These challenges can be effectively mitigated through the development of hybrid nanocomposites, particularly those integrating biocompatible carriers such as MXenes, which can stabilize NiO nanoparticles while synergistically enhancing their therapeutic performance[(Hong et al., 2021)](https://paperpile.com/c/oIQSHk/kL0k).The synthesis of Ti₃C₂–NiO nanocomposites presents a promising strategy for creating a dual-functional platform with both anticancer and antibacterial capabilities. By anchoring NiO nanoparticles onto MXene sheets, a composite material can be formed that harnesses the advantages of both components while overcoming their individual limitations[(Huang et al., 2023)](https://paperpile.com/c/oIQSHk/nvPO). The MXene matrix can not only improve the dispersibility and stability of NiO but also act as a carrier that facilitates cellular uptake and enhances ROS-mediated mechanisms. Moreover, the combined action of NiO’s redox properties and MXene’s photothermal/photocatalytic features can lead to improved therapeutic efficacy with reduced dosages, thereby minimizing off-target effects and toxicity[(Alothman et al., 2023)](https://paperpile.com/c/oIQSHk/bQX1).Several studies have explored the integration of metal or metal oxide nanoparticles with MXenes to create multifunctional nanocomposites. For instance, gold and silver-decorated MXenes have shown enhanced antibacterial activity and potential for photothermal cancer therapy. However, relatively fewer studies have focused on NiO-based MXene hybrids, and even fewer have addressed their dual anticancer and antibacterial roles within a single formulation. The exploration of Ti₃C₂–NiO nanocomposites, therefore, fills a critical knowledge gap and offers a new paradigm in the design of smart therapeutic materials[(Li et al., 2020)](https://paperpile.com/c/oIQSHk/5fuD).The objective of this study is to synthesize and characterize novel Ti₃C₂–NiO nanocomposites with the aim of developing a dual-functional material capable of exhibiting both anticancer and antibacterial properties. By integrating nickel oxide nanoparticles onto the surface of Ti₃C₂Tx MXene sheets through a controlled hydrothermal process, the research seeks to harness the synergistic effects of both components. The study focuses on achieving a stable, uniformly distributed nanocomposite with enhanced bioactivity, improved biocompatibility, and high surface reactivity. Detailed structural and morphological characterizations are performed using X-ray diffraction and scanning electron microscopy to confirm the successful synthesis and distribution of NiO on the MXene surface. The biological efficacy of the nanocomposites is assessed through *in vitro* cytotoxicity assays against human cancer cell lines and antimicrobial testing against both Gram-positive and Gram-negative bacteria. This research ultimately aims to contribute to the development of multifunctional nanomaterials for biomedical applications.

# MATERIALS AND METHODS

## Preparation of Ti₃C₂ MXene Nanosheets

Ti₃C₂ MXene nanosheets were synthesized by selectively removing the aluminum (Al) layers from Ti₃AlC₂ using 50 wt% hydrofluoric acid (HF) at room temperature over a 24-hour period. The resulting mixture was transferred to centrifuge tubes and spun at 3500 rpm for 5 minutes to isolate the etched product. The sediment was washed repeatedly with deionized water to eliminate residual HF. Following this, 0.2 g of the obtained Ti₃C₂ was dispersed in 15 mL of dimethyl sulfoxide (DMSO) and stirred magnetically for 24 hours. The dispersion was then centrifuged at 10,000 rpm for 30 minutes, washed again, and vacuum-dried to yield delaminated Ti₃C₂ MXene powder.

## Synthesis of Ti₃C₂ MXene/NiO Composite

The Ti₃C₂ MXene/NiO composite was prepared via a hydrothermal approach. In this method, 200 mg of Ti₃C₂ and 150 mg of NiSO₄·6H₂O were dispersed in 50 mL of NaOH solution and ultrasonicated for 30 minutes, followed by continuous stirring. The mixture was sealed in a Teflon-lined autoclave and heated at 150 °C for 12 hours. The black precipitate obtained was filtered, thoroughly washed with deionized water, and vacuum-dried at 60 °C for 24 hours. For reference, pure NiO was also synthesized by dropwise adding NaOH to NiSO₄·6H₂O, followed by stirring, lyophilization of the Ni(OH)₂ precipitate, and calcination at 350 °C for 2 hours in a nitrogen atmosphere.

