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Safe NIR Light for Fast Polymers Surface Sterilization using Organic Heaters

Valentin Launay^{1,2}, Frédéric Dumur³, Laurent Pieuchot^{1,2}, Jacques Lalevée*^{1,2}

¹Université de Haute-Alsace, CNRS, IS2M UMR 7361, F-68100 Mulhouse, France

²Université de Strasbourg, France

³Aix Marseille Univ, CNRS, ICR UMR 7273, F-13397 Marseille, France

e-mail: jacques.lalevee@uha.fr

Abstract

Sterilization approaches using UV radiation (e.g. 254 nm), dry heat and moist heat have been widely reported in the literature. In the present study, we proposed a most environmentally friendly approach for surface sterilization using safe Near-InfraRed (NIR) light to produce a strong photothermal effect using organic NIR dyes as heaters. Markedly, well selected organic dyes strongly absorbing in the NIR range have been used as stimuli responsive compounds. When excited upon NIR light, these heaters release the light energy to their surrounding environment in the form of heat. In this work, trimethylolpropane triacrylate (TMPTA) based polymers, used as benchmark materials for coatings, could reach high temperatures (> 120°C) in a very short time scale (~30 s). Using heaters containing coatings, sterilization experiments showed very good results with total annihilation of bacteria (*Escherichia coli*) after only 2 min of NIR irradiation. These results suggest that short irradiation flashes could be enough to sterilize contaminated surfaces. Moreover, some tests have been performed on several NIR heaters in the NIR-II range (1000-1700 nm) for a better in-depth penetration i.e. almost 200°C could be reached by polymers in less than 1 min of NIR irradiation. Therefore, NIR LED irradiation of heater-containing polymers or coatings constitutes here a robust, transportable and safe sterilization method.

1. Introduction

The sterilization process is a technique known for more than 200 years. Unlike decontamination and disinfection processes, this process consists in killing every single living microorganism from contaminated surfaces. Thus, physical approaches are useful to annihilate microorganisms. Indeed, these sterilization techniques have become essential to convert polymers or coatings, which constitute the most important class of materials, into sterile surfaces, medical devices or products. An item is considered as sterile if the theoretical probability of a viable microorganism to be alive is lower or equal to the Sterility Assurance Level (SAL).[1] For instance, medical devices are considered as sterile if there is one in a million chance for a viable microorganism to be alive (*e.g.* SAL = 10^{-6}).[2] Thus, sterilizations based on high-frequency radiation, dry heat and steam (also called moist heat) are widely used.[3-5] However, the high energy consumption of these kind of sterilization processes is a severe drawback from an environmental perspective and safer procedures for users must be established.[6] The COVID crisis has shown the interest to develop new sterilization approaches that can be transportable, robust, safe for the user and the environment.[7]

Another way to destroy microorganisms is to use light-based sterilization (mainly UV and visible light).[8] Light, and more specifically NIR light, has numerous advantages over the other physical sterilizations previously listed. Indeed, NIR light is less energetic compared to radiation and heat sterilizations which are good in an environmental standpoint. Moreover, NIR sources are safer for health due to the low photon energy. NIR devices are also cheap and compact sources. Besides, NIR wavelengths are well known for their in-depth penetration in media.[9] Furthermore, the light can be triggered on-demand with a spatial control of the irradiated area.[10] Additionally, the photothermal effect, which is the ability to convert light into heat, can be highly efficient in the NIR range for well selected heaters.[11] Finally, NIR light induced photothermal effects have been widely reported in the literature through photodynamic and photothermal therapies.[12][13] In fact, the optical window to allow a good penetration into the human skin is between 600 nm and 900 nm due to the poor absorption of some chromophores at these wavelengths such as melanin, hemoglobin and water.[14] Nevertheless, there is no work using NIR light for surface sterilization to the best of our knowledge.

