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ENHANCED ANTIBACTERIAL ACTIVITY OF $Ti_3C_2T_x$ Mxene VIA TIME-CONTROLLED PHOTOTHERMAL THERAPY

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Key words: *MXene, photothermal therapy, antibacterial activity, near-infrared irradiation, two-dimensional nanomaterials.*

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Objective *– to evaluate the antibacterial potential of two-dimensional (2D) nanomaterials, with a focus on the photothermal activity of $Ti_3C_2T_x$ MXene as an alternative to conventional antibiotic therapy.*

Material and methods. *This experimental study investigated the antimicrobial properties of $Ti_3C_2T_x$ MXene under near-infrared (NIR) laser irradiation. The photothermal antibacterial effect was assessed against Escherichia coli using continuous laser mode (4 W, 10 Hz) with varying irradiation durations. Bacterial viability was evaluated to determine the time-dependent efficacy of the treatment. The antibacterial mechanisms were analyzed based on physicochemical interactions and thermal effects induced by MXene.*

Results. *The findings demonstrated a pronounced time-dependent antibacterial effect of $Ti_3C_2T_x$ MXene under NIR irradiation. A significant bacterial reduction exceeding 99.99% was observed after 10 and 15 minutes of laser exposure. The antibacterial activity is attributed to the synergistic action of localized photothermal heating and the intrinsic physicochemical properties of MXene, leading to disruption of bacterial membranes and irreversible cellular damage. Additionally, irradiation duration and post-treatment incubation time were identified as critical factors influencing bactericidal efficiency.*

Conclusion*. $Ti_3C_2T_x$ MXene exhibits strong potential as an antibiotic-free platform for photothermal antibacterial therapy. Its effectiveness is mediated by combined thermal and material-specific mechanisms, making it particularly promising for combating drug-resistant bacterial infections. Further research is required to optimize treatment parameters, including irradiation conditions and bacterial load, to improve therapeutic outcomes and facilitate future biomedical applications.*

ПОСИЛЕНА АНТИБАКТЕРІАЛЬНА АКТИВНІСТЬ $Ti_3C_2T_x$ MXene ЗА РАХУНОК ФОТОТЕРМАЛЬНОЇ ТЕРАПІЇ З КОНТРОЛЕМ ЧАСУ

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Ключові слова: *MXene, фототермальна терапія, антибактеріальна активність, ближнє інфрачервоне випромінювання, двовимірні наноматеріали.*

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Мета роботи *– оцінити антибактеріальний потенціал двовимірних (2D) наноматеріалів з акцентом на фототермальну активність $Ti_3C_2T_x$ MXene як альтернативи традиційній антибіотикотерапії.*

Матеріал і методи*. У даному експериментальному дослідженні вивчали антимікробні властивості $Ti_3C_2T_x$ MXene під впливом лазерного випромінювання ближнього інфрачервоного (NIR) діапазону. Фототермальний антибактеріальний ефект оцінювали щодо Escherichia coli у безперервному режимі лазера (4 Вт, 10 Гц) із різною тривалістю опромінення. Життєздатність бактерій визначали для оцінки часовозалежної ефективності лікування. Механізми антибактеріальної дії аналізували на основі фізико-хімічних взаємодій та теплових ефектів, індукованих MXene.*

Результати*. Отримані дані продемонстрували виражений часовозалежний антибактеріальний ефект $Ti_3C_2T_x$ MXene під впливом NIR-опромінення. Значне зниження кількості бактерій (>99,99%) спостерігалося після 10 та 15 хвилин лазерного впливу. Антибактеріальна активність зумовлена синергічною дією локального фототермального нагрівання та власних фізико-хімічних властивостей MXene, що призводить до пошкодження бактеріальних мембран і незворотного* *клітинного ушкодження. Крім того встановлено, що тривалість опромінення та час інкубації після обробки є критичними факторами, що впливають на бактерицидну ефективність.*

