REVIEW PAPER

Nanotechnology serves the advancement of microbiology: Diagnostic and therapeutic advantages

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ABSTRACT
Today, the nanotechnology science is wide and it has entered nearly all scientific fields. One of the most important applications of nanotechnology is in medicine, especially in diagnosis of microbes (such as bacteria, fungi and viruses) and treatment of the infections caused by them. Yearly, the microbial infections lead to many medical problems such as increased duration of treatment, increased expenses of treatment, drag resistances, and even increased mortality. Since many years ago, antibiotics have been used in treatment of various infections, but unfortunately, their side effects as well as the resistance to them have always been the key problems. Thus, selection of the most suitable detection methods as well as the most appropriate treatment options for various microbial infections may reduce the crisis. It seems that nanotechnology approaches may efficiently help to diagnose many different microbial infections as well as to combat them, particularly the resistant ones, with no side effects currently caused by the traditional antibiotics.

Keywords: *Diagnosis; Microbiology; Nanotechnology; Treatment*

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INTRODUCTION
The origin of "nano" is from Greek word "nanos" that means "a dwarf". Generally, nanotechnology is used for tiny things smaller than 100 nanometers scale (nm) and for the first time was used by Norio Taniguchi in 1974, but formerly, Richard Zsigmondy used "nanometer" in a lecture in 1925. in 1959, the famous Richard Feynman gave a lecture entitled, "There's Plenty of Room at the Bottom" at an American Physical Society meeting. He might be considered as the father of modern nanotechnology [1, 2].

Today, the field of nanotechnology is wide and one can say that, nano has entered all scientific fields for example, electronics, mechanical engineering, chemistry, biomedicine, agriculture, and even clothing production [3-8]. One of the most important uses of nanotechnology is in medicine [9]. Yearly, microbial infections lead to many medical problems such as increased duration of treatment, increased expenses of treatment, drag resistances, and even increased mortality [10]. One of the key roles of nanotechnology is its application in microbiology. So, the role of nanotechnology is mainly in diagnosis of microbes and treatment of the infections caused by them. Fortunately, there are several ways to apply nanotechnology in diagnosis and treatment of the infections caused by various microbes (bacteria, viruses, fungi, …) that recently are used. The widespread use of nanotechnology in microbiology has led to many advantages including quick and cheap diagnosis that is very economical as well as the timely and efficient treatment that could reduce the course of the treatment. Moreover, the use of nanotechnology in microbiology has led to find out new methods involved in diagnosis and treatment of infectious diseases [11, 12].

Recently, nanotechnology is used in treatment and diagnosis of different gram negative and gram positive bacteria such as: *Bacillus subtilis*, *Escherichia coli*, *Nocardiopsis spp.*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Salmonella* Typhi, *Proteus mirabilis*, *Vibrio cholerae,* and methicillin-resistant *Staphylococcus*

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aureus [13-15].

Likewise, nanotechnology is useful in virology and it is used in diagnosis and treatment of infections caused by different viruses such as: Human Immunodeficiency Virus (HIV), Influenza virus, Ebola virus, Zika virus, as well as SARS-CoV-2 [16, 17].

Nanotechnology has also entered the field of mycology and has been used in diagnosis and treatment of various fungal infections caused by pathogens such as: *Candida albicans*, *Cryptococcus*, *Pneumocystis,* and *Blastomyces* [18, 19].

The purpose of this paper was to consider, review, and assess the studies applied nanotechnology in the diagnosis and treatment of various infections caused by different pathogens.

Nanotechnology and bacteria

Nanotechnology and Bacillus subtilis

Bacillus subtilis is an aerobic gram positive rod shaped bacterium capable to form spores that is found in soil. [20]. Various strains of *Bacillus* genus (especially *Bacillus subtilis* strains) are able to produce a cyclic lipopeptide biosurfactant called “surfactin” which has a cytotoxic effect on tumor types such as breast and colon cancers, leukemia, and hepatoma. Surfactin has an amphiphilic nature and this can be caused its easy incorporation into nano-formulations. Interestingly, surfactin has antibacterial, antifungal, antimycoplasmal, antiviral, and anti-inflammatory activities. Moreover, the advantages of its some nano-formulations (including surfactin in polymeric nanoparticles and nanofibers, surfactin in polymeric micelles, surfactin in microemulsions, and surfactin in liposomes), as well as nano-carrier of surfactin have so far been evidenced [21-24].

