International Journal of Nanomedicine

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REVIEW

Emerging applications of upconverting nanoparticles in intestinal infection and colorectal cancer

This article was published in the following Dove Medical Press journal:
International Journal of Nanomedicine

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Abstract: Colorectal cancer is the abnormal growth of cells in colon or rectum. Recent findings have acknowledged the role of bacterial infection and chronic inflammation in colorectal cancer initiation and progression. In order to detect and treat precancerous lesions, new tools are required, which may help to prevent or identify colorectal cancer at an early stage. To date, several different screening tests are available, including endoscopy, stool-based blood tests, and radiology-based tests. However, these analyses either lack sensitivity or are of an invasive nature. The use of fluorescently labeled probes can increase the detection sensitivity. However, autofluorescence, photobleaching, and photodamage are commonly encountered problems with fluorescence imaging. Upconverting nanoparticles (UCNPs) are recently developed lanthanide-doped nanocrystals that can be used as light-triggered luminescent probes and in drug delivery systems. In this review, we comprehensively summarize the recent developments and address future prospects of UCNP-based applications for diagnostics and therapeutic approaches associated with intestinal infection and colorectal cancer.

Keywords: near infrared, imaging, bacteria, photodynamic therapy, anti-Stokes emission, inflammation

Introduction

Colorectal cancer is the third most common cancer worldwide and accounts for almost 10% of cancer-related deaths in Western countries.[1] The growing evidence suggests that bacteria can play an important role in the initiation and progression of colorectal neoplasm by inducing chronic inflammation and by the release of carcinogenic metabolites.[2] Some of the most commonly associated bacteria with colon cancer includes *Fusobacteium nucleatum*,[3] *Bacteroides fragilis*, and *Escherichia coli*.[4] Inflammatory bowel disease (IBD)-associated colorectal cancer is a classic example of an inflammation-induced cancer form.[5]

Early detection of precancerous polyps can prevent the onset of colorectal neoplasm or increase the chances of a successful treatment. Currently, several different screening tests are available including endoscopy, stool-based blood tests, and radiology-based tests. However, these protocols lack either sensitivity or sufficient invasiveness, one of the reasons why colorectal cancer is still a major cause of cancer-related deaths in Western countries. The use of fluorescent labeling probes for cancer imaging increases the detection sensitivity. However, autofluorescence, photobleaching, and photodamage are the most commonly encountered problems with fluorescence imaging (FI). Recent reports have identified upconverting nanoparticles (UCNPs) as options to overcome these limitations.[6,7] UCNPs are light-triggered lanthanide (Ln)-doped nanocrystals that can be used to detect and treat colorectal cancer and other intestinal

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Dovepress
http://dx.doi.org/10.2147/IJN.S188887

related pathologies, as depicted in Figure 1. UCNPs convert long-wavelength near infrared (NIR) excitation light into short-wavelength emission.[8] At NIR, the nonspecific absorption and scattering of light by endogenous chromophores are at minimum.[9] This leads to deeper penetration of light and high signal-to-noise ratio (SNR). Furthermore, with different modifications, UCNPs can be used to detect bacterial infection and inflammation, which appear to precede colorectal cancer.

In this review, we provide an updated summary and future prospects of UCNP-based applications in diagnostics and therapeutics for intestinal infection and colorectal cancer.

Colorectal cancer

Cancer is defined as an uncontrolled cell proliferation induced by the accumulation of genetic or epigenetic mutations. Improvement in the average standard of living and basic health care facilities has considerably reduced the incidence of communicable diseases. These advances have led to an increase in average life expectancy in almost every region of the world. Although the incidence of communicable diseases is decreased as a result of improved medical facilities, cancer mortality rates have increased by almost 40% in the last few decades. Cancer diagnoses increased by 33% between 2005 and 2015, and in 2015, over 8.7 million people globally have died due to cancer. It is projected that the number is expected to reach 13 million people by 2030.[1] Colorectal cancer is the second most common form of cancer in women and the third most common in men, and globally, it is the third most common cancer, with 1.4 million new cases diagnosed in 2012 alone.[10]

Both genetic and environmental factors play a role in the etiology of colorectal cancer. Patients affected by hereditary colorectal cancer syndrome accounts for 5%–10% of reported colorectal cancer cases. Among these, Lynch syndrome, which is caused by a mutation in the DNA mismatch-repair genes, such as *EPCAM*, *PMS2*, *MLH1*, *MLH2*, and *MLH6*, is the most common cancer syndrome.[11,12] Familial adenomatous polyposis is the second most common hereditary syndrome, which is caused by a mutation in the adenomatous polyposis coli (*APC*), a component of the Wnt-signaling pathway.[12] Furthermore, lifestyle factors such as smoking,[13] alcohol consumption,[14] and high body mass index[15] may lead to an increase risk of colorectal cancer development. To a certain extent, these lifestyle factors can explain the geographical and socioeconomic distribution of colorectal cases.[16]

In the last decade, it has become evident that the inflammatory microenvironment is essential for tumor development and progression. Hallmarks of cancer-inducing inflammation include the presence of inflammatory cells, inflammatory mediators such as cytokines and chemokines, tissue repair and remodeling, and fibrosis.[17] Inflammation is the key component of the host immune system's fight against foreign invaders such as bacteria and viruses. Acute inflammation, if resolved properly, results in the tissue repair and healing and maintenance of homeostasis. However, uncontrolled chronic inflammation may lead to malignancy. Notably, patients with early-onset IBD develop long-term chronic

