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REVIEW

Recent Advances in Spatiotemporal Manipulation of Engineered Bacteria for Precision Cancer Therapy

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Abstract: Solid tumours possess a hypoxic and immunosuppressive microenvironment, presenting a significant challenge to anticancer treatments. Certain anaerobic microorganisms thrive in this setting, rendering them promising candidates for targeted antitumour therapy delivery. In contrast to traditional nanodrug delivery systems, bacterial-based drug delivery systems can be engineered to produce and secrete therapeutics without the need for intricate post-purification or protective delivery methods. Nevertheless, bacteria can potentially migrate beyond their intended niche, causing off-target drug release and substantial toxicity to healthy tissues. Consequently, to enhance the effectiveness of cancer treatments while minimizing side effects, it is essential to precisely manipulate bacteria for accurate and controlled drug delivery directly to the tumour site. This can be achieved by employing inducible or repressible systems that allow for precise regulation of gene expression at specific times and locations. Ideally, engineering bacteria capable of rapidly and precisely transitioning between "on" and "off" states as required will enable them to recognize and react to targeted stimuli. While various techniques such as optical, magnetic, acoustic, and hyperbaric oxygen micromanipulation have been developed for the manipulation of particles or cells, each technique boasts its unique set of pros and cons. This review article provides an updated overview of the recent progress in the spatiotemporal control of engineered bacteria via these methods and discusses the benefits and constraints of each approach.

Keywords: engineered bacteria, spatiotemporal manipulation, cancer, light, magnetic, ultrasound, hyperbaric oxygen

Introduction

Solid tumours are known to have a hypoxic and immunosuppressive microenvironment, which is a major barrier to anticancer treatment.[1] Specific obligatory and facultative anaerobic microorganisms preferentially grow in this environment,[2,3] which makes them suitable candidates for the delivery of antitumour therapies.[4,5] Unlike traditional nanoscale drug delivery systems, bacterial-based drug carriers can be engineered to produce and release medications, thus eliminating the requirement for complex postpurification or delivery protection measures.[6] However, bacteria can occasionally proliferate beyond their usual habitats, which results in off-target therapeutic release and may cause significant toxicity to normal tissues.[7,8] Thus, to boost the effectiveness of cancer treatments while reducing adverse reactions, it is essential to fine-tune bacteria for accurate and on-demand medication delivery directly to the tumour location. Depending on the requirements, exact control over gene expression can be achieved through the activation of inducible or repressible systems at precise times and locations. Ideally, designing bacteria that rapidly and precisely switch between "ON" and "OFF" modes, as needed, would allow them to identify and respond to physiological or pathological conditions in response to targeted triggers.[9] Bacteria are administered endogenous chemical inducers, which allow them to react to the acidic or hypoxic milieu of solid tumours to control bacterial activity spatially. However, these systems still cannot control bacterial activity temporally.[4,8] Exogenous chemical inducers can closely regulate the corresponding inducible promoters to manage bacterial colonization or gene expression for temporal control, but they

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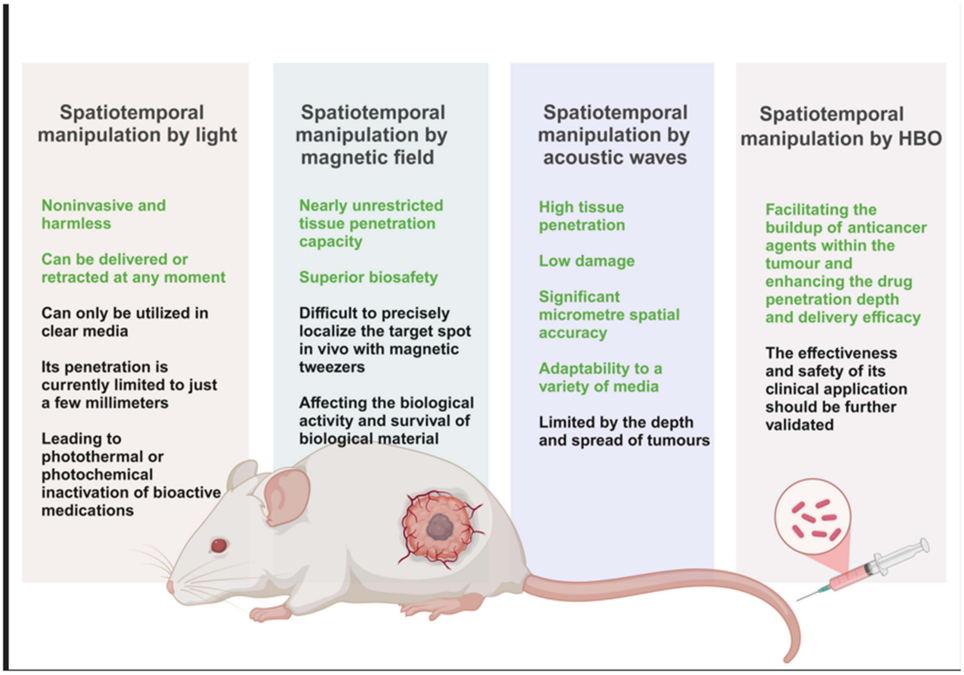
Graphical Abstract



are unable to provide spatial control of bacterial activity.[4,8] Chemical inducers cannot easily accumulate to the proper concentration in the tumour microenvironment due to the high interstitial fluid pressure, and increasing their dosages may result in unintended consequences.[10–12] Certain biological techniques, such population sensing systems for bacteria, can modify gene expression based on population size such that therapeutic gene expression only occurs when the population density reaches a certain threshold.[13,14] Nevertheless, accurately mimicking gene expression in a spatiotemporally controlled manner using biological approaches can be challenging, particularly in living animals. Compared to the two ways mentioned above, physical methods are more controllable in terms of both space and time.