In the recent decade, nanotechnology has mainly been applied to solve medicine problems. One of these applications is vaccine production by using nanoparticles, which is much more effective than conventional vaccines. Successful trials have indicated that spore nanoparticles of bacteria such as *Bacillus subtilis* may be used as vaccine which could stimulate the immune system [25-27].

Nanotechnology and Escherichia coli (E. coli)

E. coli is a gram negative and non-spore-forming bacterium found everywhere such as intestinal tract of human and other mammals. *E. coli* has many spices that cause various infections such as urinary tract infection (UTI), cholecystitis, cholangitis, bacteremia, traveler’s diarrhea as well as neonatal meningitis and pneumonia. Important spices of *E. coli* include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E.coli* (ETEC), enteroaggregative *E.coli* (EAEC), enterohaemorrhagic *E.coli* (EHEC), enteroinvasive *E.coli* (EIEC), and diffusely adherent *E.coli* (DAEC) [28-30].

Because of the importance of *E. coli* infections, diagnosis and treatment of these infections are very essential. In a study, antimicrobial activity of silver nanoparticles against *E. coli* strains has been shown [31]. Elsewhere, silver-coated Ni0.5Zn0.5Fe2O4 magnetic nanoparticles have been used against *E. coli* by researchers [32]. Moreover, in a study, nanopenicillin (the transformation of penicillin G into nano/micro-sized spheres) has been proven to showantibacterial effect on gram negative bacteria such as *E. coli* while in common, penicillin mainly affects gram positive bacteria. Fortunately, nanopenicillin has not any adverse effects on human cells in antibacterial-effective concentrations [33]. Additionally, some studies have used nanotechnology for detection of *E. coli* strains. In one of them, anti-*E. coli* antibody-bound gold nanowire arrays were used for detection of *E. coli 0157:H7* [34, 35].

Nanotechnology and Nocardiopsis spp.

The species of the genus *Nocardiopsis* are found in different ecological sites. They are aerobic, Gram-positive, catalase-positive and non-acid-fast actinomycetes. *Nocardiopsis dassonvillei* and *Nocardiopsis synnemataformans* are very important in human opportunistic infections. Important bioactive compounds such as antimicrobial agents, tumor inducers, anticancer substances, toxins and immunomodulators can be produced by *Nocardiopsis spp.* [36, 37].

Many studies have been performed about *Nocardiopsis spp.* and their functions in nanotechnology. In a study, the researchers showed that gold bionanoparticles by *Nocardiopsis sp.* MBRC-48 have different properties such as antimicrobial (against pathogenic microorganisms), antioxidant, and cytotoxic functions [38]. In another study, the supernatant of *Nocardiopsis sp.* MBRC-1 has been used in biosynthesis of silver nanoparticles (AgNPs). These researchers reported the strong antimicrobial activity of AgNPs against bacteria and fungi. Similarly, the cytotoxic effect of biosynthesized

AgNPs against *in vitro* human cervical cancer cell line (HeLa) has been evidenced [39]. Moreover, the bactericidal effect of silver nanoparticles of novel marine *Nocardiopsis dassonvillei*-DS013 on some clinical isolates has been revealed [40].

Nanotechnology and Acinetobacter baumannii

Acinetobacter baumannii is a gram-negative, rod-shaped, aerobic, and opportunistic bacterium. The emergence of multidrug-resistant (MDR) pathogens is one of the important problems in medicine and *Acinetobacter baumannii* has a key role in this subject. This pathogen can cause some serious nosocomial and community-acquired infections especially among immunocompromised individuals and it has been common case in Intensive Care Units. Also, some fatal meningitis and pneumonia are caused by *Acinetobacter baumannii* strains [41-43].