***Висновок**. $Ti_3C_2T_x$ MXene демонструє високий потенціал як платформа для антибіотик-незалежної фототермальної антибактеріальної терапії. Його ефективність зумовлена поєднанням термічних і матеріал-специфічних механізмів, що робить його перспективним для боротьби з антибіотикорезистентними бактеріальними інфекціями. Подальші дослідження необхідні для оптимізації параметрів фототермального впливу, зокрема умов опромінення та бактеріального навантаження, з метою підвищення терапевтичної ефективності та впровадження у біомедичну практику.*

Introduction. According to the World Health Organization (WHO), the rapid emergence and spread of antibiotic-resistant microorganisms represent a critical global health challenge, posing serious threats to human health, food security, and modern healthcare systems [1]. This growing crisis necessitates the development of alternative antimicrobial strategies that can effectively combat resistant pathogens while minimizing the risk of further resistance development.

In response to this challenge, significant research efforts have been directed toward nanomaterials with intrinsic antibacterial properties. These materials have demonstrated considerable potential across a wide range of applications, including water purification, food packaging, medical devices, and textile engineering [2]. Among them, two-dimensional (2D) nanomaterials have attracted particular attention due to their unique structural and physicochemical characteristics, such as high surface area, tunable surface chemistry, and mechanical sharpness, which contribute to their antimicrobial activity [3].

A fundamental understanding of the antibacterial mechanisms of 2D nanomaterials is essential for the rational design of next-generation antimicrobial systems with enhanced efficacy and reduced cytotoxicity. One of the key proposed mechanisms involves the physical interaction between sharp-edged nanosheets and bacterial cell membranes. It is hypothesized that these nanoscale sharp edges can mechanically disrupt the bacterial cell wall, leading to membrane damage, leakage of intracellular components, and ultimately cell death. In this study, we aim to experimentally investigate and validate this mechanism as a primary contributor to antibacterial activity [4].

More recently, photoresponsive nanomaterials have emerged as promising candidates for antibiotic-free antibacterial therapies. These systems can be activated by external light sources to generate localized hyperthermia or reactive oxygen species (ROS), both of which exhibit strong antimicrobial effects. However, the practical application of such approaches remains challenging, as the efficiency of ROS generation and thermal effects may decrease under physiological conditions or prolonged exposure times [5].

Photothermal therapy (PTT) represents a particularly attractive strategy, relying on photothermal agents that convert absorbed light into heat, thereby inducing bacterial inactivation through protein denaturation and thermal ablation. Among various photothermal materials, $Ti_3C_2T_x$ MXene has shown remarkable potential due to its excellent photothermal conversion efficiency, large surface area, and favorable biocompatibility. Previous studies have demonstrated that MXene exhibits antibacterial activity even in the absence of irradiation under dynamic conditions; however, its performance under near-infrared (NIR) light, particularly at a wavelength of 808 nm, remains insufficiently explored [6].

Due to their strong absorption in the NIR region, MXenes can efficiently convert light energy into heat, leading to a rapid increase in local temperature. This photothermal effect not only enhances bacterial killing but may also facilitate deeper penetration of sharp MXene nanosheets into bacterial membranes, thereby amplifying mechanical damage.

In this context, the present study aims to investigate the photothermal antibacterial performance of $Ti_3C_2T_x$ MXene under NIR laser irradiation, with a particular focus on determining the optimal exposure duration required to achieve effective bacterial inactivation. By correlating irradiation time with antibacterial efficiency and temperature changes, this work seeks to contribute to the development of optimized MXene-based photothermal antibacterial strategies.

Objective of the study: to assess the antibacterial efficacy of $Ti_3C_2T_x$ MXene as an integrative platform for photothermal therapy, taking into account the influence of irradiation duration on bacterial viability and treatment effectiveness.

Material and methods. Mueller–Hinton agar (MHA, SKU 70191) and Mueller–Hinton broth (MHB, SKU 70192) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and used as standard microbiological media for bacterial cultivation and antimicrobial susceptibility testing. All reagents were of analytical grade and used without further purification.