To manipulate particles or cells, a number of technologies, such as optical,[15,16] magnetic,[17,18] acoustic[19,20] tweezers, and hyperbaric oxygen (HBO)[21] have been developed to date. Through photon momentum transfer, optical tweezers use intensely concentrated laser beams for noncontact capture and manipulation of small objects. Optogenetics has recently advanced swiftly as a noninvasive method that is characterized by excellent spatiotemporal precision and reversibility.[22,23] However, optical tweezers can be used only in clear media, which limits their use in vivo. Moreover, biological specimens may sustain photodamage from direct exposure to high-intensity lasers, which can lead to photothermal or photochemical inactivation of bioactive medications. Magnetic fields enable the in vivo manipulation of cells through the use of magnetic tweezers, which have the ability to penetrate opaque media and boast a high level of biosafety.[24] The targets subjected to the applied magnetic field must be magnetized or associated with magnetized particles.[25,26] Owing to the unfocused nature of magnetic fields, magnetic tweezers have difficulty accurately identifying the target position in vivo. The viability and bioactivity of biological samples may be permanently impacted by magnetic labelling. Therefore, acoustic tweezers have recently become popular tools for working with biological particles because they cause low damage and have high tissue penetration, significant micron-scale spatial precision, and adaptability to a variety of environments.[27,28] Despite the numerous benefits of ultrasonic therapy, its implementation has inherent hurdles, and its efficacy in inducing the expression of genes is limited by the tumour depth and dissemination. Consequently, hyperbaric oxygen therapy techniques are employed to efficiently deplete the extracellular matrix (ECM), facilitate the buildup of anticancer agents within the tumour and enhance the drug penetration depth and delivery efficacy.[29] Nonetheless, the precise mechanism of hyperbaric oxygen therapy needs further elucidation.