The photothermal therapy (PTT) consists in using the photothermal effect to exterminate cancer cells.[15] However, the temperatures needed to exterminate cancer cells through apoptosis, necroptosis and necrosis remain quite low.[16] Nevertheless, this medicine field still remains highly interesting in order to know the most used ways to generate almost harmless photothermal effects. Inorganic materials used to generate this photothermal effect can be classified in three different classes. The first class is the noble metal nanoparticles. They are widely used because of their chemical inertness and resistance to oxidation.[17] The second class concerns carbon-based materials. This class gathers single/multi-walled carbon nanotubes and graphene-based nanomaterials.[18][19] They have been extensively used the past decade due to their low toxicity.[20] Finally, the last class concerns transition metal sulfide and oxide nanomaterials. Indeed, Fe_3O_4 , $\text{W}_{18}\text{O}_{49}$ and Cu_2MoS_4 are known for their theranostic applications and photothermal conversion efficiency.[21-23] However, these inorganic nanoparticles are expensive, have poor biocompatibility and the permanent exposure to metals compounds in the form of smoke, dust or particles can be responsible for acute or chronic respiratory pathologies which are severe drawbacks, these are the reason why metal-free compounds should be favored for surface sterilizations.[24] On the other side, NIR organic dyes possess many advantages over inorganic photothermal agents. Indeed, their modification is easily accessible, the biocompatibility and biodegradability can be better and their photophysical/chemical properties are adjustable.[25] However, the photostability of organic dyes has been widely studied and is known to be lower than inorganic photothermal agents.[26]

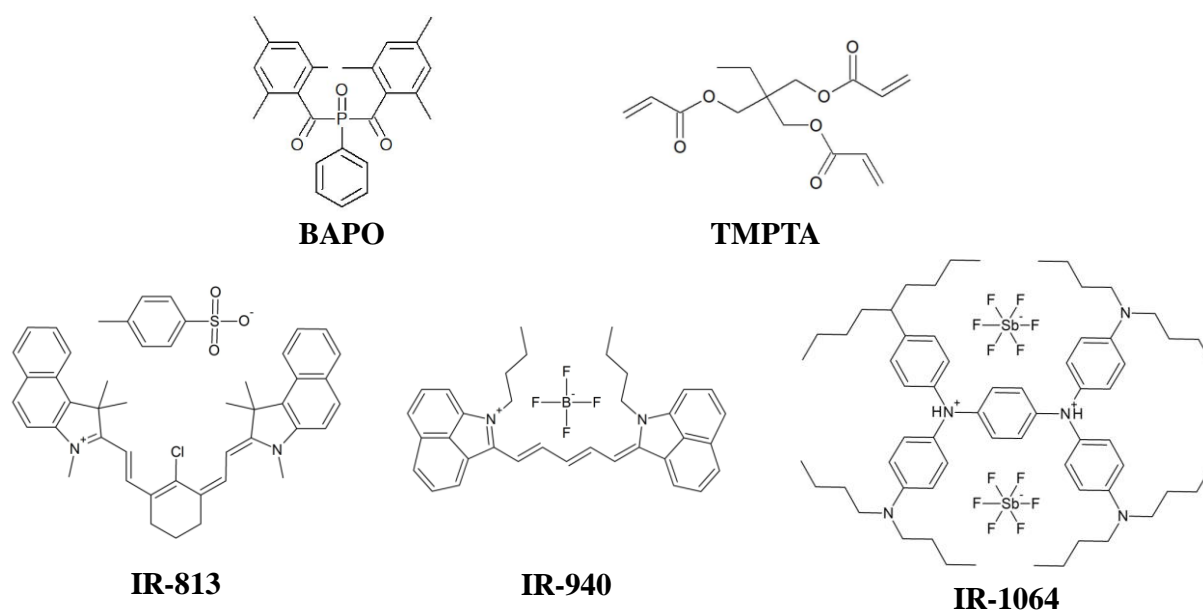
In previous studies, we proposed to use NIR organic dyes strongly absorbing in the NIR range as stimuli-responsive compounds to generate a powerful photothermal effect upon NIR irradiation.[10][27] In the present study, the photothermal effect generated upon NIR light by a NIR organic dye (hereafter called heater) is used to annihilate bacteria on polymer and coating surfaces. One of the heater used in this study, 2-[2-[2-chloro-3-[2-(1,3-dihydro-1,1,3-trimethyl-2H-benz[e]indol-2-ylidene)ethylidene]-1-cyclohexen-1-yl]ethenyl]-1,1,3-trimethyl-1H-benz[e]indolium 4-methylbenzenesulfonate (IR-813), is highly interesting since it is REACH-identified and harmless in the concentration used in this work.[28] Thus, bacteria (*Escherichia coli*) were spread on the polymer pellets which were subsequently irradiated with different irradiances and times. Finally, other NIR organic dyes have been selected to generate a strong photothermal effect in the NIR-II range (1000-1700 nm), allowing a better in-depth penetration.