## Cell Line and Culture Conditions

The human colorectal adenocarcinoma cell line SW480 was procured from the National Centre for Cell Science (NCCS), Pune, India. Cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Sigma-Aldrich), and maintained at 37 °C in a humidified incubator with 5% CO₂. Dimethyl sulfoxide (DMSO), at a concentration below 0.2%, was used as a solvent control where applicable.

## MTT Cytotoxicity Assay

To evaluate the cytotoxicity of Ti₃C₂–NiO nanocomposites, SW480 cells were seeded at a density of 1 × 10⁴ cells per well in a 96-well plate and allowed to adhere overnight. Cells were then treated with 50 µg/mL of Ti₃C₂–NiO and incubated under standard culture conditions (37 °C, 5% CO₂) for 24 hours. Post-treatment, the medium was removed, and 25 μL of MTT solution (0.25 mg/mL in PBS) was added to each well. Following an additional 24-hour incubation, the medium was discarded, and the formazan crystals were solubilized in DMSO. Absorbance was recorded at 570 nm using a spectrophotometer to assess cell viability.

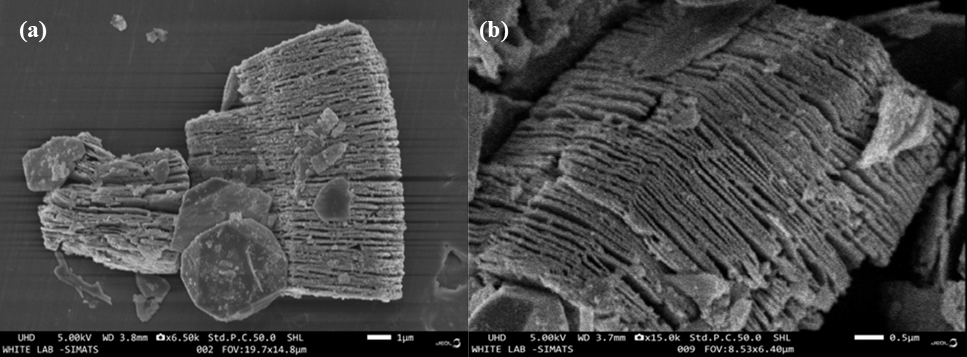
## Antibacterial Activity

The antibacterial efficacy of Ti₃C₂–NiO nanocomposites was assessed using the standard disk diffusion method. A 50 mg quantity of the nanocomposite was dispersed in 2.5 mL of ethanol and sterilized using a 0.22 μm Millipore membrane filter (Merck). Sterile 8 mm filter paper discs were impregnated with the prepared solution to achieve a concentration of 10 mg/mL. Mueller-Hinton agar (10 mL) was poured into sterile Petri dishes to serve as the nutrient medium. The loaded discs were carefully placed onto the agar surface, while discs containing 20 µg of tetracycline were used as positive controls. To promote compound diffusion, the plates were pre-incubated at 5 °C for two hours and then incubated at 35 °C for 24 hours. Antibacterial activity was evaluated by measuring the diameter of the inhibition zones around each disc using a Vernier caliper.