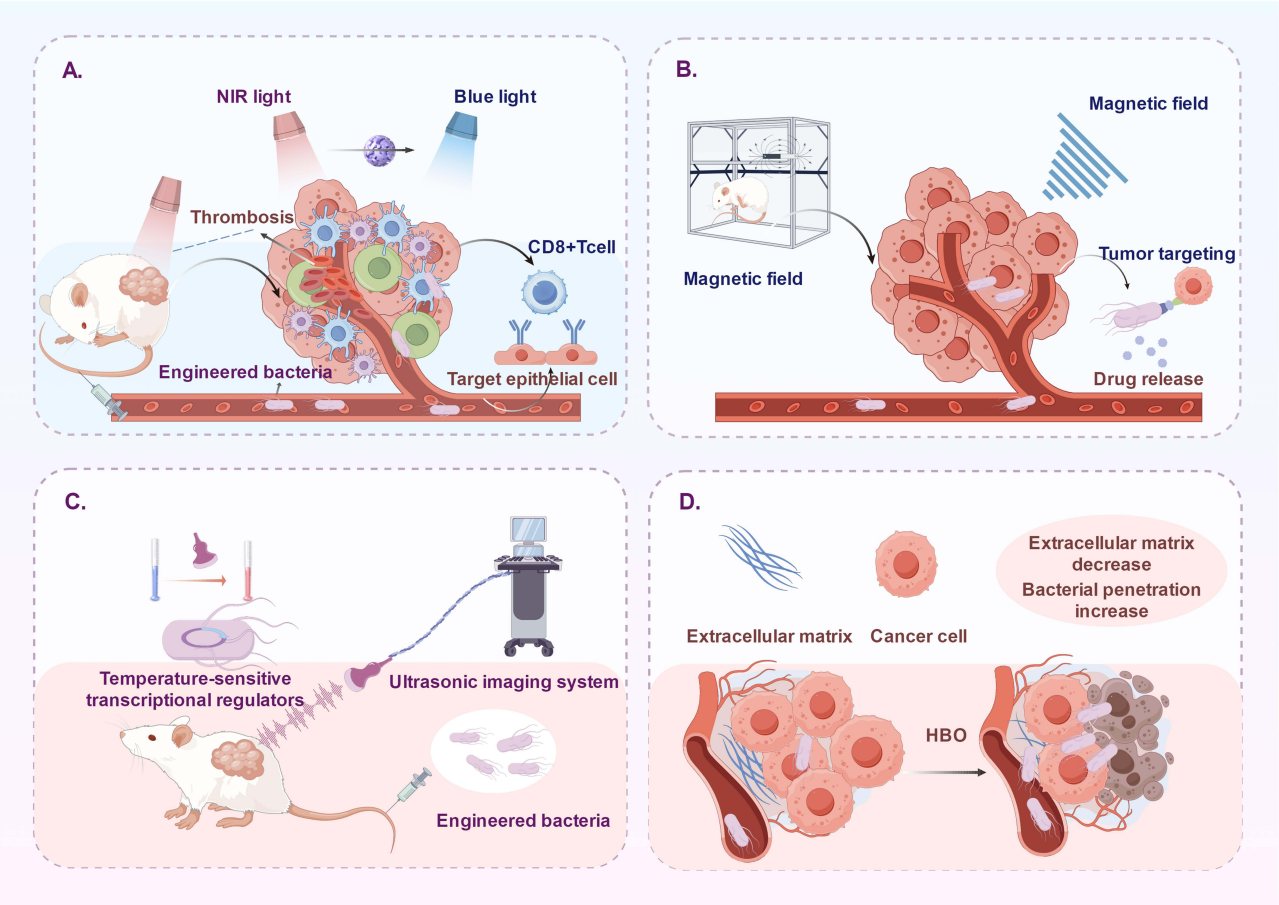


Figure 1 Spatiotemporal manipulation of engineered bacteria. (**A**) Spatiotemporal manipulation by light: Engineered bacteria can be manipulated spatiotemporally by light to reduce angiogenesis resulting from a T-cell-mediated autoimmune response. They can also convert near-infrared (NIR) light into blue light, and upon light exposure, they can induce apoptosis in tumor cells and promote the development of intratumoral thrombi. (**B**) Spatiotemporal manipulation by magnetic field: Bacteria are capable of penetrating biological matrices, specifically targeting tumours when in the presence of magnetic fields, and controlling the release of drug molecules. (**C**) Spatiotemporal manipulation by acoustic waves: Ultrasound enables the precise spatiotemporal control of anti-tumour therapy in engineered bacteria by employing temperature-sensitive transcriptional regulators. (**D**) Spatiotemporal manipulation by HBO: Hyperbaric oxygen (HBO) therapy can effectively decrease the density of the extracellular matrix, thereby promoting the accumulation of bacteria within tumours.
Abbreviations: NIR, near-infrared; HBO, hyperbaric oxygen.

The effectiveness and safety of its clinical implementation need additional validation. Each of the above particle manipulation methods has advantages and disadvantages. This review encapsulates the latest progresses in the spatiotemporal manipulation of engineered bacteria, utilizing the aforementioned techniques, and it further discusses their respective strengths and limitations. (Figure 1)

Spatiotemporal Manipulation by Light

Light is noninvasive and harmless and can be delivered or retracted at any moment. Photosensitive bacteria that react to visible light have been extensively utilized in dynamic industrial manufacturing and the modulation of signalling pathways.[30,31] (Table 1) With an aggregation-induced emission (AIE) photosensitizer MA, metabolic engineering was used to create a live therapeutic system based on attenuated *Salmonella*. The modified bacteria may settle in tumour tissues and cause the colonization and expression of VEGFR2 from outside sources. The protein encoded by the VEGFR2 gene, which can reduce angiogenesis caused by a T-cell-mediated autoimmune response and prevent tumour development, was released and expressed in tumour tissues upon exposure to light. The administered MA-engineered *Salmonella* harbouring VEGFR2 plasmids were located in the tumour tissues, and the bacteria continued to colonize and express exogenous genes since the MA labelling approach had no adverse effects on *Salmonella* reproduction. Using an AIE photosensitizer and metabolic engineering, we created a live therapeutic system based on attenuated *Salmonella* to

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Table 1 Spatiotemporal Manipulation by Light or Magnetic Field

Manoeuvring Methods	Bacteria Selection	Manipulation Components	Key Findings of Each Study	Reference
Visible light	MA-engineered *Salmonella* (strain VNP20009)	Photosensitizer MA	The encoded VEGFR2 gene was released and expressed in tumour tissues when exposed to light, which could prevent angiogenesis and slow the growth of tumours.	[32]
NIR	*E. coli* Nissle 1917	EL222 sensor	Photoactivatable cancer immunotherapy significantly reduces tumour growth and metastatic tumours.	[40]
980 nm laser	*E. coli* Nissle 1917	pDawn promoter	The upconversion optogenetic engineered bacterial system may be able to provide light-controlled cancer treatment and a precise tumour diagnosis.	[41]
NIR	*Lactococcus lactis*	pDawn promoter	PDT and immune therapy produced by optogenetic effects have shown encouraging outcomes in the treatment of local cancers and the prevention of distant cancers.	[42]
NIR	*E. coli* Nissle 1917	EL222 sensor	When exposed to NIR light, the in situ-formed thrombus greatly intensifies the photothermic ablation of malignancies.	[43]
Irradiated with 635 nm laser	*E. coli* MG1655	Black phosphorus @ YiaT protein	Utilizing surface-programmed bacteria as a photo-controlled NO producer, gas treatment and tumour immunotherapy are achieved.	[44]
NIR laser irradiation	*Rhodospirillum rubrum*	Small-size gold nanoparticles (Au NPs)	Through a photochemical transformation, R.r-Au can increase hydrogen generation and lactate consumption, which can activate the immune system to respond to tumours.	[45]
488 nm blue ligh	*E. coli* MG1655	EL222 system containing plasmids pEL222 and pBLind-GFP	Optogenetic elimination techniques were successfully used to stop the altered bacteria's survival and function after application.	[46]
Magnetic fields	*E. coli* MG1655	Magnetic nanoparticles	On-demand release of anticancer medication is made possible by magnetically steerable bacterial microrobots moving in 3D biological matrices.	[47]
Alternating magnetic field	*E. coli* BL21	Fe3O4 nanoparticles (NPs)	Modularly created engineered bacteria for precise tumour immunotherapy via magnetic field-based spatiotemporal manipulation.	[48]

Abbreviations: NIR, near-infrared; R.r-Au, nanogold-engineered *Rhodospirillum rubrum*; NPs, nanoparticles.

achieve light-controlled gene release for breast cancer treatment. Notably, the excitation wavelength of MA was in the visible spectrum, which restricted its ability to penetrate light in in vivo applications.[32] Future research will concentrate on enhancing the metabolic engineering technique with NIR or two-photon photosensitizers in order to increase the range of theranostic applications. Existing optogenetic methods are effective under certain conditions; however, they often lead to poor tissue penetration. Therefore, optogenetic manipulations frequently use intrusive fibre optic insertion.[33–35] The upconversion materials transform deep tissue-penetrating NIR light into localized visible light, thereby facilitating subsequent photoresponsive manipulations within cells. Upconversion optogenetics has been extensively used in tumour therapy and neuroscience, providing a greater spatiotemporal manipulation accuracy than conventional regulatory methods do.[36–39] To minimize trauma while penetrating deep into tissues, an optogenetic system has been developed that is responsive to NIR light. This system employs lanthanide-doped upconversion nanoparticles to transform NIR light into blue light, which indirectly stimulates the expression of optogenetic genes in bacteria that have been genetically modified to combat tumours.[40–42] This optogenetic system that is activated by NIR light effectively inhibits tumour growth but is unable to completely eradicate tumours. We engineered α-haemolysin from *Escherichia coli* Nissle 1917