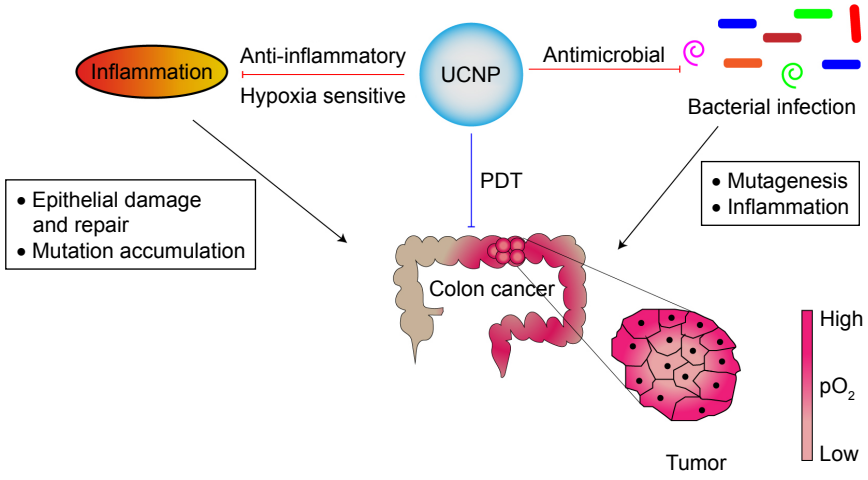

Figure 1 In vivo applications of UCNPs in intestinal infection, inflammation, and colorectal cancer.
Notes: In this figure, we depict potential (red arrows) and current (blue arrow) target areas where UCNPs can be used either for imaging or treating infection, inflammation, and colon cancer. Bacterial infection and/or inflammation leading to IBD and colorectal cancer are depicted with black arrows. UCNPs loaded with antimicrobial and anti-inflammatory compounds can be used to treat infection, inflammation, and cancer. Furthermore, hypoxia-sensitive UCNPs can be used to monitor the hypoxic state of the gastrointestinal tract. Due to the NIR excitation range, UCNPs are currently being used for localized PDT in certain cancer forms.
Abbreviations: IBD, inflammatory bowel disease; NIR, near infrared; PDT, photodynamic therapy; UCNPs, upconverting nanoparticles.

intestinal inflammation and, therefore, present an increased risk of developing colon cancer.[18]

Bacteria and colorectal cancer

The intestinal tract acts as a reservoir for various microbial species, collectively known as the intestinal microbiota.[19] In the last two decades, accumulating evidence has indicated that the gut microbiota plays a critical role in providing nutrients to the gut mucosa, in the development of the mucosal immune system, and in preventing pathogen colonization.[20] The mucosal immune system's main tasks are to mount an immune response against pathogenic microbes and to maintain tolerance against food and commensal microbial antigens. Loss of tolerance to commensal enteric microorganisms ultimately leads to uncontrolled chronic inflammation exemplified by that seen in patients with IBD. In addition, since the colon carries 10^{12} bacteria/mL, compared to 10^{2} bacteria/mL in the small intestine, the colon presents a 12-fold higher risk of developing tumors.[21] Indeed, recent findings suggest that microbes such as *F. nucleatum*,[22] enterotoxigenic *B. fragilis*, *Streptococcus bovis*, *E. coli*, and *Klebsiella pneumoniae* can play an important role in colon cancer development.[23,24] These gut-associated bacteria can increase the risk of tumor malignancy by several mechanisms including mutagenesis, secretion of mutagenetic metabolites, and the risk of promoting inflammation. Lately, a link between gut bacteria and the efficacy of anti-PD-1 immunotherapy has also been uncovered.[25–27] Collectively, these studies establish an important link between bacteria and colorectal cancer pathogenesis.

Upconverting nanoparticles

As stated previously, development of new tools to image or even treat diseases such as colon cancer are required. Among these, UCNPs have been shown to have advantageous properties compared to other available probes. UCNPs are a unique class of photoluminescent materials capable of exploiting photon upconversion (UC).[28] Two or multiple excitation photons with lower energy are converted into one emitted photon with higher energy. In general, NIR light is converted into ultraviolet (UV), visible (VIS), and anti-Stokes shifted NIR light.[29] A typical luminescent UCNP consists of an inorganic host crystal and doped Ln^{3+} ions used as sensitizers and activators, as illustrated in Figure 2A. Numerous UC mechanisms in Ln-doped crystals were recognized and formulated including excited-state absorption, energy transfer UC (ETU), photo avalanche, cooperative sensitization UC, and cross-relaxation.[30,31] Among all of these processes, ETU has the highest two-photon UC efficiency.[30] In a typical ETU process, a sensitizer ion sequentially absorbs incoming photons by donating its energy at its excited state to an activator ion. Through the sequential energy transfers