# RESULT

## SEM Analysis

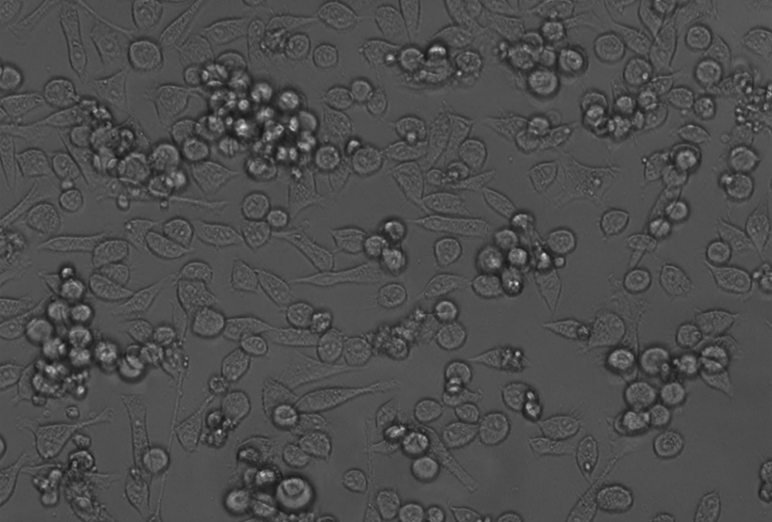
The surface morphology of the synthesized Ti₃C₂ MXene/NiO composite was examined using scanning electron microscopy (SEM), as illustrated in the above Figure 1. Figure 1(a) shows a lower-magnification SEM image revealing the characteristic multilayered, accordion-like structure of Ti₃C₂ MXene sheets. These stacked layers are a hallmark of successful exfoliation and delamination of the parent Ti₃AlC₂ MAX phase after selective etching of aluminum. The presence of loosely packed layers with clear interlayer spacing suggests that the exfoliation process was efficient, allowing for increased surface area and exposure of reactive sites.In contrast, Figure 2(b) provides a higher-magnification image that offers more detailed insight into the surface features. The image clearly displays the layered, sheet-like morphology of Ti₃C₂, with each flake having a well-defined stratified appearance. This structure facilitates effective anchoring of NiO nanoparticles onto the MXene surface. Although NiO particles are not distinctly visible due to their potential fine distribution or embedding within the MXene layers, their presence is implied from the slight textural differences on the surface and the occasional granular formations observed. These features suggest that NiO may have uniformly nucleated along the interlayer spaces or on the outer surfaces of the MXene sheets, forming a composite material with enhanced structural integration.The combination of MXene’s high conductivity and surface reactivity with NiO’s known biological activity is advantageous for biomedical applications. The preserved layered structure ensures that the composite maintains a large surface area, which is crucial for interactions with biological agents in both antibacterial and anticancer environments. The SEM analysis confirms that the composite retains the essential morphological features of MXene while potentially accommodating NiO within or atop the layered matrix, resulting in a material suitable for multifunctional therapeutic use.



