

NIR laser-activated polydopamine-coated Fe₃O₄ nanoparticles used as a novel precise photothermal insecticide

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Article

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Abstract

Chemical pesticides are widely used in prevention and control of pests. However, low insecticidal efficacy, high environmental risks and pesticidal resistance severely constrain further applications. In this study, a near-infrared (NIR) laser-activated pesticide-free Fe₃O₄@PDA nanoplatfrom with a low production cost was constructed for precise photothermal killing of housefly larvae. The polydopamine (PDA) surface-functionalized Fe₃O₄ nanoparticles (Fe₃O₄@PDA NPs) were synthesized by simply mixing dopamine with Fe₃O₄ NPs under aerobic alkaline conditions. These Fe₃O₄@PDA NPs exhibited superb photothermal performance with near-infrared (NIR) laser irradiation. Larval uptake results revealed greater *in vivo* uptake of Fe₃O₄@PDA NPs than bare Fe₃O₄ NPs when housefly larvae were fed a NP-treated diet. These NPs exhibited favorable biocompatibility and showed no obvious influence on larval development and intestinal microbial population. However, when irradiated with a NIR laser, the NPs efficiently induced death of housefly larvae with high temperature ($T_{\max} > 50\text{ }^{\circ}\text{C}$) damage to the intestinal wall and subsequent leakage of intestinal bacteria into the hemocoel. Furthermore, uneaten NPs can be collected by magnetic absorption and show excellent thermal stability, which allowed recycling of these environmentally friendly nanoparticles. The Fe₃O₄-based nanoparticles described in the present study integrate the independence of pesticides, pronounced photothermal control of pests, and environmental friendliness, making this nanoplatfrom promising for controlling vector pests and diseases.

Introduction

Musca domestica L. (Diptera: Muscidae), a synanthropic fly, can be found in poultry, livestock and human facilities. As a major worldwide pest, they interfere with human life and spread human diseases.

House flies are carriers of more than 65 human and animal intestinal diseases and are responsible for bacterial protozoan and helminthic infections as well as viral and rickettsial infections^{1,2}. These flies also transmit eye diseases such as trachoma, infect wounds and skin with diseases such as cutaneous diphtheria, mycoses, yaws and leprosy^{3,4}. Larvae can invade the human umbilical cord, oral cavity, nasal cavity, intestinal tract, ear canal, eye, pelvic organ and other parts, causing myiasis^{5,6,7,8}.

The annual cost for insecticides used to control house flies in poultry farms in the United States has been estimated to be over 1.6 million (US dollars)⁹. Adult house flies are often controlled with insecticides when adult fly populations exceed acceptable abundance or activity levels¹⁰. However, overuse of insecticides for house fly control has resulted in development of house fly resistance to nearly all major insecticide classes¹¹.

Traditional control methods are currently limited to the use of physical patting, electrocuting light traps, odor baited traps and chemical insecticides (such as carbamates, organophosphates and pyrethroids) to kill house fly adults and larvae¹². However, the low efficiency and rapid accumulation of dust on sticky materials for these physical control methods severely limit their application. Chemical control is the most

efficient and common strategy used to suppress house fly populations. For example, insecticide-mixed sugars attract house flies and then kill them. However, abuse of chemical pesticides can cause consequent environmental disruption, ecological imbalance, mortality of nontarget organisms and development of pesticide resistance^{13, 14}. Furthermore, less than 0.1% of pesticides ultimately act on harmful biological targets due to evaporation, runoff and degradation¹⁵. Therefore, researchers have sought alternative control strategies.

In recent years, nanopesticide carriers based on organic polymers or inorganic nanoparticles have been widely developed. These nanopesticide carriers reduce the toxic effects of pesticides on the surrounding environment and improve pesticide utilization efficiency by suppressing evaporation and degradation and prolonging the effective duration. For example, Yan *et al.* developed a star polymer (SPc) for delivering a pesticide (osthole) to control both pests and diseases of strawberry. This nanosized osthole/SPc complex was delivered to the cytoplasm of insect cells, and the efficacy for control of strawberry pests and diseases was significantly improved. Moreover, the osthole/SPc complex exhibited no influence on strawberry fruit quality and nontargeted predators, which fulfilled the need for green control of pests¹⁶. To improve insecticidal efficacy and reduce the environmental risks of pesticides, Luo *et al.* constructed self-assembled degradable nanogels featuring foliar affinity and pinning for pesticide delivery. These pesticide-loaded nanogels increased the foliar protection area and improved both the pesticide exposure area and target contact efficiency. Due to their flexibility and viscosity, the nanogels largely enhanced washing resistance and the retention rate of the pesticide. Moreover, the degradability of nanogels make these nanocarriers environmentally friendly and promising for delivering pesticides¹⁷. However, chemical control may not be an effective strategy for controlling this double-wing house fly with a wide range of activities, especially outdoors. Furthermore, this nanoscale pesticide still did not break away from classical biological or chemical control techniques and showed potential for pesticide resistance. Therefore, it is necessary to explore new disruptive and precise control strategies.