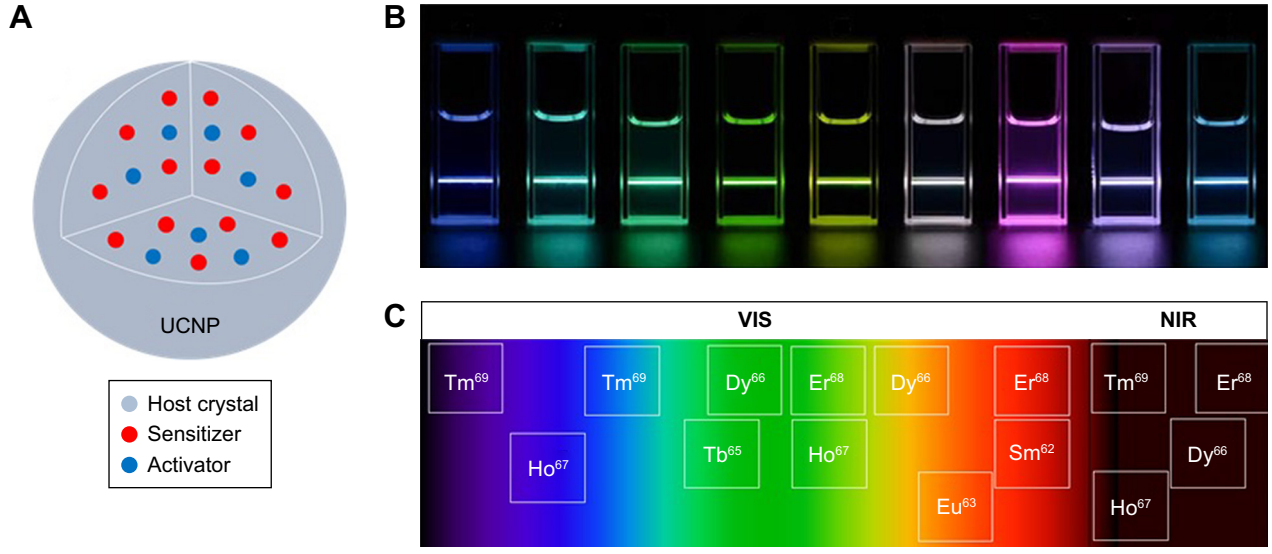

Figure 2 UCNP characteristics.

Notes: (**A**) A cross-sectional schematic representation of a typical UCNP. This includes an optically inert host crystal where optically active Ln^{3+} activator and sensitizer ions are embedded by replacing the cations of host matrix in the crystallization process. (**B**) Lanthanide-based UCNPs in colloidal solution are able to convert NIR light into different wavelengths in the VIS region of the EM. Reproduced from Zhong Y, Tian G, Gu Z, et al. Elimination of Photon Quenching by a Transition Layer to Fabricate a Quenching-Shield Sandwich Structure for 800 nm Excited Upconversion Luminescence of Nd^{3+}-Sensitized Nanoparticles. *Adv Mater*. 2013;26(18):2831–2837.[29] Publisher: John Wiley and Sons. © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (**C**) Ln^{3+} ions exhibit different emission spectral bands under NIR excitation, spanning from NIR to VIS and UV regions as each Ln^{3+} has its unique energy level structure.

Abbreviations: EM, electromagnetic; NIR, near infrared; UCNP, upconverting nanoparticle; UV, ultraviolet; VIS, visible.

from the sensitizers, the activator ion undergoes multi-step excitation, which results in UC luminescence.

To date, several different crystal compositions have been investigated as host crystals, including oxides, fluorides, vanadates, sulfides, and chlorides.[32–37] Among the reported host crystals, fluoride-based $NaREF_4$ crystals (in particular $NaYF_4$) are the most favorable host materials due to their low photon energy and high chemical stability.[38,39] Lns, a special group of elements in the periodic table, offer unique energy level structure, which are beneficial for use in UCNP. UCNPs embedded with Ln^{3+} ions produce sharp emission spectral peaks varying from UV to NIR region of electromagnetic (EM) spectrum, as shown in Figure 2B.[29]

Among all Lns, trivalent thulium (Tm^{3+}), erbium (Er^{3+}), and holmium (Ho^{3+}) are the most favorable activators used as luminescent centers due to their higher UC emission efficiency when they are doped with trivalent ytterbium (Yb^{3+}), an efficient NIR sensitizer. Another commonly used sensitizer is trivalent neodymium (Nd^{3+}) ion, which has relatively high molar extinction coefficient in the NIR region. Due to the unique energy level structure of each Ln^{3+} ion, UC emission wavelength is dependent on the type of Ln^{3+} ion used as an activator ion.[40] Typical activator Ln^{3+} ions with their observed UC emission wavelengths in the EM are shown in Figure 2C. Moreover, UC emission varies depending on the host crystal type, concentration ratio between activator and sensitizer dopants, and the crystalline phase of the nanocrystal.[40–42]

Inexpensive low power continuous wave NIR light sources are adequate to generate UC emission in Ln-based UCNPs. NIR excitation of UCNPs enables deeper tissue penetration.[43,44] Most of the intrinsic tissue biomolecules are relatively weak scatters and absorbers in the NIR region.[45] Thus, tissue autofluorescence is minimized, which yields superior sensitivity and higher SNR. Furthermore, narrow emission bandwidths of Ln^{3+} ions originating from the electronic transitions within 4f energy states have relatively long emission lifetime ranging from microseconds to milliseconds.[46] Unlike quantum dots (QDs), another class of photoluminescent materials, Ln-based UCNPs do not display photoblinking and UC emission wavelength is not susceptible to changes in particle size[47] – thus long-term continuous imaging is feasible. Moreover, Ln-based UCNPs do not suffer from photobleaching and offer high photochemical stability as compared to conventional organic fluorophores and QDs.[48]