(EcN) to be expressed upon exposure to blue light, which is emitted by lanthanide-doped upconversion nanoparticles, to overcome this limitation. As an in situ photothermal agent in tumours, the αHL not only kills tumour cells but, more significantly, damages endothelium to cause thrombosis and facilitate a cascade of photogenetic and photothermal therapeutic effects, which can result in the complete eradication of surface tumours. Thrombi with strong NIR absorbance like this allow precise photothermal therapy to totally stop tumour growth with very little off-target toxicity, which is uncommon in purely genetically engineered bacterial systems. Furthermore, αHL secretion triggers an immune response and inhibits 4T1 tumour metastasis. Therefore, the in situ PTT generated by bacteria-based optogenetic systems has a lot of potential for precise cancer treatment.[43]

Another photocontrolled engineered bacterium exerts an antitumor effect by synthesizing antitumour agents and metabolizing compounds that facilitate tumour proliferation. People have engineered the gram-negative *E. coli* strain MG1655 and genetically programmed the outer membrane of the bacterial cells with therapeutic TRAIL using the YiaT surface display system. The therapeutic protein might be supplied straight to the intended tumour areas using this tactic after bacterial targeting of tumors. Additionally, the surface-programmed bacteria act as light-driven nitric oxide generators that reduce nitrate to nitric oxide, which is released locally in the tumour region, increases treatment efficiency, and polarizes tumour-associated macrophages towards the M1 phenotype. The simultaneous production of reactive oxygen species causes the death of immune cells, enhancing the anticancer effect. This study highlights the use of microbial metabolism and programmable bacteria with functional materials to create living, multipurpose anticancer systems.[44] In addition, a study used the transdermal therapeutic cryomicroneedle patch to deliver bacteria to the tumour site. Following laser irradiation at a set time, *Rhodospirillum rubrum* (R.r-Au) efficiently produced hydrogen, depleted lactate, and enhance the activation of anticancer immunity. This study shows the potential of cryomicroneedle delivery of nanogold-engineered *Rhodospirillum rubrum* as a minimally invasive in situ delivery method for living bacterial drugs and provides new opportunities for nanoengineering bacteria to convert tumour metabolites into useful compounds for tumour optical biotherapy.[45]

Another study developed a photogenetic clearance approach for engineered bacteria. Initially, a photogenetic elimination gene circuit that utilizes the blue light-responsive system EL222, which incorporates the dark-induced antitoxic protein ccdA and the blue light-induced toxin ccdB, was developed. The engineered bacteria perished upon exposure to 488-nm laser irradiation, thereby enabling the engineered bacteria to function for the desired period of time.[46]

Light-induced control elements provide high spatiotemporal precision[49–51]and show great potential in basic research and clinical applications, but they still have some significant limitations in practice. First, because optical tweezers are only used in clear media, their in vivo usefulness is limited. Furthermore, biological samples may be photodamaged and biologically active medications may be inactivated by photothermal or photochemical methods when the target is directly exposed to high-intensity laser radiation.[52] Therefore, in clinical applications, these treatments may damage healthy tissues in the irradiation pathway or affect the effect of biologically active drugs.[53] Therefore, reducing the intensity or duration of light required and reducing damage to cells and tissues may be possible in the future by developing genetic light-responsive tools that are sensitive to a low light intensity.[54,55] The introduction of antioxidants or photothermal protectants during light exposure may attenuate the damage to cells and active drugs caused by light exposure.[56] Another significant limitation lies in the restricted penetration depth of light,[57] which consequently constrains its accessibility to deep-seated tumour tissues.[58] Implantable luminescent technology may represent a potentially viable solution.[59,60]

Spatiotemporal Manipulation by Magnetic Field

Alternating magnetic fields serve as ideal signals for the manipulation of microorganisms because of their nearly unrestricted tissue penetration capacity and superior biosafety.[61–63] (Table 1) Recently, *Escherichia coli* cells were effectively bound to magnetic nanoparticles and nanoliposomes, which were loaded with photothermolysin and chemotherapeutic medicines. In order to locate the bacterial biohybrids in tumour tissues, magnetic fields align and direct them across three-dimensional porous microenvironments. Activated by pH and NIR light, photothermal and pH-sensitive liposomal carriers on biohybrids provide a flexible and on-demand delivery platform that enables the spatiotemporal release of chemotherapeutic molecules at the target. A multifunctional microrobotic platform has been developed for directed navigation inside three-dimensional biological networks and for delivering stimulus-responsive medicines for diverse medical applications.[47] Afterward, Ma X et al created a prototype of a bacterium-based micro-biorobot called AMF-manipulated tumour-homing bacteria (AMF-Bac), which has five

modules: active navigation, signal decoding, signal feedback, signal processing, and signal output. The active navigation module gives AMF-Bac active tumor-targeting to orthotopic colon cancers when administered colon-specifically. Once orthotopic colon tumours are targeted, the Fe3O4 nanoparticles in the engineered bacteria allow AMF-Bac to receive and transform magnetic signals into heat signals. This process triggers the expression of lytic proteins in the bacteria, which leads to bacterial lysis and the release of the antitumor protein CD47nb, which was previously stored and pre-expressed in the AMF-Bac. The AMF-Bac also permitted continual magnetic field-controlled mobility, which improved tumour targeting and dramatically increased the therapeutic efficacy. These findings not only represent a technique for the noninvasive, spatiotemporal, real-time AMF manipulation of tumour-homing bacterial gene expression but they also integrate the idea of modular design into the development of bacterial systems.[48]