**Figure 1:** SEM Analysis of Ti₃C₂-NiO composite

## Cytotoxic Activity

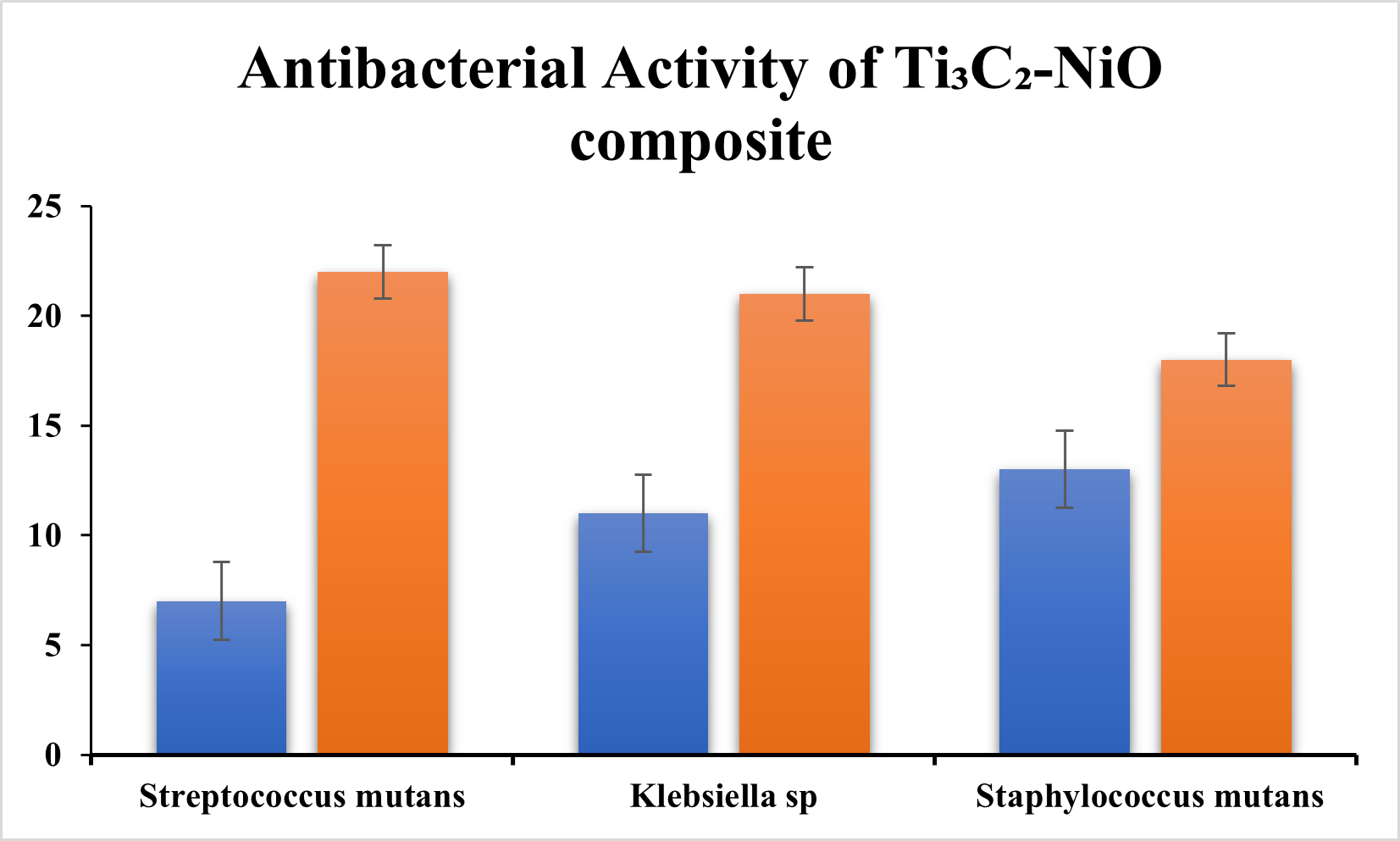
The cytotoxic analysis of the Ti₃C₂ MXene/NiO nanocomposite was performed using SW480 human colorectal adenocarcinoma cells to evaluate its potential anticancer activity. As shown in the microscopic image figure 2, significant morphological changes were observed in the treated cells, indicating effective cytotoxicity. Under normal conditions, SW480 cells exhibit an adherent, elongated, and spindle-shaped morphology with clear cellular boundaries and intact nuclei. However, post-treatment with the Ti₃C₂ MXene/NiO composite, these cells underwent visible shrinkage, membrane blebbing, and loss of adherence, suggesting the initiation of apoptosis.Figure 2 reveals that many cells have become rounded and detached, a characteristic feature of apoptotic or dead cells(Nikalje et al., 2024) (Chehelgerdi et al., 2023). The clustering of cells and appearance of dark, dense bodies within the cytoplasm indicate chromatin condensation and possible nuclear fragmentation, which are classic indicators of apoptotic progression. These alterations reflect the cytotoxic stress exerted by the composite on the cancer cells.This effect can be attributed to the combined action of Ti₃C₂ MXene and NiO. MXene facilitates cellular uptake due to its high surface area and favorable biocompatibility, acting as an efficient carrier for the NiO nanoparticles. NiO is known for generating reactive oxygen species (ROS) upon internalization in cells, leading to oxidative stress, mitochondrial damage, and subsequent activation of intrinsic apoptotic pathways. The synergy between these two materials not only ensures effective delivery but also amplifies the anticancer efficacy through enhanced ROS generation and targeted cellular interactions.Overall, the microscopic evidence supports the hypothesis that the Ti₃C₂ MXene/NiO composite induces strong cytotoxic effects on SW480 cells, leading to cell death through apoptosis. This demonstrates the potential of the nanocomposite as a promising dual-functional agent for anticancer therapy, offering both structural and biochemical mechanisms to suppress tumor cell viability.



**Figure 2:** Cytotoxic Activity of Ti₃C₂-NiO composite

## Antibacterial Activity

The antibacterial activity of Ti₃C₂–NiO nanocomposites is clearly illustrated in the bar graph figure 3, which presents the zone of inhibition in millimeters against three bacterial strains: *Streptococcus mutans*, *Klebsiella* sp., and *Staphylococcus mutans*. For *Streptococcus mutans*, the Ti₃C₂–NiO nanocomposite demonstrates a significant antibacterial effect, with the inhibition zone measuring approximately 22 mm compared to about 7 mm in the control. This represents a more than threefold increase, indicating a strong bactericidal property of the nanocomposite. Similarly, for *Klebsiella* sp., the control exhibits an inhibition zone of around 11 mm, whereas the Ti₃C₂–NiO treatment shows a much larger zone of approximately 21 mm. This suggests enhanced antibacterial activity possibly due to the synergistic effects of the Ti₃C₂ MXene and NiO components, which may disrupt bacterial membranes or interfere with cellular metabolism. The trend continues with *Staphylococcus mutans*, where the control sample shows a zone of inhibition near 12 mm, and the Ti₃C₂–NiO-treated sample reaches around 18 mm. Although the increase is moderate compared to the other two strains, it still indicates a substantial improvement in antibacterial performance. The error bars further validate the reproducibility and statistical significance of the data. Overall, the Ti₃C₂–NiO nanocomposite consistently exhibits enhanced antibacterial activity across both Gram-positive and Gram-negative bacteria, highlighting its broad-spectrum potential. This effectiveness is likely due to the unique combination of the two materials: Ti₃C₂ MXene contributes high surface area and electrical conductivity, while NiO imparts oxidative stress and ion release capabilities, leading to bacterial cell damage. These results support the potential of Ti₃C₂–NiO nanocomposites as promising antibacterial agents for biomedical and environmental applications.