Although UCNPs with their unique optical properties offer numerous advantages in bioimaging, an ideal Ln-based UCNP also requires various physical and chemical criteria for efficient use in biological applications. Hydrophilicity and high colloidal stability are critical functionalities. Functionalization of UCNPs surface is crucial for further interaction with various bio-entities. The introduction of functional groups such as carboxyl (–COOH), thiol (–SH), hydroxyl (–OH), maleimide (MA), amine (–NH), and amino ($–NH_2$) to the surface of UCNPs provides capabilities for covalent attachment and bio-conjugation with a variety of biomolecules including proteins, antibodies, oligonucleotides, and cell receptors.[49]

There are still some hurdles to overcome to allow a wider use of UCNPs. For example, quantum yield (QY), a parameter proportional to brightness, is relatively low, particularly at the low optical power regime that is required to avoid any photodamage to biological specimens. The UC QYs of different kinds of UCNPs are summarized deftly by Wilhelm.[50] In addition, although no or some toxicity of UCNPs has been reported in rodents,[51,52] no reports in human studies are yet available and there are still no adequate research studies to evaluate the biodistribution of UCNPs in vivo in humans.[53] In addition, nonspecific accumulation of UCNPs in reticuloendothelial system including liver and spleen is still a big hurdle in the field.[54]

Surface modification of UCNPs for biological applications

There are a number of methods to synthesize UCNPs, including co-precipitation, thermal decomposition, hydro(solvo) thermal, and microwave-assisted synthesis, all of which have been reviewed in details in various articles.[55–58] In general, hydrophobic ligands, oleylamine or oleic acid, are favored during the synthesis of UCNPs to control the crystal growth, phase, shape, and size, in order to yield high-quality monodispersed UCNPs with uniform size and shape. These hydrophobic ligands form a layer upon the surface of UCNPs, hence they become dispersible in nonpolar organic solvents such as hexane and toluene. However, UCNPs capped with hydrophobic surfactant molecules lack biocompatibility. They cannot be utilized directly in biomedical applications, as they cannot disperse in water.

Biocompatibility of UCNPs in vivo and in vitro is governed by their surface properties. Fortunately, the surface nature of Ln-based UCNPs can be changed by the following surface modification approaches. Ligand interaction, ligand oxidization, layer-by-layer deposition, and ligand exchange are the most common strategies to alter the surface characteristics of UCNPs[59] and result in the gain of hydrophilic characteristics by hydrophobic UCNPs. Moreover, as a consequence of these modifications, the surface of UCNPs

hosts functional groups, which enables conjugation with other biomolecules such as antibodies.

UCNPs functionalized with carboxylic acid group are particularly suitable for conjugating with biomolecules containing amine group through covalent linkage. Kumar et al[60] demonstrated the conjugation of mercaptopropionic acid-capped $NaYF_4$:Gd^{3+}, Yb^{3+}, Er^{3+} UCNPs with anti-claudin-4 antibody for imaging cancer cells. Similarly, the amine group present on the UCNPs' surface can bind to molecules containing the carboxylic group. Zhan et al[61] successfully targeted carcinoembryonic antigen (CEA) on the surface of HeLa cells in vitro by employing polyallylamine hydrochloride-coated mercaptosuccinic acid-capped $NaYbF_4$:Yb^{3+}, Ho^{3+} and $NaYbF_4$:Yb^{3+}, Tm^{3+} UCNPs immunolabeled with anti-CEA8 antibodies.

Furthermore, Raphaela et al reported that MA-functionalized PEG-UCNPs can conjugate with γ-globulin via thioether linkage.[62] Also, Xiong et al[63] reported the binding of MA group to amine-capped $NaYF_4$:Yb^{3+}, Er^{3+} (Tm^{3+}) UCNPs. In addition, Feng et al[64] reported a method to synthesize chitosan-capped LaF_3:Eu^{3+}, which is water soluble and contains hydroxyl and amine groups. Additionally, Li et al[65] modified the surface of hydrophobic OM-capped $NaYF_4$:Yb^{3+}, Er^{3+} by using thioglycollic acid (TGA), which results in hydrophilic UCNPs containing carboxyl and thiol groups.

Therefore, surface modifications play a crucial role to resolve biocompatibility issues arising from conventional synthesis methods of UCNPs. As a consequence of appropriate modifications, UCNPs can gain new surface properties, such as hydrophilicity, biocompatibility, colloidal stability, and bio-targeting, which are crucial for UCNPs to be more extensively used in biological applications.

Optical imaging (OI)

Molecular imaging plays a significant role in medicine, providing essential diagnostic and prognostic information of a disease by the visualization and characterization of biological processes at cellular and/or subcellular levels. The benefit of molecular imaging techniques is the evaluation of molecular changes rather than morphological abnormalities.