$Ti_3C_2T_x$ MXene (where T_x represents surface terminations such as –OH, –Cl, and –F groups) was synthesized via selective etching of the aluminum layer from the Ti_3AlC_2 MAX phase precursor. The etching process was performed using a minimally intensive layer delamination (MILD) method, involving a mixture of hydrochloric acid (HCl) and lithium fluoride (LiF), as previously described [7]. Following etching, the multilayered MXene was delaminated to obtain few-layer $Ti_3C_2T_x$ flakes, which were subsequently dispersed in deionized water to form a stable colloidal suspension. The final concentration of the MXene dispersion used in this study was 4.4 mg/mL. Prior to use, the suspension was sonicated to ensure homogeneous dispersion and prevent agglomeration.

The antibacterial activity of MXene was evaluated against Escherichia coli (ATCC 25922), a model Gram-negative bacterium. The bacterial strain was cultured overnight on MHA plates at 37 °C. A fresh bacterial suspension was prepared and adjusted to approximately 10^6 colony-forming units (CFU)/mL. For the experimental setup, 100 µL of the bacterial suspension was added to each well of a sterile 96-well plate. Subsequently, 100 µL of MXene solution (final concentration: 50 µg/mL) was introduced into the wells. Control wells without MXene were included for comparison. The plates were incubated at 37 °C for 6 hours to allow interaction between bacteria and MXene. After incubation, samples were subjected to near-infrared (NIR) laser irradiation in continuous mode (4 W, 10 Hz) for 5, 10, and 15 minutes. To evaluate antibacterial efficacy, samples were analyzed at two time points: 30 minutes and 24 hours after irradiation. At each time point, 10 µL aliquots were taken from each well and plated onto MHA using the streak plate method. The plates were incubated at 37 °C for 24 hours, after which bacterial colonies were counted, and CFU/mL values were calculated. All experiments were performed in triplicate to ensure reproducibility.

Scanning electron microscopy (SEM) was employed to investigate the interactions between MXene materials and bacterial cells. Observations were carried out using a Phenom ProX (Phenom-World BV, Eindhoven, The Netherlands) and a JEOL JSM-7001F microscope, both equipped with an energy-dispersive X-ray spectroscopy (EDX) system for elemental analysis. In addition, transmission electron microscopy (TEM) analysis was performed using a JEOL 2100F to further examine structural details at higher resolution.

To evaluate the photothermal ablation effect of MXene on bacterial biofilms, glass substrates (0.2 × 0.3 cm) were initially prepared and incubated with a standardized bacterial suspension according to the previously described protocol, allowing the formation of a uniform biofilm layer on the surface. After biofilm establishment, the samples were subjected to near-infrared (NIR) laser irradiation under the previously defined experimental conditions in order to activate the photothermal properties of the MXene nanosheets.

Following irradiation, the samples were gently rinsed with phosphate-buffered saline (PBS, pH 7.4) to remove non-adherent or weakly attached bacterial cells. The remaining biofilm structures were then fixed using 1% glutaraldehyde in PBS for 15 minutes, with the fixation step repeated twice to ensure adequate preservation of both cellular components and the extracellular matrix. Subsequently, the samples were washed twice with PBS (15 minutes each) to eliminate residual fixative.

Dehydration of the biofilms was performed using a graded ethanol series (25%, 50%, 80%, and 96%), ensuring gradual solvent exchange and minimizing structural collapse of the biofilm architecture. After complete dehydration, the samples were air-dried and mounted onto carbon adhesive tape. Prior to SEM imaging, a thin gold coating (~5 nm) was applied by sputtering to enhance electrical conductivity and improve image resolution.

All experiments were performed in triplicate. Data for normally distributed variables are expressed as mean ± standard deviation (SD). Comparisons among three or more groups were conducted using one-way ANOVA with Tukey's post hoc test. Non-normally distributed data were analyzed using the Kruskal–Wallis or Friedman test. Statistical analysis was carried out with GraphPad Prism 10.3.1, and $p < 0.05$ was considered statistically significant.

Results and Discussion. The antibacterial performance of $Ti_3C_2T_x$ MXene under NIR laser irradiation was systematically evaluated using *E. coli* as a representative Gram-negative microorganism. The results demonstrate a clear dependence of antibacterial activity on irradiation time and subsequent incubation period (Figure 1).