Nanotechnology has been implicated in treatment of resistant strains of *Acinetobacter baumannii*. For example, in a recent study, researchers have used the bacteriagenic silver nanoparticles (AgNPs) to eradicate *Acinetobacter baumannii* AIIMS 7 in planktonic and biofilm form and showed the synergistic interaction of AgNPs with doxycycline, tetracycline, and erythromycin to eliminate *Acinetobacter baumannii* AIIMS 7 [44, 45]. Similarly, in the other studies the effect of silver nanoparticles on resistant strains of *Acinetobacter baumannii* has been evaluated and proven [46-48]. Furthermore, nanotechnology has been involved in diagnosis of *Acinetobacter baumannii* strains in some surveys [49, 50]

Nanotechnology and Klebsiella pneumoniae

For the first time, *Klebsiella pneumoniae* is described by Carl Friedlander in 1882. *Klebsiella pneumoniae* is a gram-negative, non-motile, encapsulate, and opportunistic bacterium that belongs to *Enterobacteriaceae* family. Some infections such as nosocomial and community-acquired pneumonia as well as bacteremia can be caused by *Klebsiella pneumoniae*. Importantly, bacteremia has a high mortality rate in neonates. Furthermore, *Klebsiella pneumoniae* isolates have become resistant to different antibiotics and the treatment of their infections has become difficult [51-53].

Many studies have used different bacteria to produce some nanoparticles for example, gold nanoparticles may be produced by *Klebsiella pneumoniae* [54].

Carbapenem-resistant *Klebsiella pneumoniae* (CRKPN) are very resistant and can cause various important infections. Interestingly, in a study, CRKPN has been used against itself. In this method, the outer membrane vesicles from CRKP in addition to nanoparticles were used as a vaccine to produce CRKPN-specific antibody titers [55].

In the other studies, many researchers have recorded the positive effects of silver nanoparticles on the resistant strains of *Klebsiella pneumoniae* [56-59].

Nanotechnology and Pseudomonas aeruginosa

Pseudomonas aeruginosa is a Gram-negative, rod shaped, aerobic, and opportunistic bacterium. Today, *Pseudomonas aeruginosa* has become one of the important health-threatening bacteria in human. Unfortunately, increasing of antibiotic resistant strains of *Pseudomonas aeruginosa* has been a major problem. This pathogen can cause serious diseases such as urinary tract infections, bacteremia, pneumonia, and skin soft tissue infections especially in immunocompromised individuals. Additionally, much of morbidity and mortality can be caused following nosocomial infections by *Pseudomonas aeruginosa* in cystic fibrosis (CF) patients [60-62].

Recently, some strategies have been utilized to combat *Pseudomonas aeruginosa* by exploiting nanotechnology. In one protocol, the effect of silver nanoparticles against *Pseudomonas aeruginosa* was evaluated and the good results of antibacterial activity of silver nanoparticles in elimination of resistant *Pseudomonas aeruginosa* was reported [63, 64].

One of the important problems encountered in *Pseudomonas aeruginosa* infections, is biofilm formation and the destruction of this structure is very important in management of the treatment. In one approach, a study has shown that the application of nanotechnology may help to prevent the formation of biofilms by *Pseudomonas aeruginosa* [65]. The role of nanoparticles in inhibition of biofilm formation by multidrug-resistant *Pseudomonas aeruginosa* has also been indicated in the other studies [44, 66].

According to the importance of *Pseudomonas aeruginosa* in medicine, the fast and accurate diagnosis of this bacterium in clinical specimens is significant. In this regard, many studies have reported positive results about diagnosis of

Pseudomonas aeruginosa using nanotechnology approaches [67-71].

Nanotechnology and Mycobacterium tuberculosis

Mycobacterium tuberculosis can cause tuberculosis that is still one of the scariest diseases with thousands people die in a year, worldwide. After entrance to the body, *Mycobacterium tuberculosis* stays latent until create tuberculosis. Long detection process and increasing rates of antibiotic resistance in *Mycobacterium tuberculosis* strains have cause difficulties in management of the disease. As a result, fast detection and subsequently, an appropriate and timely treatment of *Mycobacterium tuberculosis* infection can reduce tuberculosis dies and the burden of the diseas [72-76].