OI is a rapidly emerging molecular imaging method where light–tissue interactions such as absorption, scattering, and fluorescence are investigated. Due to the cost-effectiveness and simplicity, FI has been a method of choice for biological researchers. This technique relies on the endogenous or exogenous fluorescent characteristics of biomolecules. A fluorescent biomolecule is able to absorb excitation light at different wavelengths, with varying strengths, and re-emit light at a longer wavelength than that of the excitation light, which is known as fluorescence emission. The biomolecules with this ability are considered as promising molecular contrast agents for in vivo and in vitro applications. However, endogenous contrast agents generally exhibit inadequate spectroscopic characteristics. Therefore, exogenous fluorescent contrast agents including organic dyes (eg, indocyanine green [ICG], fluorescein sodium, and methylene blue), fluorescent proteins (eg, green fluorescent protein), and QDs (eg, $CdSe_2$, Ag_2, and $CuInS_2$) are externally administered to enhance image contrast. Despite the remarkable optical properties of the exogenous fluorescent contrast agents, their use in molecular imaging is limited. This is due to several reasons. First, they suffer from relatively poor spatial resolution due to the overlap of excitation and fluorescence emission spectra of major fluorescent tissue components (eg, nicotinamide adenine dinucleotide, collagen, elastin, and flavins), ie, tissue autofluorescence. Second, exogenous fluorescent contrast agents generally require UV or VIS light excitation, which limits the light penetration depth due to a relatively high absorption and scattering nature of bio-entities inside the tissue. Hence, deep tissue imaging with high sensitivity becomes difficult. While employing QDs, cytotoxicity appears a major challenge due to the presence of toxic heavy metals commonly used including cadmium, silver, and copper. They also exhibit photoblinking effects upon continuous irradiation, which hinders long-term imaging. Moreover, organic dyes and fluorescent proteins suffer from photobleaching effect; the fluorescence emission fades after relatively short time as the molecule starts to degrade under continuous irradiation, which also impedes long-term imaging. To date, among all reported exogenous fluorescent contrast agents, only two NIR fluorescent molecules, ICG and IRDye800CW conjugate, are approved by Food and Drug Administration (FDA).[66] Noura et al showed the feasibility of a lateral region sentinel node biopsy of lower colorectal rectal cancer guided by ICG.[67] Additionally, Gong et al[68] used IRDye800CW to image the tumors in colorectal cancer.

Considering the aforementioned issues associated with the existing fluorescent contrast agents, Ln-based UCNPs with their previously stated remarkable optical features can be considered as promising photoluminescent contrast markers. Ln-based UCNPs with their NIR excitation ability can avail of optical transparency window, thus maximizing high light penetration depths. In addition, their photon UC ability gives them anti-Stokes shifted characteristics, which minimizes tissue autofluorescence. As they show high photochemical stability, they do not create photoblinking and photobleaching effects under long-term continuous irradiance.

In the next sections, we will be focusing on UCNPs' applications of bacterial, anti-microbial detection as well as tumor imaging.

Bacterial detection using UCNPs

The well-known bacterial detection techniques, culture-based or DNA-based using PCR, present both benefits and deficiencies. Various drawbacks in these methodologies, which make them less suitable for on-site diagnosis, include enrichment steps and inability to simultaneously detect two or more bacteria or amplification steps, which makes process more time-consuming.[69] Recently, bacterial detection using UCNPs (Table 1 and Figure 3A) has been shown to be successful by improving the sensitivity and the speed of the detection process.

Ong et al reported the first in vitro study showing that UCNPs coupled with an anti-*E. coli* antibody successfully detected *E. coli* as a consequence of superior photostability of the UCNP-labeled bacteria as compared to the GFP-expressing bacteria.[70] Moreover, using a dendritic cell and bacteria co-culture infection system, the potential of UCNPs' imaging for long-term bacterial trafficking was also demonstrated. Additionally, Pan et al[71] were able to reduce the detection level of *E. coli* as low as 10 CFU/mL, when using UCNPs functionalized with an anti-*E. coli* antibody. In an in vitro study, Wu et al,[69] improved the existing technology using multicolor UCNPs, doped in various rare-earth metals to obtain different emission peaks, coupled with bacteria-specific aptamers, and confirmed simultaneous detection of three different bacterial strains, such as *Staphylococcus aureus*, *Vibrio parahemolyticus*, and *Salmonella typhimurium*. The authors also tested if their UCNPs' conjugates were able to detect the three bacteria in real food samples, providing evidence for the potential use of UCNPs in bioassays for food safety. Dai et al[72] also measured the level of ochratoxin, a group of mutagenic and teratogenic compounds produced by *Aspergillus* and *Penicillium* fungal species, in beer samples by using UCNPs–aptamer complexes. More recently, the periodontal pathogen *Porphyromonas gingivalis* was also detected by UCNPs and, similar to *E. coli* studies, the *P. gingivalis* detection limit was 10 CFU/mL.[73] Furthermore, Cheng et al designed a luminescence energy transfer system, where anti-*Salmonella* aptamer-conjugated UCNPs served as energy donor and gold nanorods served as acceptor allowing the detection of *Salmonella* in an aqueous buffer with a detection limit of 11 CFU/mL. In the absence of *Salmonella*, the electrostatic interaction between the negatively charged UCNPs and the positively charged gold particles shorten the distances between these two, leading to luminescence quenching. However, addition of *Salmonella* into the system