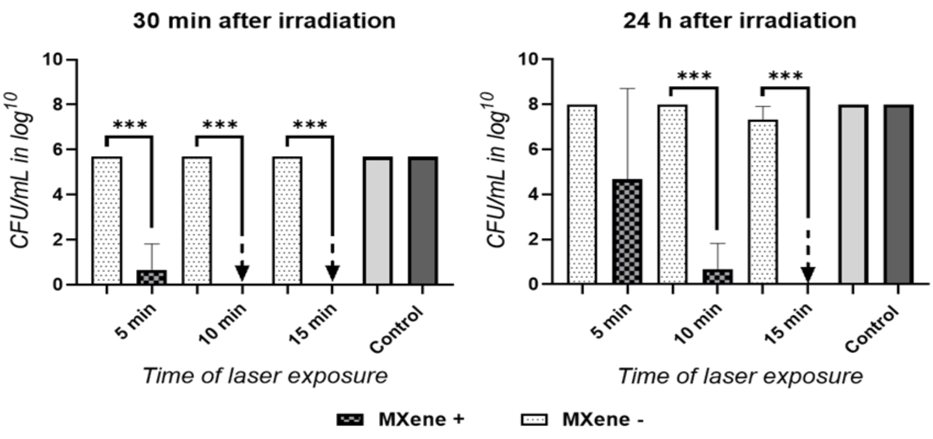

Fig. 1. Time-dependent antibacterial activity of MXene after varying irradiation exposure times. Control samples correspond to non-irradiated wells

Short-term analysis (30 minutes post-irradiation) revealed that even brief exposure (5 minutes) resulted in a noticeable bacteriostatic effect, characterized by suppression of bacterial growth rather than complete eradication. This indicates that MXene, even at relatively short irradiation durations, can interfere with bacterial proliferation.

After 24 hours of incubation, a more pronounced effect of irradiation duration was observed. Samples exposed to 10 and 15 minutes of NIR irradiation exhibited significant antibacterial activity, with bacterial reduction reaching up to 99.9%. In contrast, shorter irradiation times showed limited long-term bactericidal effects, suggesting insufficient thermal damage to ensure irreversible bacterial inactivation.The observed antibacterial behavior strongly correlates with temperature elevation during laser exposure, as illustrated in Figure 2. Prolonged irradiation resulted in higher localized temperatures within the wells, confirming the photothermal nature of MXene. This temperature increase is a critical factor contributing to bacterial cell damage.

The antibacterial mechanisms of MXene under photothermal activation can be attributed to several factors. First, localized heat generation disrupts bacterial membrane integrity, leading to leakage of intracellular components. Second, elevated temperatures can cause protein denaturation and enzyme inactivation, ultimately impairing essential cellular processes. Additionally, the potential generation of reactive oxygen species (ROS) may further enhance oxidative stress within bacterial cells [8].

Importantly, the photothermal conversion efficiency of MXene plays a central role in its antibacterial performance. Upon NIR irradiation, MXene absorbs light and converts it into heat through non-radiative relaxation processes. This localized hyperthermia creates a hostile microenvironment for bacterial survival [9].

A widely accepted explanation of MXene antibacterial activity is the “nano-knife” mechanism, in which sharp edges of 2D flakes penetrate bacterial membranes, causing physical disruption and cell death. To verify this, SEM analysis was performed after incubation of *E. coli* with $Ti_3C_2T_x$ MXenes. To prevent MXene sedimentation and ensure uniform dispersion, *E. coli* were co-incubated with Ti-based MXenes under continuous rotation. This maintained homogeneous suspension and maximized potential membrane contact required for the “nano-knife” mechanism. Despite improved dispersion and prolonged exposure, no significant differences in bacterial growth or colony formation were observed compared to static controls, indicating negligible antibacterial activity under these conditions. No evidence of membrane damage was observed (Fig. 3, A). Instead, MXene nanosheets adhered to or coated bacterial surfaces, with smaller flakes attaching to membranes and larger sheets enveloping the cells. TEM confirmed intact membrane structures while showing MXene localization on the cell surface (Fig. 3, B), and EDX (Fig. 3, C) detected titanium, consistent with $Ti_3C_2T_x$ presence. These results indicate that mechanical membrane disruption does not occur under the tested conditions, questioning the relevance of the “nano-knife” mechanism in this case.