Magnetic field-based spatiotemporal manipulation shows great potential for clinical applications. Unfortunately, magnetic tweezers cannot easily or accurately locate target sites in vivo due to the unfocused nature of the magnetic field, but the use of imaging techniques, such as magnetic resonance imaging or magnetic particle imaging, to precisely locate deep tumours may be possible in the future.[64] Magnetic labelling can have irreversible effects on the viability and biological activity of biological samples. Engineered bacteria are difficult to modify and require the integration of specific magnetic materials or receptor genes into the bacterial genome. This process is complex and costly, and the success rate is not always high. In addition, a potential risk of an immune response to the introduced exogenous material may exist. Prolonged exposure to magnetic fields may also have an impact on the physiological functions and metabolism of the bacteria themselves.[65] Therefore, much more work is needed to achieve clinical translation. Continuous efforts are needed to improve the related technologies, develop more efficient and safe magnetic materials and methods, increase the precision and convenience of gene editing tools, and explore innovative ways of multidisciplinary cross-fertilization in order to further improve the safety and anti-tumour efficacy.

Spatiotemporal Manipulation by Acoustic Waves

Recently, acoustic tweezers have become useful tools for working with biological particles because of their high tissue penetration, low damage, significant micrometre spatial accuracy, and adaptability to a variety of media.[27] (Table 2) Recently,

Table 2 Spatiotemporal Manipulation by Acoustic Waves or HBO

Manoeuvring Methods	Bacteria Selection	Manipulation Components	Key Findings of Each Study	Reference
FUS	*E. coli* Nissle 1917	TcI42 repressor	In situ activation of US-activated therapeutic microorganisms results in the significant inhibition of tumour growth.	[66]
FUS	*E. coli* MG1655	TcI repressor	FUS-induced hyperthermia increases IFN-γ gene expression, enhancing the anti-tumour effectiveness of URB.	[67]
US	*Salmonella typhimurium*, VNP20009	Thermosensitive transcriptional repressor TlpA39	The local expression and release of therapeutically effective anti-tumour payloads are activated by brief US-triggered heat stimulation.	[68]
Holographic acoustic tweezers	*E. coli* BL21	GVs	Genetically engineered bacteria can be manipulated acoustically in vivo.	[69]
Hyperthermia high-intensity FUS	*E. coli* MG1655	GVs and lambda promoters	Utilizing US and visible microorganisms, tumour chemo-immunotherapy is accomplished.	[64]
US	*E. coli* BL21	sonosensitizers	Due to the secreted tumour-associated antigens and the innate immunogenicity of bacteria, SDT can induce strong anticancer immune responses.	[70]
HBO	*E. coli* Nissle 1917	ECM	ECM depletion combined with a hyperbaric oxygen treatment approach promote bacterial growth inside tumours and trigger immunogenic cell death.	[21]

Abbreviations: FUS, focused ultrasound; GVs, submicron gas vesicles; HBO, hyperbaric oxygen; SDT, Sonodynamic therapy; ECM, extracellular matrix.