Table 1 List of published studies where UCNPs were used to detect bacteria

Type of UCNPs' system used	UCNPs' conjugation	Bacteria/bacterial metabolite detected	Advantage	Reference
Multicolor UCNPs (NaYF4:Yb, Tm NaYF4:Yb, Ho NaYF4:Yb, Er/Mn)	Antibacteria aptamer	*S. aureus*, *Vibrio parahemolyticus*, and *S. typhimurium*	High specificity, simultaneous detection	Wu et al[69]
NaYF4:Yb, Er	Anti-*E. coli* antibody	*E. coli*	High photostability	Ong et al[70]
NaYF4:Yb, Er	Anti-*E. coli* antibody	*E. coli*	Low detection limit – 10 CFU/mL	Pan et al[71]
NaYF4:Yb0.2, $Tm_{0.02}$	Anti-ochratoxin A aptamer	Ochratoxin A	High sensitivity	Dai et al[72]
NaYF4:Tm@SiO_2, NaYF4:Er@SiO_2	Anti-*P. gingivalis* antibody	*P. gingivalis*	Low detection limit – 10 CFU/mL	Qin et al[73]
LET, Mn^{2+}-doped NaYF4:Yb, Tm as donor and Au nanorod as acceptor	Anti-*Salmonella* aptamer	*S. typhimurium*	Low detection limit – 11 CFU/mL	Cheng et al[74]
FRET, Au NP as donor and NaYF4:Yb/Er as acceptor	Antiaptamer cDNA	*E. coli*, *B. subtilis*, and *S. aureus*	Detect *E. coli* in real food and water samples	Jin et al[75]
Optical trapping, KLu2F7:Yb^{3+}, Er^{3+}	None	*E. coli*	Single bacteria detection	Xin et al[76]
Bio-microlens to enhance the upconversion fluorescence of NaYF4:Yb^{3+}/Tm^{3+}	None	*E. coli*	Single bacteria detection	Li et al[77]

Abbreviations: *B. subtilis*, *Bacillus subtilis*; *E. coli*, *Escherichia coli*; FRET, fluorescence resonance energy transfer; LET, luminescence energy transfer; NP, nanoparticle; *P. gingivalis*, *Porphyromonas gingivalis*; *S. aureus*, *Staphylococcus aureus*; *S. typhimurium*, *Salmonella typhimurium*; UCNPs, upconverting nanoparticles.

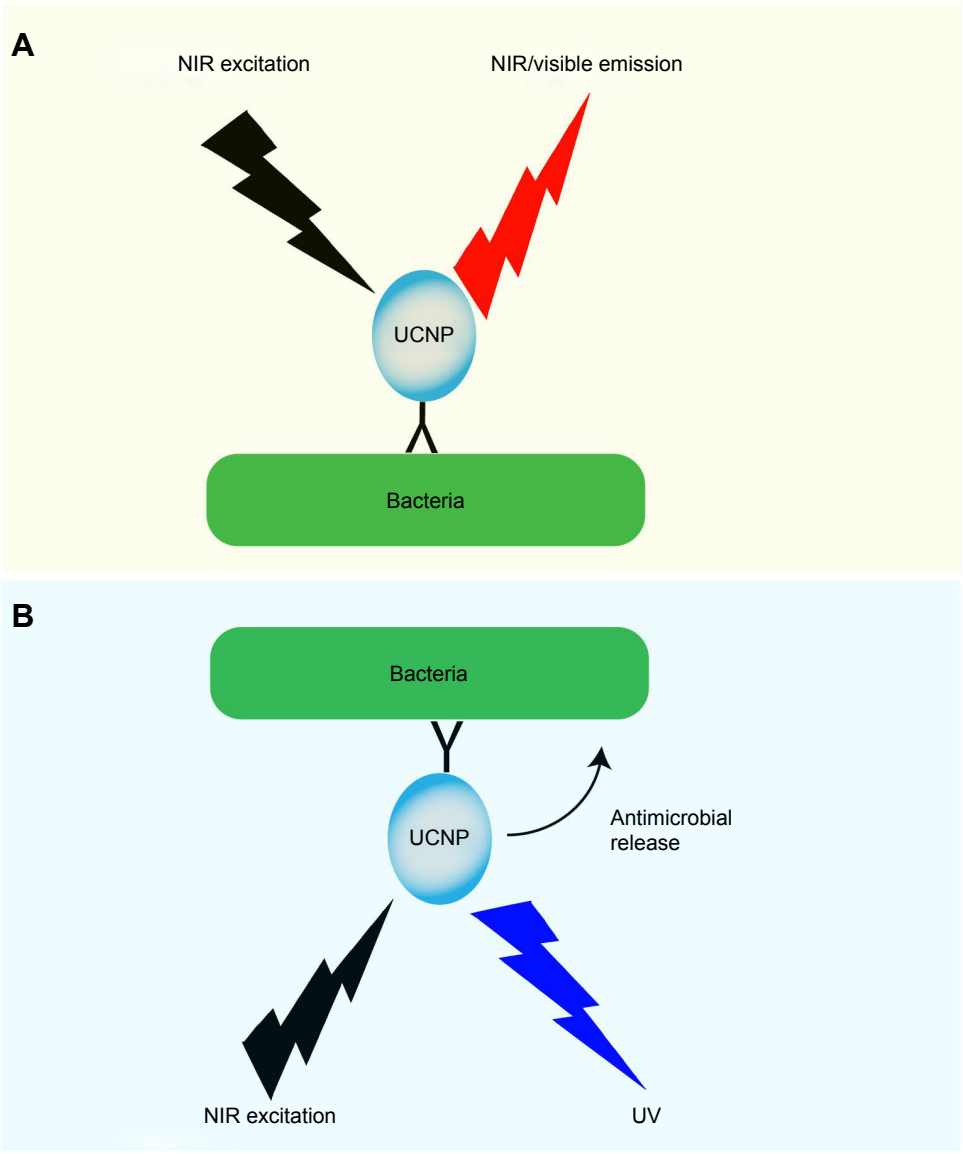

Figure 3 Mode of action of UCNPs in visualizing and killing bacteria.
Notes: In this figure, we summarize published work on UCNPs' role in visualizing and killing bacteria. (**A**) Functionalized UCNPs targeting bacteria with NIR/VIS emission range is used to image the bacteria. (**B**) UCNPs loaded with antimicrobial compounds or with an UV emission range are used for localized antimicrobial activity.
Abbreviations: NIR, near infrared; UCNPs, upconverting nanoparticles; UV, ultraviolet; VIS, visible.