This study showed very promising results and could be applied for the sterilization of surfaces using transportable, robust, safe for the user and the environment NIR LED light.

2. Experimental section

2.1. Chemical compounds

IR-813 was purchased from Tokyo Chemical Industry (TCI-Europe), *bis*(2,4,6-trimethylbenzoyl)phosphine oxide (BAPO) was obtained from Lambson Ltd (UK), trimethylolpropane triacrylate (TMPTA) was ordered from Allnex. 1-Butyl-2-((1*E*,3*E*,5*Z*)-5-(1-butylbenzo[*cd*]indol-2(1*H*)-ylidene)penta-1,3-dien-1-yl)benzo[*cd*]indol-1-ium tetrafluoroborate (IR-940) and *N*¹,*N*¹,*N*⁴,*N*⁴-tetrakis(4-(dibutylamino)phenyl)benzene-1,4-diaminium hexafluoroantimonate (IR-1064) were provided by Lotchem. Chemical structures of the different products are presented in the Scheme 1.



Scheme 1. Chemical structures of the monomer (TMPTA), photoinitiator (BAPO) and NIR heaters (IR-813, IR-940 and IR-1064) used in this study.

2.2. Preparation of samples

The multifunctional acrylate monomer (TMPTA) was mixed with 1%_{wt} of photoinitiator (BAPO) and 0.1%_{wt} of heater (IR-813/IR-940/IR-1064). Then, the sample was stirred for one week. Afterwards, photopolymerization experiments were carried out at room temperature upon laser diode irradiation at 405 nm (110 mW.cm⁻²) for polymers and coatings preparation.

2.3. Colony forming unit (CFU) assays

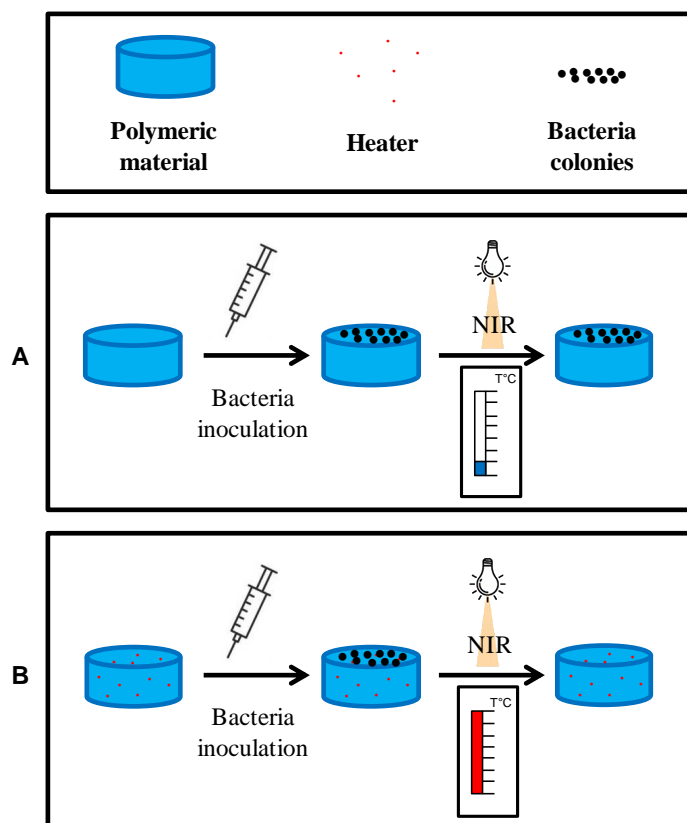
Escherichia coli (E. coli) strain was pre-grown overnight in lysogeny broth (LB) medium at 37°C (200 rpm). The bacterial culture was diluted to 0.1 optical density units (OD, measured at $\lambda = 600$ nm) and incubate at 37 °C (incubator shaker, 200 rpm) until it reached 0.6 OD. Disc-shaped polymer samples were inoculated with 50 μ L drops of a 1/10 dilution of the bacterial solution (1cm radius discs, one drop per disc) and incubated at RT for 10 min. The bacterial solution was removed and the samples were subsequently submitted to irradiation treatments. Irradiated samples were plunged into sterile tubes containing 10 mL of LB for 10 min on a rotary shaker. Serial dilutions from the resulting culture were plated onto LB agar plates in triplicate. The plates were then incubated at 37°C for 24 h and CFUs were counted on the adequate dilution (25-250 CFU per plate).