In contrast to direct MXene–bacteria co-incubation, photothermal therapy (PTT) provides an effective route for MXene-based antibacterial action. $Ti_3C_2T_x$ MXenes (50 µg/mL) were irradiated with NIR for 5 - 15 min. A strong antibacterial effect was already observed after 5 min, with complete eradication after 10 and 15 min (Fig. 3, E). Temperature measurements showed significant heating in the presence of MXenes, reaching 62.7 °C after 15 min, compared to 31.1 °C for the control. SEM analysis revealed clear bacterial damage after irradiation, with *E. coli* exhibiting surface deformation and leakage of intracellular contents, confirming photothermal-induced structural disruption.

Furthermore, the intrinsic physicochemical characteristics of MXene, including high specific surface area, hydrophilicity, and abundant surface terminations, are essential for interactions with bacterial membranes. While pristine MXene nanosheets exhibit limited intrinsic antibacterial activity, their efficacy is significantly enhanced under near-infrared (NIR) laser irradiation, which induces localized photothermal effects and membrane disruption [10]. These properties also allow for functional tunability, and strategic surface modifications or synergistic combination with other antimicrobial agents may further improve antibacterial performance, providing promising avenues for future investigation.

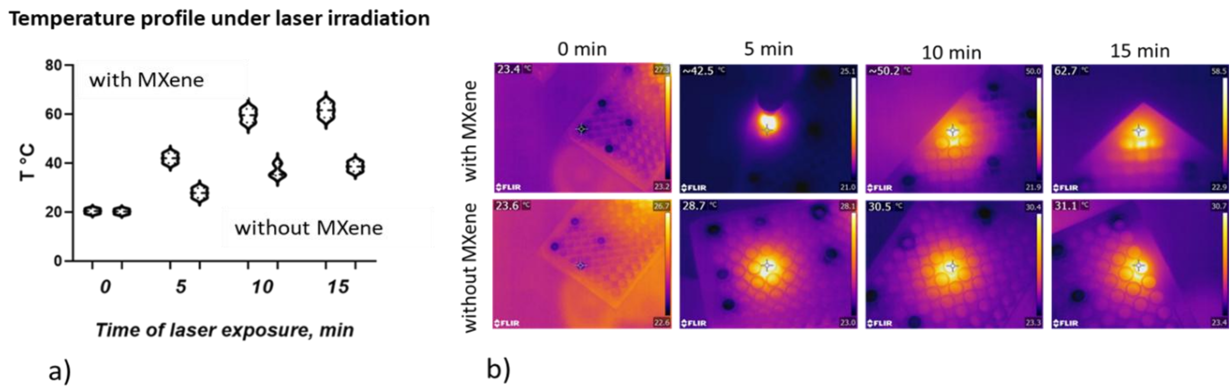

Fig. 2. Average temperature recorded in the wells following laser irradiation at different time intervals