restores the luminescence by increasing the distance between the donor and the acceptor due to the binding of *Salmonella* to the UCNPs through anti-*Salmonella* aptamer.[74] Jin et al[75] also developed a similar fluorescence resonance energy transfer (FRET) system by using gold nanoparticle aptamers as the donor and UCNPs coupled with cDNA as the acceptor. Recently, Xin et al[76] and Li et al[77] reported that a single bacteria resolution was achieved by enhancing the UC fluorescence of UCNPs using the trapped optical fiber on yeast and human cells, respectively, as form of a microlens. These collective data demonstrate that UCNPs can be used as a luminescent probe to detect bacterial signal as low as 1 CFU, which can be of relevance for a number of different areas including diagnostics and food industry.

Antimicrobial applications of UCNPs

As illustrated in Figure 3, the use of UCNPs has not only been limited to bacterial detection. For example, NIR-triggered release of the antibiofilm agent D-amino acid and other bactericides was used to eradicate bacteria and biofilms.[78] UCNPs coated with a thin layer of TiO_2, with an emission frequency in the UV region, is linked to D-amino acid through a UV cleavable linkage. Upon excitation at NIR, UCNPs emit photons in UV range, which subsequently lead

Table 2 Antimicrobial actions of UCNPs

Type of UCNPs' system used	Antimicrobial	Target bacterium	Reference
β-NaYF4:Tm^{3+} coated with TiO_2	D-amino acid (D-tyrosine) and ROS	Individual *B. subtilis* and biofilm	Wei et al[78]
β-NaYF4:Yb, Tm	PFVCN	*E. coli*	Li et al[79]
Oleic acid capped β-NaYF4:23%Yb, 2%Er@NaYF4 zinc phthalocyanine as photosensitizer	OC and ROS inducing zinc phthalocyanine	Multidrug-resistant *S. aureus* and β-*lactamase*-producing *E. coli*	Li et al[80]

Abbreviations: *B. subtilis*, *Bacillus subtilis*; *E. coli*, *Escherichia coli*; OC, *N*-octyl chitosan; PFVCN, poly(9,9-bis[6,6-{*N*,*N*,*N*-trimethylaminium}-fluorene]-2,7-ylenevinylene)-co-alt-2,5-dicyano-1,4-phenylene]; *S. aureus*, *Staphylococcus aureus*; UCNPs, upconverting nanoparticles.

to the controlled release of ROS from TiO_2 and D-amino acid. Furthermore, Li et al[79] developed a FRET system using UCNPs as energy donors and poly[9,9-bis(6,6-(*N*,*N*,*N*-trimethylaminium)-fluorene-2,7-ylenevinylene)-co-alt-2,5-dicyano-1,4-phenylene] (PFVCN), a conjugate polymer (CP), as energy acceptor. CPs are organic polymers that have emerged as alternative antibiotics and therefore can led to the generation of ROS by absorbing UV or VIS light. However, UV light in itself can damage the cells and VIS light is too weak to penetrate deeper into the tissue. To overcome these limitations, the authors used the UCNPs/PFVCN FRET system. In this system, UCNPs convert the NIR light into localized UV light, which is subsequently absorbed by PFVCN and generates ROS. In addition, to kill multidrug-resistant *S. aureus* and β-lactamase-producing *E. coli*, a photodynamic system, where UCNPs were coated with *N*-octyl chitosan loaded with ROS producing zinc phthalocyanine as photosentsitizer, was developed.[80] Collectively, these reports highlight further the use for UCNPs as a light-triggered antimicrobial agent (Table 2 and Figure 3B), which could potentially replace conventional antimicrobial drugs.[81]

Detection and treatment of colon cancer by UCNPs

Due to the low fluorescence background in the NIR region, NIR fluorescent nanoparticles and dyes in conjugation with tumor-targeting ligands have been preferentially used for in vivo imaging of colorectal cancer (Table 3). However, commonly encountered issues with dyes or other luminescent materials include low penetration depth, photobleaching, autofluorescence, and high background signal. To overcome these limitations, tumor-targeting UCNPs have been developed (Table 3). The feasibility of UCNPs to act as a contrasting agent was initially reported by Liu et al[82] who successfully imaged the mouse gastrointestinal tract using UCNPs. Next, tumor-targeting UCNPs were developed with the aim to detect primary gastric tumors and lymphatic metastasis using an orthotopic tumor model.[83] Biocompatible tumor targeting UCNPs were used to detect primary colorectal cancer in Kunming mice.[84] To date, UCNPs are commonly administered intravenously for colorectal imaging in mice. However, a study by Gao et al[85] comparing biodistribution and accumulation of UCNPs in cancerous and normal tissues demonstrated that intraperitoneal administration of UCNPs in a colorectal cancer model appeared to be a superior route when compared with intravenous administration.

Recently, UCNPs loaded with an immune adjuvant, a toll-like receptor-7 agonist were used with the FDA-approved check-point inhibitor anti-cytotoxic T lymphocyte-associated protein 4 antibody to treat a primary tumor in an animal model of colorectal cancer.[86] NIR exposure triggers photodynamic destruction of the primary tumor. Subsequently to photodynamic therapy (PDT), the cancer-associated antigens released from the cancerous tissue induce an anticancer immune response with the help of the immune adjuvant. The antitumor response is further potentiated by the check-point inhibitor.