External/internal stimulus-responsive smart nanoagents featuring precise theranostics have been widely used in different fields. For example, in our previous work, we reported that pH-, GSH- and H₂O₂-responsive MnO₂-based nanoparticles exhibited superb potential for tumor-targeted theranostics^{18, 19}. Bo *et al.* developed polydopamine (PDA)-based nanoparticles (NPs) for H₂O₂ removal in the treatment of oxidative stress-induced periodontal disease²⁰. Specifically, photothermal materials convert visible to near-infrared (NIR) light into heat, leading to thermal ablation of cells and tissues, and this is a minimally invasive and controllable method that has drawn widespread attention^{21, 22}. Moreover, houseflies generally lay eggs in polluted feces, organic waste and other environments, and these develop into housefly larvae. Housefly larvae have a limited range of motion and take up their food through broad scraping, allowing them to take up nanoscale photothermal materials.

In this study, we constructed an NIR-activated Fe₃O₄@PDA nanoplatforM for precise photothermal control of housefly larvae to eliminate pesticide dependence. As shown in Scheme 1, environmentally friendly Fe₃O₄ NPs were chosen as the photothermal precursor. Then, a thin PDA layer (also showing high

photothermal conversion efficiency)²³ was coated on the Fe₃O₄ NPs. We evaluated the photoconversion efficiency of Fe₃O₄@PDA NPs *in vitro* and *in vivo*. NIR thermal imaging and inductively coupled plasma-optical emission spectrometry (ICP-OES) were used to investigate the uptake of NPs by housefly larvae. Additionally, the biosafety of NPs for larval development of intestinal microbial populations was studied. Finally, NIR laser-activated thermal control of larvae and its mechanism were systematically studied. The results demonstrated that Fe₃O₄@PDA NPs are promising candidates for controlling vector pests and diseases.

Results And Discussion

Synthesis and characterization of Fe₃O₄@PDA NPs

PDA-functionalized Fe₃O₄ NPs were synthesized *via* dopamine oxidation and self-polymerization. Scanning electron microscopy (SEM) images showed that these organic PDA-coated NPs have rough and flexible surfaces, while the surfaces of bare Fe₃O₄ NPs are relatively smooth and rigid (Fig. 1a, b). Transmission electron microscopy (TEM) images revealed that a thin layer was coated onto the surface of Fe₃O₄ NPs and showed a core/shell morphology (Fig. 1c, d). The average size of the nanoparticles was increased from 183.8 ± 27.5 nm to 201.2 ± 17.0 nm (Supplementary Figure 1). These nanoscale NPs had negligible effects upon uptake by housefly larvae when mixed with feeding materials.

The chemical characteristics of the NPs were examined by Energy-dispersive X-ray spectroscopy (EDS). As shown in Supplementary Figure S2, the signals for Fe were derived from the Fe₃O₄ core, and those for C and N were ascribed to the PDA shell, while elemental Cu in the EDS spectrum probably arose from the copper grid. This result confirmed the presence of PDA. Element mapping indicated the presence of PDA and a uniform distribution in the as-prepared NPs (Fig. 1e-i). FTIR spectroscopy of Fe₃O₄@PDA NPs revealed the appearance of new peaks between 1,000 cm⁻¹ and 1,600 cm⁻¹ (Supplementary Figure 3): the peak at 1280 cm⁻¹ was assigned to aromatic ring absorption; the peaks at 1,500 and 1,600 cm⁻¹ were ascribed to N-H shearing vibrations; and a peak at 3,450 cm⁻¹ was assigned to intermolecular O-H stretching vibrations or aromatic secondary amine N-H stretching vibrations; these data indicated the presence of PDA²⁴. Overall, these results confirmed successful coating of PDA onto the Fe₃O₄ NPs. Finally, the weight percent of Fe₃O₄ in Fe₃O₄@PDA NPs was quantified by ICP-OES and found to be 73.7 ± 2.1%.