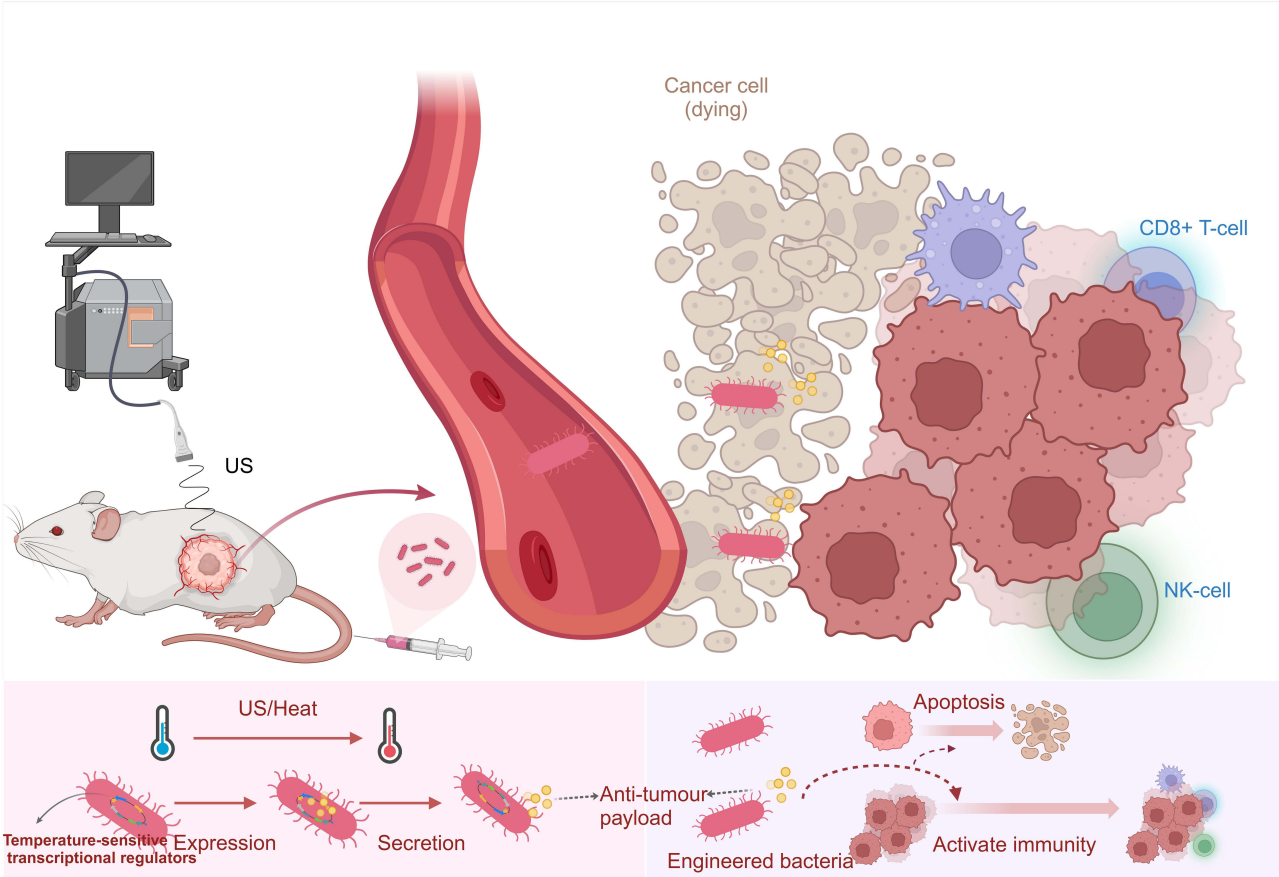

Figure 2 Spatiotemporal manipulation based on temperature-sensitive transcriptional regulators. Ultrasound stimulation-induced temperature increase activates thermosensitive transcriptional repressors, which in turn prompt the release of anti-tumor payloads from engineered bacteria. This action can lead to tumor cell apoptosis or stimulate an anti-tumor immune response.bacteria by employing temperature-sensitive transcriptional regulators. (Created in BioRender. Chang, x. (2025) https://BioRender.com/a36h404).
Abbreviation: US, ultrasound.

two US-inducible transgenic expression methods for cancer therapy have been developed in bacteria using the thermosensitive bacteriophage λ repressor TcI.[66,67].(Figure 2) The pR-pL tandem promoter was activated to boost the expression of therapeutic outputs for triggering the immune system after the tumours were exposed to targeted ultrasonic irradiation to reach a temperature of 42°C to 45°C after bacterial colonization. The integrase-based state switch is uncontrollable once it is activated. Thus, immune checkpoint inhibitor synthesis over an extended period of time may be harmful to the immune system.[66] Another bacterium that responds to ultrasound (US) was created by incorporating a US-activated therapeutic circuit into the *E. coli* MG1655 laboratory strain.[66,67] In conclusion, by combining URB with FUS, we were able to induce highly spatiotemporally controllable gene expression in deep-seated tumours. Healthy tissue can be subjected to thermal damage in the range of temperatures from 42°C to 45°C. Therefore, it is essential to create US-activated transgenic expression systems that are more sensitive and manageable. These systems should be activated at temperatures of 39°C to 40°C and should be constructed by using safer, nonpathogenic, or weakened bacterial strains that are suitable for therapeutic purposes. Thus, the team developed an US-controlled activatable gene circuit, termed SINGER, which employs the heat-sensitive transcriptional regulator TlpA39. (Figure 2) The modified bacteria containing this system can be activated by US and locally heated to 39°C to precisely trigger efficient expression of designated genes. This study successfully achieved US -triggered protein expression and secretion in specifically engineered bacteria with minimal disruption and increased induction rates through promoter modification and the tuning of a ribosome-binding site.[68] Designing therapeutic bacteria to be controlled by FUS, a form of energy that can be applied noninvasively to specific anatomical sites (eg, solid tumours), may address limitations such as the release of the payload into healthy tissue outside of the tumour resulting from systemic drug delivery and the transplantation of

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small numbers of bacteria. This control is provided by temperature-driven genetic state switches that respond to brief bursts of FUS-induced hyperthermia to generate a sustained therapeutic effect that successfully initiates and induces significant inhibition of tumour growth in situ. This technique provides a key tool for the spatiotemporal targeting of potent bacterial therapies in a variety of biological and clinical scenarios.

Nevertheless, manipulating normal cells directly is challenging because of their minute sizes and a close acoustic impedance match with the adjacent medium. Heterologous gene cluster expression has enabled the creation of bacteria that are capable of producing a multitude of submicron gas vesicles (GVs) within their cytoplasm. The incorporation of GVs significantly reduces the average bacterial density and enhances compressibility, which in turn increases acoustic responsiveness, making bacterial cells controllable via US. (Figure 3) By employing phased-array acoustic tweezers, it is possible to trap and manoeuvre clusters of these engineered bacteria, both inside and outside living organisms, by manipulating acoustic beams electronically. This method allowed for controlled, reverse, or on-demand circulation of bacteria within the vascular network of mice, thus facilitating targeted tumour therapy in preclinical models. This work also shows that this method can be used to increase the aggregation efficiency of engineered bacteria in malignancies. This work offers a framework for manipulating living cells in vivo, which will advance cell-based biomedical applications.[69] Recently, an acoustic reporter gene (ARG1) has been engineered to produce gas vesicles in bacteria, which facilitates real-time imaging guidance for FUS. The operator would receive instant feedback when the tissue was exposed to hHIFU irradiation since the contrast signals would vanish as a result of GVs collapsing under the strong