The importance of the tumor microenvironment has been recently recognized as an attractive target for cancer treatment. Hypoxia, low pH, extracellular matrix, the tumor vascular system, immune cells, and tumor-associated fibroblasts collectively form the tumor microenvironment. UCNPs can be used to target the tumor microenvironment for therapeutic purposes. Indeed, a recent in vitro study reported a targeted killing of tumor cells by affecting specific tumor markers such as EGF by Ln-UCNPs.[87] Overall, these findings identify a potential use of UCNPs in colorectal cancer diagnosis and treatment.

Conclusion and future perspectives

In vitro and preclinical studies to date indicate that, for colorectal cancer, UCNPs are an enhanced and better imaging strategy as compared with luminescent or fluorescence probes. Additional analyses have demonstrated that UCNPs can indeed target-specific bacteria strains and even exert antimicrobial actions upon NIR triggering, suggesting their

Table 3 Colorectal cancer detection and treatment using UCNPs

Type of UCNPs	UCNPs' conjugation	Ligand specificity	Tumor model	Route of UCNPs' administration	Type of tumor detected	Anticancer property used	Reference
NaGdF4:Yb, Er@NaGdF4 coated with PEG	FA	Folate-receptor overexpressing melanoma cells	Chemically induced primary colorectal cancer model	Intravenous injection	Primary colorectal cancer	None	Liu et al[84]
NaLuF4:Yb, Tm@NaLuF4 nanocrystals modified with citrates	None	Passive targeting	Kunming mice injected with human colorectal cancer LOVO cell line	Intraperitoneal and intravenous injected	Colorectal tumor	None	Gao et al[85]
PEGylated NaYF4 (Y/Yb/Er=78:20:2)	Ce6, a photosensitizer, and imiquimod (R837), a toll-like-receptor-7 agonist and an anti-CTLA-4, checkpoint inhibitor	None	BALB/c mice, subcutaneously injected CT26 cells	Intratumor injection	Colorectal tumor	PDT with checkpoint inhibitor	Xu et al[86]

Abbreviations: Ce6, Chlorin e6; FA, folic acid; PDT, photodynamic therapy; PEG, polyethylene glycol.

utilities as new potential treatment strategies in colorectal cancer and other intestinal disorders.

The healthy gut maintains a state of hypoxia on and around the mucosal surface of the colon. However, in a chronically inflamed intestine (which often precedes a colorectal cancer finding in IBD patients), the concentration of oxygen on the colonic surface increases due to an increase in blood flow around the inflamed tissue. By utilizing a hypoxia-sensitive UCNPs' imaging, the inflammatory state of the gut could be imaged with minimal invasiveness (Figure 1). Earlier identification of individuals predisposed to develop colorectal cancer or intestinal inflammation (eg, IBD) may result in better clinical outcomes.

Although several studies have demonstrated the involvement of microbes in IBD and cancer progression, the mechanistic insights into how these bacteria actually lead to these conditions or their potential role in relapse of disease are yet to be discovered. UCNPs can therefore be used to visualize host–microbe interactions at the cellular and molecular levels to establish a cause and effect relationship for these chronic conditions.

Photodynamic therapy involves the generation of ROS resulting from the interaction of photosensitizer and VIS light. However, VIS light is too weak to penetrate deep into the tissue. Also, ROS production is limited due to the hypoxic environment of tumor and the colon tissue. Furthermore, ROS production consumes most of the oxygen available to an induced hypoxic environment in the tissue, which further potentiates tumorigenesis. Talaporifin sodium (TS), a light-activated drug/photosensitizer, has been approved in Japan for the treatment of early-state endobronchial cancer.[88] Activation of TS with a 664 nm VIS range light generates a single oxygen species, resulting in the induction of apoptotic cell death.[88] In a Phase II trial of TS in patients with colorectal cancer and metastasis to the liver, the efficacy of the treatment depended on the number of excitation sources used to activate the drug.[89] This study shows that the treatment efficacy depends on the penetration of cancer tissue by the excitation light with enough photons to activate the photosensitizer. UCNPs with emission at UV range could be used to overcome these limitations associated with VIS light and ROS production. Deep penetrating NIR light can be used to excite the UCNPs, and the localized emission of UV can be used to kill the surrounding carcinogenic cells.

However, even though NIR is nontoxic for biological tissues, high intensity NIR can indeed overheat cells and result in photodamage[90] of not only cancerous cells but also healthy and noncancerous cells. A way to reduce these issues is using

800 nm excited Nd^{3+}-based UCNPs, which leave cells intact due to less absorption of water[90] or by lowering the laser power.[91] In summary, we are confident that future studies will resolve the gap between the bench and clinical translation, resulting in the beneficial use of UCNPs in humans in several biological applications including diagnostics and treatment purposes.

Acknowledgment

The authors thank Dr Amanda Lohan for proofreading the article.

Disclosure

Professor Stefan Andersson-Engels, Mr Raminder Singh, and Mr Gokhan Dumlupinar receive financial support from Science Foundation Ireland (SFI) (Research Professor Award), grant number SFI/15/RP/2828, and the APC Microbiome Ireland, and Dr Silvia Melgar receives financial support from SFI, grant number SFI/12/RC/2273. The authors report no other conflicts of interest in this work.

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