2.4. NIR irradiation followed by thermal imaging camera

In order to kill the bacteria, polymers/coatings surfaces were irradiated upon NIR light at 785 or 1064 nm. Excitations with the NIR light source were performed at a distance of 2 cm. Three polymer/coating samples per parameter were irradiated as replicates. A Fluke TiX500 infrared thermal imaging camera was used to follow in real time the temperature reached by the polymer/coating surfaces.

3. Results and discussion

Scheme 2 summarizes the idea of using a NIR heater in-situ embedded in a polymer material for bacteria annihilation. Indeed, NIR heaters are not involved in the polymerization process since they have no or low absorption in the near-UV range (wavelength used for the polymer preparation step by photopolymerization). Thus, they can be used to generate on demand a powerful photothermal effect upon NIR light. In fact, the polymeric material temperature should not increase a lot upon NIR light in absence of heater whereas high temperature should be reach in the presence of heater. Therefore, we can expect the photothermal effect produced to be sufficient enough in the presence of heater to annihilate bacteria.



Scheme 2. Bacteria annihilation using NIR irradiation. (A) Polymer sample without heater. (B) Polymer sample with heater.

Irradiations of TMPTA-based polymers upon NIR light were recorded by thermal imaging camera. Polymers containing heaters were irradiated with an irradiance of 2.55 W.cm^{-2} (Figure 1A) or 0.4 W.cm^{-2} (Figure 1B) whereas heater-free polymers were irradiated at 2.55 W.cm^{-2} (Figure 1C) as a reference. Markedly, the photothermal effect produced is really high for polymers containing heater than heater-free polymers ($+143^\circ\text{C}$ vs. $+11^\circ\text{C}$). Furthermore, the light irradiance also plays a key role in the photothermal effect generated. Indeed, the polymer sample only reached 60°C at 0.4 W.cm^{-2} against 167°C at 2.55 W.cm^{-2} . Therefore, we can expect a slight bactericidal effect under the conditions presented in Figure 1B and a very strong one under the conditions shown in Figure 1A. **Indeed, the most thermophilic microorganisms are entirely destroyed after 1 h at 170°C for dry heat sterilizations and 20 min at 121°C for steam sterilization (18 min at 134°C for prions), as extensively used methods for sterilization.[29][30] E. coli being a mesophilic microorganism (growth temperature near the human body temperature), the conditions to observe a bactericidal effect are milder.**