Nanotechnology may help in rapid diagnosis and treatment of tuberculosis. In a review, Nasiruddin *et al*, have shown various nanotechnology-based approaches in treatment of tuberculosis [77]. In another study, nanotechnology methods has been applied in detection of mycobacterial strains; moreover, nanotechnology-based drug delivery systems used for effective treatment of mycobacterial infections [78]. The other studies have also revealed the high value of nanotechnology methods in diagnosis and treatment of tuberculosis [79-82].

Nanotechnology and salmonella typhi

Salmonella enterica serotype Typhi is a gram-negative, motile bacterium belonged to *Enterobacteriaceae* family. Importantly, this bacterium can cause typhoid fever disease for which human is the only reservoir. *Salmonella enterica* was first identified by Karl Eberth in 1880 and it was cultured by Georg Gaffky in 1884. Yearly, typhoid fever has affected many people and caused thousands of death especially in children and young adults. However, one of the important problems regarding typhoid fever is carrier patients that can transfer the infection to others [83, 84].

Maybe, quick and on time identification of typhoid fever and its carrier state could reduce the rate of mortality and infection transfer, respectively. Fortunately, nanotechnology has recently been used to fact and accurate both treatment and identification of *Salmonella enterica* serotype Typhi. In many studies, nanoparticles (such as nanogold) and nanobiosensors have been used in diagnosis of *Salmonella enterica* serotype Typhi. Moreover, in some studies researchers have benefited from nanotechnology (for example, using nanosilver and nano antibiotic) in treatment of the infections caused by *Salmonella spp.* specially serotype Typhi [85-93].

Nanotechnology and Proteus mirabilis

Proteus species belong to *Enterobacteriaceae* and are found in water, soil and human intestine (as normal microbiota). *Proteus mirabilis* is a gram-negative, rod-shaped, and motile bacterium (having swarming motility) with peritrichous flagella involved in motility as well as biofilm formation. Using different virulence factors, *Proteus mirabilis* can cause catheter-associated urinary tract infections and bacteremia. Therefore, fast and accurate treatment of the infections caused by this bacterium is necessary [94-96].

As mentioned above, nanotechnology has recently been used to diagnose and treat the infections caused by *Proteus mirabilis*. For example, Parveen *et al*. could evaluate the antibacterial activity of silver nanoparticles on *Proteus mirabilis* in 2018 [97]. Similarly, other researchers have shown the antibacterial and antibiofilm activity of some nanoparticles such as TiO2, selenium, and so on [96, 98-101].

Nanotechnology and vibrio cholerae

Vibrio cholerae is a gram-negative, highly motile, comma-shaped pathogen. Human is the natural host of this bacterium. Cholerae disease is caused by this bacterium and it is a watery diarrhea transmitted by fecal-oral route through contaminated water. So far, *Vibrio cholerae* has caused several pandemics and killed many people. Unfortunately, this deadly bacterium can cause more than 95 thousand deaths yearly [102, 103].

Today, the use of new methods is essential to diagnose and treat the infectious diseases. Conventional methods used in determination of *Vibrio cholerae* are costly and time-consuming. Hence, in a study Herfehdoost *et al*. have developed a new method for detection of *Vibrio cholerae* based on nanotechnology using polymerase chain reaction (PCR) and recorded valuable results [104]. Some researchers believe that the sole antibiotic therapy is not drastic in treatment of diarrhea caused by *Vibrio cholerae* and using of neutralization methods to neutralize the *Vibrio cholerae* toxin is necessary. In this regard, they have used GM1-polymer hybrid nanoparticles to neutralize the *Vibrio cholerae* toxin [105]. The other studies have also indicated the using of nanotechnology-based methods in detection of *Vibrio cholerae* [106, 107]. The positive effects of some nanoparticles has also been evidenced in other papers [108, 109].

Nanotechnology and methicillin-resistant staphylococcus aureus (MRSA)

Staphylococcus aureus is a Gram-positive bacterium capable to colonize the human, naturally. When *Staphylococcus aureus* receives the resistance gene (*mecA*), it is changed to Methicillin-Resistant *Staphylococcus aureus*. Unfortunately, MRSAs are resistant to many antibiotics and could create different nosocomial infections especially in people with predisposing conditions, such as burns, surgical wound infections, pneumonia, mastitis, infections of skin, osteomyelitis, endocarditis and bacteremia [110, 111].