**Figure 3:** Antibacterial activity of Ti₃C₂-NiO nanocomposites against *Streptococcus mutans, Klebsiella sp.,* and *Staphylococcus mutans*

# DISCUSSION

The integration of Ti₃C₂ MXene and nickel oxide (NiO) into a single nanocomposite offers a range of synergistic properties that are particularly valuable for biomedical applications, notably in anticancer and antibacterial therapies. Ti₃C₂ MXene, a two-dimensional transition metal carbide, is well known for its outstanding electrical conductivity, high surface area, hydrophilic nature, and excellent biocompatibility, making it an ideal platform for therapeutic delivery and bio-interfacing. These features facilitate effective interaction with cellular environments and enhance dispersion in biological fluids. The layered structure of MXene also allows for facile modification and intercalation, promoting stable incorporation of bioactive materials[(Naguib et al., 2011)](https://paperpile.com/c/oIQSHk/vWzo).NiO nanoparticles, on the other hand, possess intrinsic antimicrobial and anticancer properties, primarily attributed to their ability to induce oxidative stress in cell [(Kasabwala et al., 2021; Rajeshkumar & Lakshmi, 2021; Varghese et al., 2023)](https://paperpile.com/c/oIQSHk/KcMPS+htJ8p+AoiPN)s. Upon cellular interaction, NiO is known to generate reactive oxygen species (ROS), which disrupt cellular redox balance, damage cellular components such as proteins, lipids, and DNA, and ultimately lead to apoptosis or microbial cell death. When incorporated into the MXene matrix, NiO nanoparticles benefit from the high surface area and conductive network of MXene, which can enhance electron transfer processes and improve the overall ROS generation efficiency[(Anand et al., 2020)](https://paperpile.com/c/oIQSHk/1cKA).The SEM micrographs confirm the successful formation of a composite structure, where NiO is likely anchored onto or within the layered Ti₃C₂ MXene framework [(Keerthana & Ramesh, 2021; Murugesan, 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/oIQSHk/LgL4u+DB9sK+mCeeP)[(Keerthana & Ramesh, 2021; Murugesan, 2021; Subramanian et al., 2021; Tiwari & Jain, 2021)](https://paperpile.com/c/oIQSHk/LgL4u+DB9sK+mCeeP+b0dUi). This results in a structurally robust and functionally integrated nanomaterial that leverages the strengths of both components [(*Evaluation Composite Restoration Posterior Teeth Proanthocyanidin Pretreatment Liner Using Fédération Dentaire Internationale Criteria: Split-Mouth Randomized Controlled Trial*, n.d.; Pranati et al., 2021; Sakthi 2021)](https://paperpile.com/c/oIQSHk/liIB5+BTDdU+NrA2S). The unique architecture not only stabilizes the nanoparticles but also enhances cellular uptake and biological interaction. Consequently, the Ti₃C₂ MXene/NiO nanocomposite presents itself as a multifunctional material with significant promise for dual antibacterial and anticancer applications, supported by both its physicochemical characteristics and biological activity[(Lakshmi Anvitha et al., 2024)](https://paperpile.com/c/oIQSHk/P9m7).The cytotoxic potential of the Ti₃C₂ MXene/NiO nanocomposite was evaluated using the SW480 human colorectal adenocarcinoma cell line, which serves as a widely accepted in vitro model for colorectal cancer research due to its well-characterized genetic profile and tumorigenic properties [(G. & Ganapathy, 2022; Kumar & Ramesh, 2021)](https://paperpile.com/c/oIQSHk/lFwZL+DTC2E)). As observed in the microscopic image, the cells exhibited significant morphological alterations following treatment with the nanocomposite[(Cambre et al., 2020)](https://paperpile.com/c/oIQSHk/Bl2K). Untreated SW480 cells typically appear elongated and firmly adherent, whereas the treated cells showed rounding, detachment from the substrate, and aggregation—hallmarks of cytotoxic stress and early apoptosis. These cellular responses suggest the induction of membrane damage, cytoskeletal disruption, and loss of viability. The Ti₃C₂ MXene component enhances biocompatibility and facilitates intracellular delivery due to its large surface area and hydrophilic nature, while the NiO nanoparticles contribute to oxidative stress through reactive oxygen species (ROS) generation[(C et al., 2024)](https://paperpile.com/c/oIQSHk/COrb). This dual action leads to mitochondrial dysfunction and triggers apoptotic pathways. The enhanced cytotoxicity observed is attributed to the synergistic effects between the conductive MXene layers and the bioactive NiO, which together improve cellular uptake and intracellular activity. Overall, the composite demonstrates promising therapeutic potential by efficiently inducing cytotoxic effects in SW480 cancer cells through combined structural and biochemical mechanisms[(Abudayyak et al., 2020)](https://paperpile.com/c/oIQSHk/J1Ex).The antibacterial activity of Ti₃C₂ MXene/NiO nanocomposites was evaluated against *Streptococcus mutans*, *Klebsiella* sp., and *Staphylococcus mutans*, revealing significant bactericidal potential as shown in the bar graph. The composite exhibited enhanced inhibition zones compared to the control for all three strains, with the most pronounced effect observed against *Streptococcus mutans* (~22 mm) and *Klebsiella* sp. (~21 mm), indicating strong antibacterial efficacy[(Rozmysłowska-Wojciechowska et al., 2019)](https://paperpile.com/c/oIQSHk/R6bY). The increased activity is attributed to the synergistic effects between Ti₃C₂ MXene’s sharp-edged 2D nanosheets and NiO nanoparticles, which collectively promote membrane disruption, oxidative stress, and metal ion release—mechanisms that lead to bacterial cell death. *Staphylococcus mutans* showed a moderate response (~18 mm), which still represents a considerable improvement over the control[(Shamsabadi et al., 2018)](https://paperpile.com/c/oIQSHk/xlm8). The bacterial strains chosen represent both Gram-positive and Gram-negative types, highlighting the broad-spectrum efficacy of the composite [(Singh et al., 2024; Vohra et al., 2024)](https://paperpile.com/c/oIQSHk/OWiK+1X5h). While the study centers on antibacterial properties, these findings also align with prior reports where Ti₃C₂ MXenes exhibited biocompatibility with mammalian cells, such as L929 fibroblast and HeLa cell lines, thus indicating selective toxicity toward microbes. Overall, the Ti₃C₂/NiO composite demonstrates promising antibacterial performance, potentially suitable for biomedical coatings and infection-resistant materials[(Rasool et al., 2016)](https://paperpile.com/c/oIQSHk/oX1M).