Both Fe₃O₄ NPs and Fe₃O₄@PDA NPs absorb broadly at UV and NIR wavelengths (Supplementary Figure 4), so efficient light-to-heat conversion should occur. As expected, both Fe₃O₄ NPs and Fe₃O₄@PDA NPs exhibited strong photothermal performance and displayed temperature increases that depended on the laser power density (Fig. 2a, b). Specifically, the temperature of the Fe₃O₄@PDA NPs (0.5 mg/mL) increased to 73.3 °C ($\Delta t = 45.5$ °C) after irradiation (2.0 W/cm²) for 300 s, which was higher than that

of Fe₃O₄ NPs (T = 68.8 °C; Δt = 41.9 °C), while the temperature of DI water was increased by only 8.7 °C under the same conditions (Supplementary Figure 5). At low concentrations (≤ 0.5 mg/mL), these two NPs showed concentration-dependent temperature increases with a laser power density of 1 W/cm² (Fig. 2 c, d). However, when the concentrations reached 1 mg/mL, the temperatures of the Fe₃O₄ NPs and Fe₃O₄@PDA NPs reached 50 °C (Δt = 23.4 °C) and 52.8 °C (Δt = 26.2 °C), respectively, which were lower than 53.5 °C (Δt = 26.3 °C; Fe₃O₄ NPs) and 57.8 °C (Δt = 30.7 °C; Fe₃O₄@PDA NPs) seen at a concentration of 0.5 mg/mL. This may be ascribed to the tendency for high concentrations of NPs to aggregate at high temperatures, since the thermocouple probe mainly measured the temperature of the supernatant solution. This can also be seen from the results of photothermal heating photographs. As shown in Fig. 2e, the Fe₃O₄@PDA NP dispersion (0.5 mg/mL) showed excellent photothermal stability with no obvious aggregation over five minutes of illumination, while the Fe₃O₄ NP dispersion (0.5 mg/mL) aggregated substantially from the first minute. This excellent photothermal performance indicated high potential for killing housefly larvae.

Biosafety evaluation

These pesticide-free NPs were designed to fulfill the need for green control of pests, so the effects of NPs on body length, body weight, pupal weight, pupation rate and emergence rate of housefly larvae were first systematically evaluated. Varied volume (1, 3, 5, 10 mL) of Fe₃O₄ NP suspension (10 mg/mL, in DI water) was mixed with wheat bran and milk powder (Wheat bran (g): DI water (mL): milk powder (g) = 1:1:0.4), this giving feeding materials, and termed **F1**, **F3**, **F5**, **F10**, respectively. Fe₃O₄@PDA NPs-included feeding materials was mixed used the same mixture ratio, and termed **FP1**, **FP3** and **FP5**, **FP10**. Ten normal-breeding, good-growing and uniform-sized 1-day-old housefly larvae were fed with F1, F3, F5, F10, FP1, FP3 and FP5, FP10 in gauze covered porous centrifuge tube (10 mL), respectively. During the evaluation period, all housefly larvae were alive. Specifically, compared with the control group, FP1, FP3, FP5 and PF10 showed no effect on the body weights and body lengths of housefly larvae (Fig. 3a, b). Moreover, the pupal weight, pupation rate and emergence rate of larvae fed different doses of Fe₃O₄@PDA NPs were similar to those in the control group (Supplementary Table 1). These results showed that Fe₃O₄@PDA NPs had no significant effects on the development of housefly larvae.

The effect of FP5 on the intestinal microbial population of housefly larvae before and after NIR laser irradiation (808 nm, 1 W/cm²) was further studied. A total of 14 genera were identified in all housefly larva samples, of which *Providencia* and *Klebsiella* were the dominant genera. The relative abundances of the dominant genus in the intestines of housefly larvae fed FP5 without/with NIR laser irradiation were basically the same as that in the control group (Fig. 3c). Compared with the control group, the relative abundances of *Morganella*, *Weissella* and *Serratia* increased less in the Fe₃O₄@PDA NP (Laser-FP5, Laser+FP5) group, while the relative abundances of *Lactococcus*, *Enterobacter*, *Bordetella* and *Myroides* decreased less (Fig. 3d). According to the heatmap, the compositions of the intestinal flora in different samples of housefly larvae were dynamic. Compared with the nonirradiated group (Laser-CT, Laser-FP5), the relative abundance of *Providencia* increased with Laser + FP51d, Laser + FP52d, and the relative

abundance of *Proteus* and *Myroides* increased with Laser + FP52d, Laser + FP53d. The relative abundances of *Klebsiella*, *Leuconostoc* and *Lactococcus* decreased with Laser+FP51d (Fig. 3d). Principal component analysis (PCA) showed that samples of housefly larvae fed at the same time clustered together, and there were differences in intestinal flora among samples fed at different times (Fig. 3e). UPGMA evolutionary tree analysis further proved that there were differences in intestinal flora between the nonirradiated group (Laser-CT, Laser-FP5) and the irradiated group (Laser + CT, Laser + FP5) (Figure 3f) of housefly larvae. The above results showed that Fe₃O₄@PDA NPs had no effect on the intestinal flora of housefly larvae, but NIR laser irradiation had different effects on the intestinal flora of housefly larvae.