Today, MRSA is one of the important challenges in medicine, therefore, the fast diagnosis and treatment of their infections are very important. Fortunately, in many studies the proper effects of functional nanomaterials and nanoparticles have been documented against MRSA strains [112-116]. Interestingly, in the study of Balcucho *et al*, polycaprolactone and copper oxide nanoparticles have been used as wound dressings against MRSA [117]. Moreover, Gill *et al*. have recently denoted the nanomaterial-based optical and electrochemical techniques for detection of MRSA [118]. In the other studies both nanoparticles and nanostructures in conjunction with natural or synthetic receptors have been used as sensitive and/or specific tools for detection of MRSA, as well [119].

Nanotechnology and viruses

Nanotechnology and human immunodeficiency virus (HIV)

Human Immunodeficiency Virus types 1 and 2 (HIV-1 and HIV-2) are belonged to lentiviruses and can cause acquired immunodeficiency syndrome (AIDS). Originally, both HIV-1 and HIV-2 may be created from simian immunodeficiency viruses (SIVs). Unfortunately, AIDS kills many people throughout the entire world yearly. Moreover, according to the World Health Organization (WHO) report, thousands of new cases are reported especially in young people from the least-developed countries, annually [120-123].

The early diagnosis along with the effective treatment can definitely reduce AIDS-related mortality. Recently, the use of nanotechnology in treatment and diagnosis of HIV infections has been started. In a series of studies, researchers have used nanoparticle biosensor, DNA nano-machines, as well as nano-sensor for appropriate detection of HIV [124-126]. Macchione *et al.* have completely reviewed the different nano-systems as potential candidates for prevention and treatment of HIV infections [127]. There have also been many other studies regarding the prevention, detection, and treatment of as well as vaccines for HIV/AIDS [128-132].

Nanotechnology and influenza virus

Influenza virus is from the family Orthomyxoviridae. Influenza types A, B, C, and Thogotovirus are in this family and only types A and B are clinically relevant in humans. Influenza virus has an envelope and it is sensitive to different conditions such as, humidity, temperature, lipid solvents, detergents, heat and a low pH. Influenza virus can be easily transferred to other people by infectious aerosol through talking, coughing, sneezing, and etc. This virus has developed many pandemics and caused millions people death so far. One of the important problems with this virus is causing disease in older patients with primary disease for example chronic heart or lung disease, metabolic disorders such as diabetes, immune disorders, and/or superinfection with some bacteria [133-135].

There are many papers regarding the use of nanotechnology for detection or treatment of Influenza virus infection. In an interesting study, Lauster *et al.* showed that virus infection can be inhibited *in vitro*, ex *vivo*, and *in vivo* using phage capsid nanoparticles [136]. Furthermore, several studies have drastically linked the nanotechnology with the detection, treatment, prevention of and vaccine production for some viruses, especially Influenza virus [137-140].

Nanotechnology and ebola virus

Ebola virus is from the genus *Ebolavirus* and the Filoviridae family. This virus may cause severe, fatal (rates range from 30% to 90%), and zoonotic infections. Transmission of this infection is either person to person (direct contact) or through contact with body fluids of infected persons. Importantly, WHO classified the 2013–16 outbreak of this virus as a Public Health Emergency of International Concern. Unfortunately, diagnosis and treatment of this infection is not easy and new methods are required for diagnosis and treatment of the disease [141-143].

Like other viruses noted above, nanotechnology may be useful in treatment of Ebola virus infections. In a study, the effect of nanoparticle formulations on RNAi has been shown to be beneficial in treatment of Ebola virus infection [17]. Accordingly, there are studies indicating the successful treatment of Ebola virus infections by use of nanotechnology [137, 139].

Nanotechnology and COVID-19

COVID-19 is a new emerged infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The pandemic of SARS-CoV-2 has been started in 2019 in China and it has caused thousands people death so far. SARS-CoV-2 is a member of the genus *Betacoronavirus* in the family of *Coronaviridae*. The origin of this contagious disease is not specified exactly but what is clear that, COVID-19 is a dangerous disease and it needs prompt diagnosis and appropriate treatment [144-147].