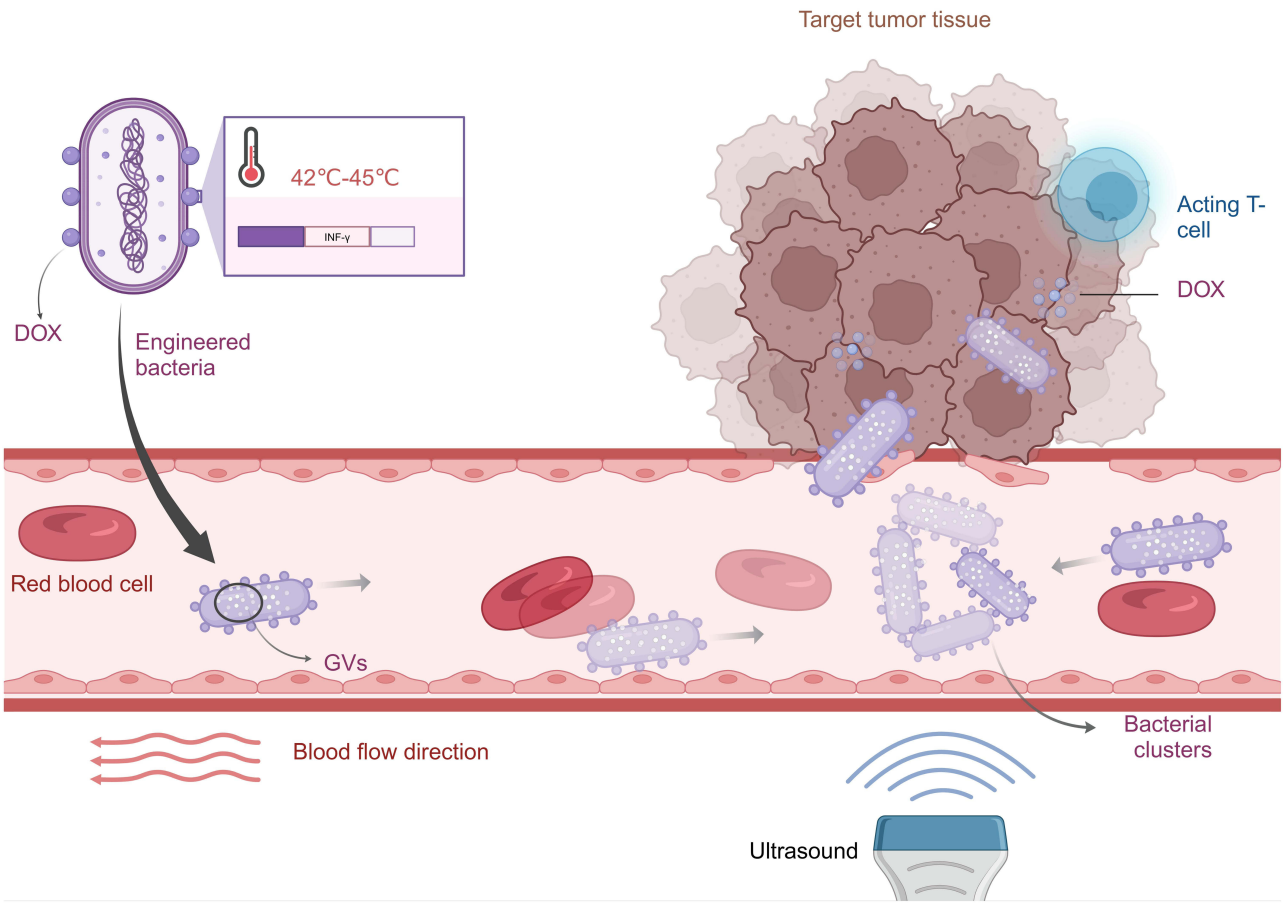

Figure 3 Spatiotemporal manipulation based on the combination of biosynthetic GVs and acoustic tweezers. Ultrasound enabled the controlled, reversible, or on-demand circulation of bacteria within the vascular network of mice; it also stimulated the release of IFN-γ and DOX from engineered bacteria, which in turn promotes tumour-specific T-cell responses. (Created in BioRender. Chang, x. (2025) https://BioRender.com/j04y108).
Abbreviations: GVs, gas vesicles; DOX, doxorubicin.

acoustic pressure. This advancement allows for the precise localization of the US focal point to the engineered bacteria within the tumour, prompting the bacteria to express and secrete IFN-γ locally within the tumour. The simultaneous application of IFN-γ and DOX on engineered bacteria stimulates tumour-specific T-cell responses, yielding a synergistic effect that significantly amplifies the antitumour response. Notably, the IFN-γ gene could be induced and therapeutic effects produced with just 25 minutes of high-intensity FUS irradiation at 42°C to 45°C. This process significantly increases safety for potential clinical applications.[64]

Sonodynamic therapy (SDT) utilizes US to activate sonosensitizers, which generate reactive oxygen species (ROS) to kill cancer cells. This method is emerging as a promising cancer treatment strategy because of its desired tissue penetration capacity, noninvasiveness, and low toxicity.[71–74] A novel in vivo biotherapeutic drug (*E. coli*-pE@PCN) was developed by electrostatic adsorption of a nanoacoustic sensitizer onto engineered bacteria to be used with SDT. It was found that *E. coli*-pE@PCN could effectively accumulate in and penetrate the entire tumour owing to the instinctive bacterial targeting ability and active mobility. Engineered bacteria not only sustainably produce catalase to relieve tumour hypoxia but also facilitate the enriched and expanded distribution of the carried sonosensitizer at tumour sites. By utilizing the potency of sonodynamic therapy in promoting the release of tumour-associated antigens and bacterial pathogen-associated molecular patterns, *E. coli*-pE@PCN-based SDT could stimulate strong anti-tumour immune responses against cancer progression, metastasis, and recurrence. Thus, the biohybrids based on programmable bacteria created here open the door to developing next-generation sonodynamic immunotherapeutics to eradicate cancer and stop its spread and recurrence.[70]

While many benefits associated with the use of US for antitumor therapy have been noted, it also has inherent difficulties, especially when dealing with deep-seated or metastatic malignancies. Its efficacy in modulating gene expression is impeded by the tumour spread and depth, which necessitate more accurate heat transfer techniques. Potential remedies for more specialized treatment include external beam radiation and high-intensity FUS. Additionally, additional toxicological investigations are necessary to thoroughly assess the harmful effects of bacteria that have been created. The anticancer efficacy of engineered bacteria in patient-derived xenograft models requires further research in order to yield more insightful findings for clinical use.