***In vivo* uptake evaluation**

Larval uptake experiments were performed to explore the influence of the PDA functional coating on inorganic Fe₃O₄ NPs on uptake by housefly larvae. ICP–OES quantification results (Fig. 4a) showed a concentration-dependent uptake increase for both Fe₃O₄ NPs and Fe₃O₄@PDA NPs. It was very encouraging to see that the uptake of NPs was significantly increased after coating PDA on the Fe₃O₄ NPs. This was also confirmed by comparing the body colors of larvae. As shown in Fig. 4b–f, the larvae fed FP5 exhibited the darkest color (representing the highest level of uptake), which corresponded to the ICP-OES results. The mechanism may be ascribed to the relatively flexible and adhesive surfaces provided by PDA^{25,26}, which helped larvae scrape these NPs.

***In vivo* photothermal imaging and insecticidal activity evaluation**

Inspired by the excellent photothermal performance and enhanced uptake of Fe₃O₄@PDA NPs *in vitro*, *in vivo* photothermal imaging was assessed. Housefly larvae were fed F5 and FP5 and then exposed to an 808 nm NIR laser (1.0 W/cm²) at 2 d post-feeding. NIR images were captured with an infrared thermal imaging system. As shown in Fig. 5a and Supplementary Figure 6, F5 quickly elevated the temperature of the larvae to 43.6 °C within 30 s, and the temperature reached 45.1 °C after NIR irradiation for 3 min; the temperature of larvae fed NP-free materials increased by no more than 1 °C. More encouragingly, FP5 achieved the most potent light-to-heat conversion *in vivo*. The temperature of FP5-fed larvae increased to 51.1 °C in the first 30 s, and by the end of irradiation, the temperature reached 52.7 °C. These results indicated that the Fe₃O₄@PDA NPs have potent *in vivo* photothermal activity and could be used to ablate larval tissue.

The *in vivo* thermal ablation activities occurring in NP-fed larvae were then assessed. After feeding different doses of Fe₃O₄ and Fe₃O₄@PDA NPs, the larvae were irradiated with an NIR laser (1 W/cm²) at predetermined times (1, 2, 3, or 4 days post-feeding), and the time to death (t_d) was recorded. Generally, in the first two days, the NPs showed feeding time- and dose-dependent insecticidal efficacy, and FP5 achieved the most potent insecticidal efficacy (Fig. 5b, c), while NIR laser irradiation alone did not have any significant insecticidal effects. Specifically, with NIR laser irradiation at 1 day post-feeding, the t_d of F1-, FP1-, F3-, FP3-, F5-, and FP5-fed larvae were 240 s, 240 s, 240 s, 238.6 s, 171.6 s, 149.8 s, and 105 s,

respectively, which were longer than the t_d for 2-day-old larvae after feeding with the corresponding NPs. This may be ascribed to the enhanced uptake ability of 2-day-old larvae, which produced more heat to kill larvae. However, NPs-fed 3-day-old larvae showed weaker insecticidal efficacy (Fig. 5d, e), which was ascribed to decreasing uptake and increasing excretion by 3-day-old larvae. As a result, we chose 2 days of F5- and FP5-fed larvae to further study the effect of power densities on insecticidal efficacy.

With increasing larval feeding time, the death time of larvae was shortened by NIR laser irradiation, and the shortest time of larval death was 2 d (Fig. 5f, g). Similarly, because the uptake amounts for NPs decreased and the excretion amount increased with further development of larvae, the t_d was prolonged for 3-day-old and 4-day-old larvae (Fig. 5h, i).

Insecticidal mechanisms study

To better understand the insecticidal mechanism, a [histological analysis](#) were carried out. H&E-stained images of F5- and FP5-fed larvae without NIR laser irradiation showed no obvious intestinal tissue damage compared with those of the control group without/with NIR laser irradiation (Fig. 6a). In sharp contrast, with NIR laser irradiation, F5 caused serious intestinal tissue damage as a result of excellent light-to-heat conversion by Fe_3O_4 -based NPs. Moreover, after PDA coating, FP5 further damaged the intestinal tissue, persuasively demonstrating enhanced uptake of NPs by larvae.