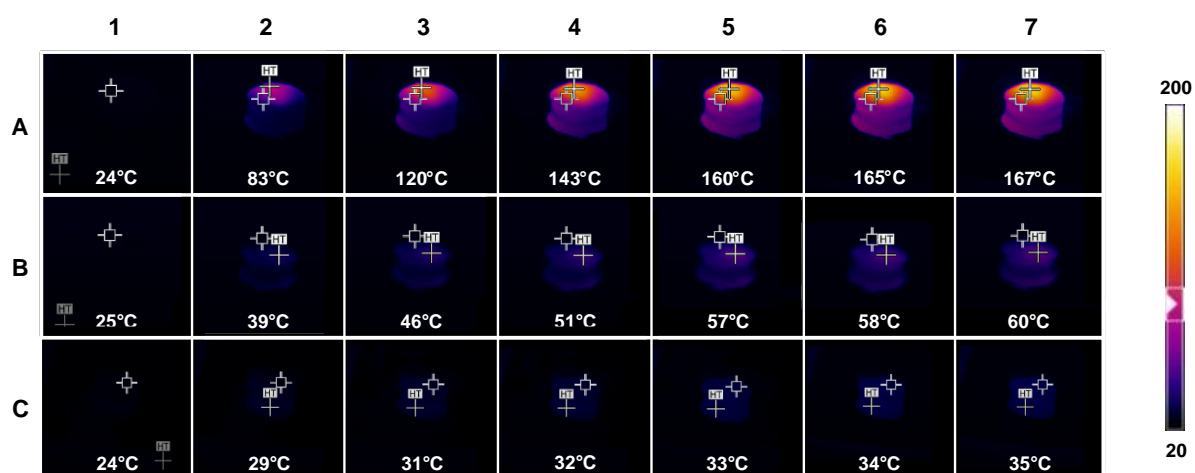


Figure 1. Thermal imaging camera pictures taken during NIR irradiation. Pictures are taken after (1) 0 s ; (2) 10 s ; (3) 20 s ; (4) 30 s ; (5) 60 s ; (6) 120 s ; (7) 180 s of NIR irradiation at 785 nm. (A) Polymer containing heater irradiated at 2.55 W.cm^{-2} . (B) Polymer containing heater irradiated at 0.4 W.cm^{-2} . (C) Polymer without heater irradiated at 2.55 W.cm^{-2} .

Afterwards, the temperatures of these polymers were still recorded by thermal imaging camera to learn more about the cooling speed after the irradiation step (i.e. when the NIR light is switched off). Thus, the cooling of heater-containing polymer after 3 min of irradiation at 2.55 W.cm^{-2} (Figure 2A) and 0.4 W.cm^{-2} (Figure 2B) are presented as well as the cooling of heater-free polymer after 3 min irradiation at 2.55 W.cm^{-2} (Figure 2C). We can see on the Figure 2 that the cooling is quite fast. Indeed, the temperature of samples B and C are back to their normal temperature after only 90 s whereas the sample A is almost back to the temperature before the irradiation after 120 s. However, the temperature of this latter is still valuable 10 s after the NIR irradiation (90°C), offering good prospects for short irradiation flashes to maintain the temperature in the desired range for surface sterilization.