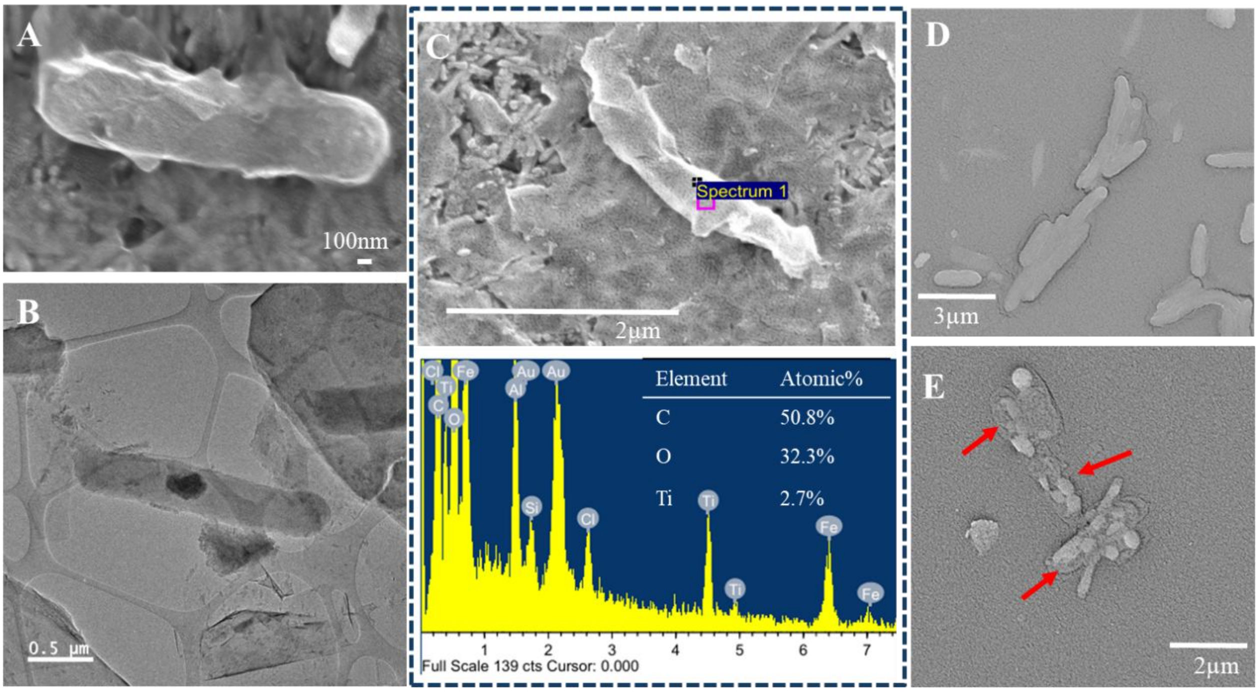

Fig. 3. (A) SEM and (B) TEM images of E. coli treated with $Ti_3C_2T_x$ MXenes, (C) the squares indicate the areas of EDX analysis for determining the presence of element distribution (at.%), where Au originates from sputter coating and Fe from the electrically conductive substrate, of E.coli treated with $Ti_3C_2T_x$ MXene; SEM images of the E. coli after $Ti_3C_2T_x$ Mxene-based photothermal therapy (D) control samples, (E) strains treated with Ti3C2T$_x$. The red arrows indicate the morphology changes

Overall, the results highlight that irradiation duration is a critical parameter governing the transition from bacteriostatic to bactericidal effects. Achieving sufficient thermal exposure is essential for irreversible bacterial damage.

Conclusions. This study demonstrates that $Ti_3C_2T_x$ MXene exhibits significant antibacterial activity when activated by NIR laser irradiation, primarily through a photothermal mechanism. The antibacterial effect was strongly dependent on irradiation duration, with optimal bactericidal performance observed at 10–15 minutes of exposure. These findings position MXene as a promising candidate for photothermal antibacterial applications, particularly in the context of wound healing and treatment of drug-resistant infections. The ability to achieve high antibacterial efficiency without inducing resistance further enhances its biomedical potential. However, to fully translate this approach into clinical applications, further investigations are required. Future studies should focus on optimizing key parameters such as irradiation mode, power density, exposure time, and MXene concentration. Additionally, in vivo studies and biocompatibility assessments are essential to validate safety and therapeutic efficacy. A deeper understanding of dose-dependent effects and interaction mechanisms will be critical for developing standardized protocols for MXene-based photothermal antibacterial therapy.

Prospects for further research to optimize photothermal treatment parameters of $Ti_3C_2T_x$ MXene, including irradiation conditions, exposure time, and bacterial load, as well as to investigate its antibacterial efficacy against a broader spectrum of clinically relevant and drug-resistant microorganisms.

Conflict of interest. The authors declare no conflict of interest.

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