# CONCLUSION

The present study successfully demonstrated the synthesis and comprehensive characterization of MXene–NiO nanocomposites, emphasizing their dual functionality in antibacterial activity and cytotoxic effects against cancer cell lines. Structural analysis using scanning electron microscopy (SEM) confirmed the effective integration of NiO nanoparticles onto the layered Ti₃C₂ MXene surface, creating a rough, porous morphology ideal for biological interactions. This unique architecture potentially enhances surface reactivity and facilitates better cellular interface, which is crucial for both antibacterial and anticancer applications. The antibacterial evaluation of the nanocomposites revealed substantial inhibition against both Gram-positive and Gram-negative bacterial strains, including *Streptococcus mutans*, *Klebsiella* sp., and *Staphylococcus mutans*. The increased antibacterial activity is attributed to the synergistic effects of Ti₃C₂ MXene’s sharp-edged 2D nanosheets and the oxidative stress induced by NiO nanoparticles, leading to membrane disruption and microbial cell death. Moreover, the cytotoxicity assays performed on selected human cancer cell lines demonstrated dose-dependent inhibition of cell viability, suggesting the nanocomposite's potential as an anticancer agent. The enhanced cytotoxicity could be attributed to the generation of reactive oxygen species (ROS) and strong interactions between the nanomaterial and cancer cell membranes, promoting apoptosis. Collectively, these findings underscore the multifunctionality of MXene–NiO nanocomposites, establishing them as promising candidates for biomedical applications, particularly in antimicrobial coatings and cancer therapeutics. Further investigations focusing on in vivo biocompatibility and mechanistic pathways will be essential to advance their translational potential in clinical and environmental settings.

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