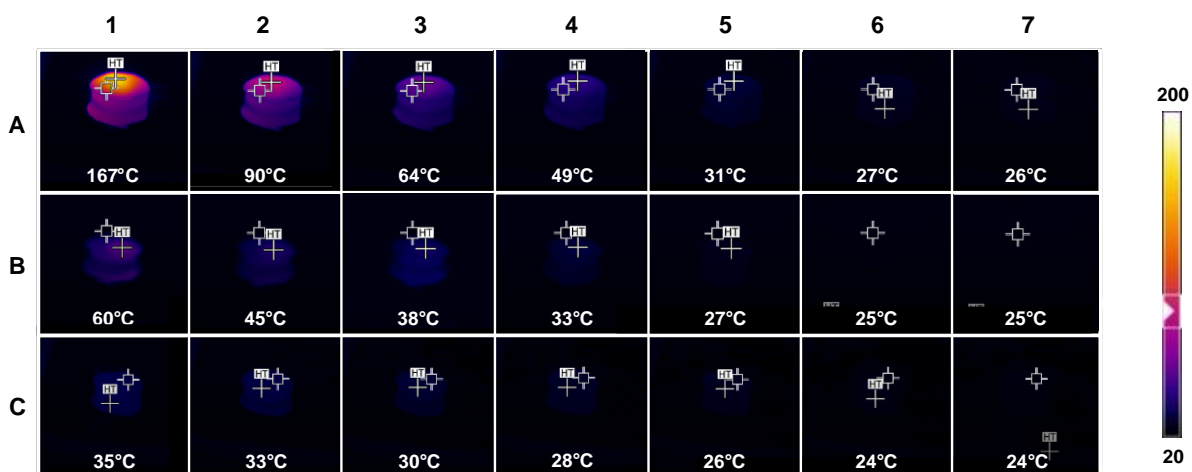


Figure 2. Thermal imaging camera pictures after NIR irradiation. Pictures are taken (1) 0 s ; (2) 10 s ; (3) 20 s ; (4) 30 s ; (5) 60 s ; (6) 90 s ; (7) 120 s after NIR irradiation at 785 nm. (A) Polymer containing heater irradiated at 2.55 W.cm^{-2} . (B) Polymer containing heater irradiated at 0.4 W.cm^{-2} . (C) Polymer without heater irradiated at 2.55 W.cm^{-2} . (D) Polymer without heater irradiated at 0.4 W.cm^{-2} .

Figure 3 introduces the temperature curves of the three different irradiation conditions. We can observe a huge and fast photothermal effect produced when the TMPTA-based polymer in presence of IR-813 (0.1%_{wt}) is irradiated at 785 nm (2.55 W.cm^{-2}). Indeed, less than 20 s are required for the polymeric material to reach 100°C and a plateau at $\sim 160^\circ\text{C}$ is reached in less than a minute. This huge and fast photothermal effect can bode well for short irradiation flashes in order to keep a determined temperature into the polymer.

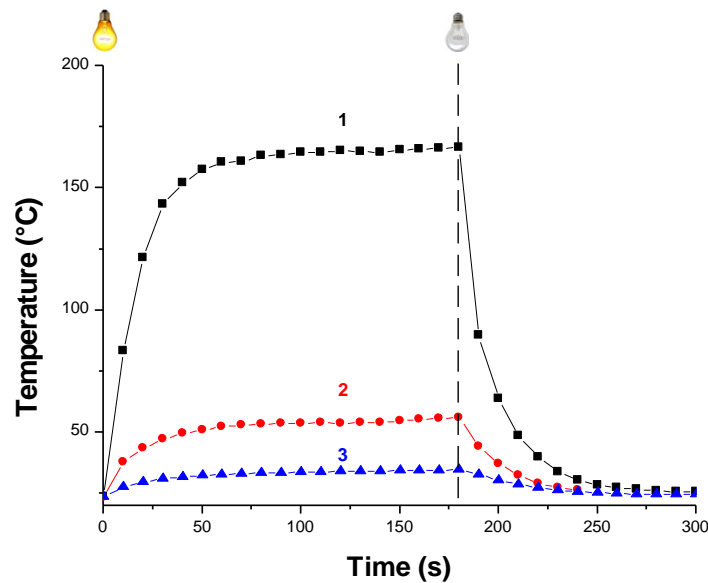


Figure 3. Temperature versus time of TMPTA-based polymers. Irradiations are performed at 785 nm from $t = 0$ s to $t = 180$ s and stopped from $t = 180$ s. (1) TMPTA-based polymer in presence of IR-813 (0.1%_wt) at 2.55 W.cm^{-2} . (2) TMPTA-based polymer in presence of IR-813 (0.1%_wt) at 0.4 W.cm^{-2} . (3) TMPTA-based polymer without heater at 2.55 W.cm^{-2} .