Spatiotemporal Manipulation by HBO

It has been found in patients that HBO, a noninvasive treatment method, can effectively reduce the compact extracellular matrix, thereby promoting bacterial buildup inside tumours. (Table 2) Inspired by this finding, Xu et al modified EcN by incorporating cypate molecules to create EcN-cypate, a PTT tool that can initiate immunogenic cell death (ICD). HBO significantly boosts the accumulation of EcN-cypate within tumours and encourages the penetration of immune cells. When EcN–cypate is exposed to near-infrared (NIR) laser radiation, it achieves PTT and stimulates immunogenic cell death (ICD), which in turn triggers systemic immunological responses, including dendritic cell (DC) maturation, to destroy malignancies. HBO treatment dramatically increases the intratumoural accumulation of EcN–cypate and promotes the intratumoural infiltration of immune cells to achieve the desired tumour elimination. This work provides a straightforward and noninvasive way to boost the intratumoural transport efficiency of natural or engineered bacteria, which may be useful for the clinical application of bacteria-mediated synergistic cancer therapy.[21]

However, the therapeutic use of hyperbaric oxygen treatment is more complicated and expensive due to the need for specialist equipment and strict operation. Moreover, long-term efficacy may be limited by the stability and activity of engineered bacteria under hyperbaric oxygen. Therefore, we must use genetic engineering to improve the stability and targeting of modified bacteria in a hyperbaric oxygen environment. And the effectiveness and safety of its clinical application should be further validated. In addition, to further understand the mechanism of action of hyperbaric oxygen, we need to do further basic research.

Challenges and Outlook

Engineered bacteria have been combined with optical, magnetic, and acoustic tweezers and hyperbaric oxygen to precisely control multiple key steps in the process of bacterial treatment of tumours and to spatiotemporally control the release of therapeutic drugs, which can greatly improve treatment effectiveness and reduce costs for future cancer

patients. In particular, focused ultrasound (FUS)-actuated bacterial medicines provide a route to eventual clinical implementation thanks to the expanding corpus of research on bacteria-based therapies[75–77] and the growing clinical acceptance of FUS.[78,79] More effort will be required to optimize the timing, dosage, and molecular identity of FUS-activated treatment release for every application. Combining FUS-activated bacterial treatments with other molecular or cellular therapies may improve therapeutic success. For instance, tumor entrance and engraftment patterns of immune cells and engineered microbes are different and frequently complementary. An environment that is more accessible to modified T cells may be created by creating bacteria that can enter immunosuppressed tumor areas and release cytokines or checkpoint inhibitors. In this manner, the bacteria and T cells can work together to fulfill their respective therapeutic roles.[80] Despite notable advancements in achieving the spatiotemporal manipulation of engineered bacteria, critical challenges must be overcome to enable their successful clinical translation.

The clinical translation of engineered bacteria necessitates addressing critical biosafety and stability challenges. These microorganisms pose inherent risks due to their pathogenic potential, immunogenic properties, and robust colonization capacity, which may precipitate adverse outcomes, including sepsis and a systemic inflammatory response. Furthermore, while engineered bacterial vectors demonstrate biocompatibility and immune evasion capabilities, these attributes may inadvertently drive uncontrolled proliferation, genetic instability, and chronic toxicity.[81] These properties necessitate implementing fourfold safeguards: (1) the selection of virulence-attenuated strains; (2) the development of novel materials for engineered bacterial delivery;[46] (3) real-time antibiotic therapeutic management;[82] and (4) real-time infection monitoring systems. In the future, we may be able to improve the biosafety and stability of engineered bacteria by delivering them to tumor sites with degradable materials that can persist in the body for long periods and develop genetic circuits for physical control of engineered bacteria—enabling engineered bacteria to die after a corresponding physical stimulus (eg, laser irradiation) so that the engineered bacteria can function for a desired period and degrade in a timely manner.[46] The use of materials that degrade at specific triggers to manage the breakdown of delivered materials after the death of these bacteria, as well as improved targeting of materials, are key to improving the reliability and efficiency of this approach.

Developing intelligent engineered bacterial systems has become imperative to address the evolving therapeutic demands and enhance treatment efficacy. Contemporary synthetic biology approaches enable the precise spatiotemporal control of therapeutic payload release through bacterial–environment interactions. Notably, recent advancements have established synergistic platforms combining engineered bacteria with multimodal anticancer strategies, including radiotherapy,[83–85] chemotherapy,[86] and immunotherapy,[87,88] thereby expanding the therapeutic landscape. Future developments may integrate engineered bacteria with multimodal antitumor therapies and spatiotemporal manipulation techniques through advanced synthetic biology platforms. This paradigm could increase therapeutic precision while optimizing tumour targeting efficacy. In addition, the synergistic actions of numerous spatiotemporal manipulation techniques may transcend the limitations of each technique alone and provide fresh perspectives for the area.[89] Furthermore, combinatorial approaches utilizing multiple engineered bacterial consortia may potentiate antitumor responses through interactions.[90]

To further improve the controllability and targeting of spatiotemporally manipulated engineered bacteria, it is crucial to further construct visual bacteria[64] and to add termination switches to engineered synthetic cargoes.[25] In addition, exploring new methods of spatiotemporal manipulation (eg electric current) may shed new light on further development.

The antitumor efficacy of the above strategies must be tested in future studies, which may provide valuable clinical translation lessons.

Abbreviations

HBO, hyperbaric oxygen; ECM, extracellular matrix; NIR, near-infrared; AIE, aggregation-induced emission; MA, MeTTPy-D-Ala; NIR, near-infrared; EcN, *Escherichia coli* Nissle 1917; PTT, photothermal therapy; AMF, alternating magnetic field; AMF-Bac, AMF-manipulated tumor-homing bacteria; GVs, gas vesicles; IFN-γ, interferon γ; DOX, doxorubicin; SDT, sonodynamic therapy; US, ultrasound; FUS, focused ultrasound; ROS, reactive oxygen species; ICD, immunogenic cell death.

Data Sharing Statement

No data were used for the research described in the article.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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