Since the end of 2019, many efforts have been made for detection, treatment, and/or prevention of COVID-19. Among these studies, the use of nanotechnology has been one of the most important approaches. More recently Rangayasami *et al.* have discussed the impact of nanotechnology researches on COVID-19, the capability of using nanotechnology against COVID-19, and the progressions in nanotechnology for construction of vaccines against COVID-19 [148]. likewise, in an another study the role of nanomedicine on COVID-19 has been evaluated [149].

A recent study proposed the possibility of the application of interference phenomenon as an alternative option for prophylaxis against COVID-19 [150]. Nanotechnology approaches may help to achieve this likely purpose professionally. Many other papers have been published about the impact of nanotechnology strategies on diagnosis, treatment of, and vaccine creation for COVID-19 [151-153].

Nanotechnology and fungi

The fungi are the biggest and the most diverse microorganisms in all ecosystems on the Earth. Fungi could play different roles in the world. Just as they can cause disease; they also may cause health and this is amazing. Additionally, some fungi may be commensals (common members of the microbiomes), and they may act as pathogens too. However, the fungal diseases and the drug resistant fungi are increasing. Therefore, the accurate identification and the appropriate treatment of fungal infections seems to be very important [154-157].

Fortunately, there are many studies regarding the importance of nanotechnology in detection and treatment of the infections caused by the different fungi such as *Candida albicans*, *Cryptococcus*, *Pneumocystis,* and *Blastomyces*. Widely, nanoparticles (for example silver, nitric oxide-releasing, chitosan-containing, and metal-containing nanoparticles) have been used in treatment of some fungal infections. Nanoparticles have also been used in prevention of microbial biofilm formation as well as in drug delivery systems for some fungal infections [158-167].

Nanotechnology and other microorganisms

In this review, we tried to search and record the application of nanotechnology in detection and treatment of infections caused by many important microbes. However, nanotechnology approached has also been used for many other microorganisms and fortunately yielded promising results (Table 1).

Table 1. Nanotechnology and some of the other microorganisms

Microorganism	Description	Reference
Shigella dysenteriae	Use of nanoparticles as antibiotics	[168]
Neisseria gonorrhoeae	Use of nanotechnology for rapid detection	[169]
Campylobacter jejuni	Use of nanocarriers as antimicrobials	[170]
Epstein–Barr Virus (EBV)	Use of nano-medicine for treatment	[171]
Human Papilloma Virus (HPV)	Use of nanotechnology-based strategies for detection, prevention, and treatment	[172]
Hepatitis C Virus (HCV)	Use of nanotechnology for diagnosis	[173]
Histoplasma capsulatum	Use of nanoparticles for treatment	[174]
Paracoccidioides brasiliensis	Use of a gold nano-probe for detection	[175]

Moreover, recently some possible alternatives to traditional antibiotics (non-antibiotic techniques) have been introduced in order to combat the pathogens and prevent the development of antibiotic-resistant strains. These possible alternatives may include quorum quenching, application of bacteriophages, and using of herbal medicine [176, 177]. In this regard, it is recommended to exploit the possible nanotechnological methods to achieve the purposes.

CONCLUSION

Finally, the contemporary medicine needs to be updated and new more effective approaches are required to combat the infectious diseases. It seems that nanotechnology is one of the important approaches. As mentioned above, fortunately, many researches have shown good results regarding the use of nanotechnology in detection and treatment of microbial infections. Many important microbes have been detected using some various nanotechnological methods. Moreover, many infections caused by these microbes have been treated by use of nanotechnology. Nanotechnology-based approaches have also been used in eradication of biofilm formation (a serious problem in medicine), and magically the formulation of new drugs in nano-sized carriers applied to combat some global infectious diseases such as: malaria, tuberculosis, and AIDS.

Since many years ago, antibiotics were used in treatment of infections, but unfortunately, their side effects as well as the resistance to them have always been the key problems. Thus, selection of the most suitable detection methods as well as the most appropriate treatment options for various microbial infections may reduce the crisis. Nowadays, having the less side effects, the use of nano-antibiotics in treatment of wide range of microbial infections, particularly the resistant ones are increasing. Altogether, the above mentioned facts highlight the importance of applying nanotechnological approaches in microbiology.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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