We assessed the bactericidal effect of **Near Infrared (NIR)** treatments on our heater-containing polymers/coatings by performing colony forming unit (CFU) assays. Disc-shaped polymer samples were inoculated with an *E. coli* bacterial culture suspension and subsequently submitted to **NIR** treatments. In the absence of heater, **near** infrared irradiation has no effect on colony forming activity: the samples exhibit similar CFU numbers as untreated samples. This shows that the **NIR** treatment by itself has no effect on bacteria viability and suggests that the presence of heater within the polymer is not toxic for bacteria. **It also shows that NIR alone does not exhibit antibacterial properties (contrary to UV light) due to the long and safe wavelength involved.** Strikingly, no CFUs were observed after **NIR** treatment of the polymers containing heater, suggesting that all bacteria were killed by the irradiation (Figure 4A). Interestingly, we did not observe any bacterial growth from the heater-containing treated samples even after overnight incubation in **lysogeny broth** culture medium. This demonstrates the strong **NIR**-inducible bactericidal properties of the heater-containing polymer. A significant decrease in colony forming activity was also observed for short irradiation period (Figure 4C), suggesting that short irradiation flashes can be enough to sterilize contaminated surfaces.

These results show the huge importance of reaching high temperatures. Indeed, the temperature reached for 0.4 W.cm^{-2} (see Figure 3) is not high enough to observe a significant decrease in CFU numbers. However, we can observe a significant decrease in CFU numbers as soon as the reached temperature is high enough even for short irradiation times. **The decrease in CFU number can be approximately found with the formula $N = N_0 \cdot 10^{(-t/D)}$ with N the number of viable microorganisms at time t, N_0 the number of viable microorganisms at time 0, t the heating time and D the decimal reduction time.[31] This decimal reduction time depends on the microorganism, the environment and the temperature.** Thus, a great part of bacteria is eliminated after only 30 s of NIR irradiation at 2.55 W.cm^{-2} ($T_{\text{max}} = 143 \text{ }^\circ\text{C}$) and there is almost no bacterium left after 1 min ($T_{\text{max}} = 160 \text{ }^\circ\text{C}$). Finally, all bacteria are annihilated after 2 min of NIR irradiation. Therefore, NIR irradiation of heater-containing polymer clearly constitutes a robust sterilization method.

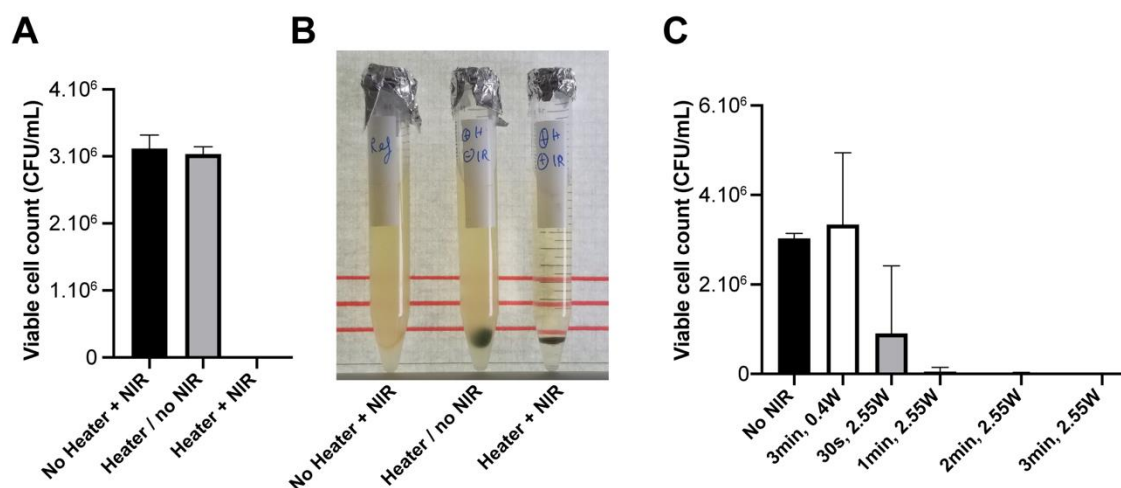


Figure 4. Near-Infrared-induced sterilization of heater-containing polymer samples. (A) Quantification of viable bacterial cells on contaminated polymer samples after Near-Infrared (NIR) treatment, in the presence or absence of heaters. (B) Overnight grown LB medium cultures containing the polymer samples used in A. (C) Effect of various NIR treatments on sterilization efficacy of contaminated heater-containing polymer samples.

As said above, surface sterilizations in the NIR-II range (1000-1700 nm) are really attractive since they allow a better in-depth penetration into the polymeric surface. Thus, different NIR organic dyes have been selected to generate a photothermal effect upon irradiation to 1064 nm light (Figure 5). As expected, the reference system and the TMPTA-based polymer in presence of IR-813 exhibit a low photothermal effect due to its low absorption in the NIR-II range. However, polymers in presence of IR-940/IR-1064 generate a powerful

photothermal effect in this NIR region. Indeed, TMPTA-based polymers in presence of IR-940 reached more than 140°C whereas the one in presence of IR-1064 almost reached 200°C. Moreover, the high temperature of this latter is obtained under less than one minute of irradiation. Furthermore, there is a good reproducibility in the temperatures reached by polymeric materials. This experiment demonstrates the high potential of NIR organic dyes for surface sterilization even in the highly penetrating NIR-II region.

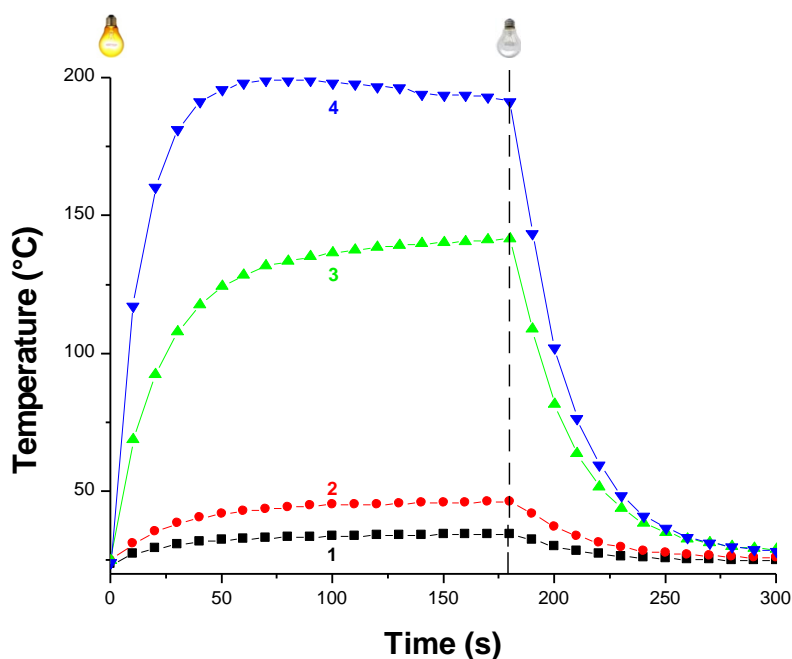


Figure 5. Temperature versus time of TMPTA-based polymers. Irradiations are performed at 1064 nm (2.5 W.cm^{-2}) from $t = 0$ s to $t = 180$ s and stopped from $t = 180$ s. (1) TMPTA-based polymer without heater. (2) TMPTA-based polymer in presence of IR-813 (0.1% wt). (3) TMPTA-based polymer in presence of IR-940 (0.1% wt). (4) TMPTA-based polymer in presence of IR-1064 (0.1% wt).

4. Conclusion

The photothermal effect generated by extremely low NIR heater content (0.1% wt) increased the local temperature of TMPTA-based polymers, allowing the sterilization of these latter. The NIR-I range organic dye used in this study is REACH-identified and is safe under the low concentration used in this study. High temperatures were quickly reached followed by significant decreases in colony forming activity, suggesting that short irradiation flashes can be enough to sterilize contaminated surfaces. Moreover, two NIR-II range organic dyes were selected to exhibit a strong photothermal effect for longer irradiation wavelengths (>1000 nm),

allowing a better in-depth penetration in the polymeric material and higher photothermal effect. These results are highly interesting since the NIR light sterilization constitutes an environmentally friendly approach over heat and other radiation sterilization procedures. Very compact and portable irradiation devices can be used for rapid sterilizations. The extension of this work to 3D printed devices will be carried out in forthcoming papers.

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