Intestinal bacteria have a profound influence on the growth of larvae^{27, 28} and may leak into hemocoel when intestinal tissue is damaged. The spilled body fluids of F5- and FP5-fed larvae with/without NIR laser irradiation were cultured by the plate method. As expected, no colonies grew on nutrient agar plates for any of the groups without NIR laser irradiation and the group treated with NIR laser irradiation alone, meaning that no intestinal bacteria leaked into the hemocoel (Fig. 6b). However, the nutrient agar plates from the irradiated housefly larvae groups (Laser+F5, Laser+FP5) were covered with different colonies, indicating leakage of bacteria from the intestinal tract into the hemocoel. Collective thermal ablation of the intestinal tract followed by leakage of intestinal bacteria contributed to the deaths of larvae.

Recovery of NPs

Good biocompatibility and recoverability play key roles in green prevention and control of insect pests. With the revelation that Fe_3O_4 -based NPs possess various advantages, including good biosafety and magnetic properties, we recovered Fe_3O_4 @PDA NPs by magnetic absorption. The recovery rates were $50.2 \pm 7.5\%$ and $27.9 \pm 8.0\%$ for F5 and FP5, respectively, further confirming the enhanced uptake of PDA-coated Fe_3O_4 NPs. The photothermal properties and stabilities of the recycled Fe_3O_4 @PDA NPs were investigated. As expected, the temperature of the Fe_3O_4 @PDA NPs (0.5 mg/mL) also increased to ~ 59 °C after irradiation (1.0 W/cm^2) for 300 s (Supplementary Figure 7). Furthermore, the recycled Fe_3O_4 @PDA NPs possessed desirable photothermal stability for at least three cycles of laser irradiation/cooling

(Supplementary Figure 8), suggesting that Fe₃O₄@PDA NPs may be suitable for use as photothermal insecticides.

To date, the use of pesticides is the most effective and valuable way to decrease the population of house flies. However, the consequent environmental disruption, ecological imbalance, and mortality of nontarget organisms caused by excessive abuse of chemical pesticides severely limits their use. Furthermore, this chemical control effect on adult flies is relatively nonideal, and houseflies easily develop pesticide resistance. Nanopesticide carriers could improve pesticide utilization efficiency and improve the bioavailability of pesticides in the surrounding environment, thus providing an effective, safe, and environmentally friendly way to use pesticides^{29,30}. Based on this nanoscale platform and breaking out the dependence on pesticides, we constructed an NIR laser-activated pesticide-free Fe₃O₄@PDA nanoplatform with a low production cost for precise photothermal control of housefly larvae and provided a new concept for transforming pest control.

Conclusion

In this study, we constructed an NIR-activated pesticide-free Fe₃O₄@PDA nanoplatform for precise photothermal control of housefly larvae. The Fe₃O₄@PDA NPs showed a core/shell morphology with an average size of 201.2 ± 17.0 nm. These Fe₃O₄@PDA NPs exhibited superb photothermal performance with NIR laser irradiation. Larval uptake results revealed greater *in vivo* uptake of Fe₃O₄@PDA NPs than bare Fe₃O₄ NPs when housefly larvae were fed the NP-treated diet. These NPs have favorable biocompatibility and showed no obvious influence on larval development and the intestinal microbial population. However, when irradiated with a NIR laser, the NPs efficiently eliminate housefly larvae due to intestinal wall damage caused by hyperthermia ($T_{\text{max}} > 50\text{ }^{\circ}\text{C}$) and subsequent leakage of intestinal bacteria into the hemocoel. Furthermore, uneaten NPs can be collected by magnetic absorption and show excellent thermal stability, thus allowing recycling of these environmentally friendly nanoparticles. The Fe₃O₄-based nanoparticles described in the present study integrate the independence of pesticides, pronounced photothermal control of pests, and environmental friendliness, making this nanoplatform promising for controlling vector pests and diseases.

Declarations

Data availability. Experimental procedure and characterization data are available in Supplementary Information.

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Author contributions

H.W. and Q.Z. contributed equally to this work. H.W. and Z.Z. conceived the project. H.W. prepared and characterized the Fe₃O₄@PDA NPs. H.W., Q.Z., Z.Z. analyzed the data and drafted the manuscript. X.Z., K.Z., X.Z., and R.Z. assisted with the *in vitro* and *in vivo* experiments. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

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Schemes

Scheme 1 is in the supplementary files section.

Figures

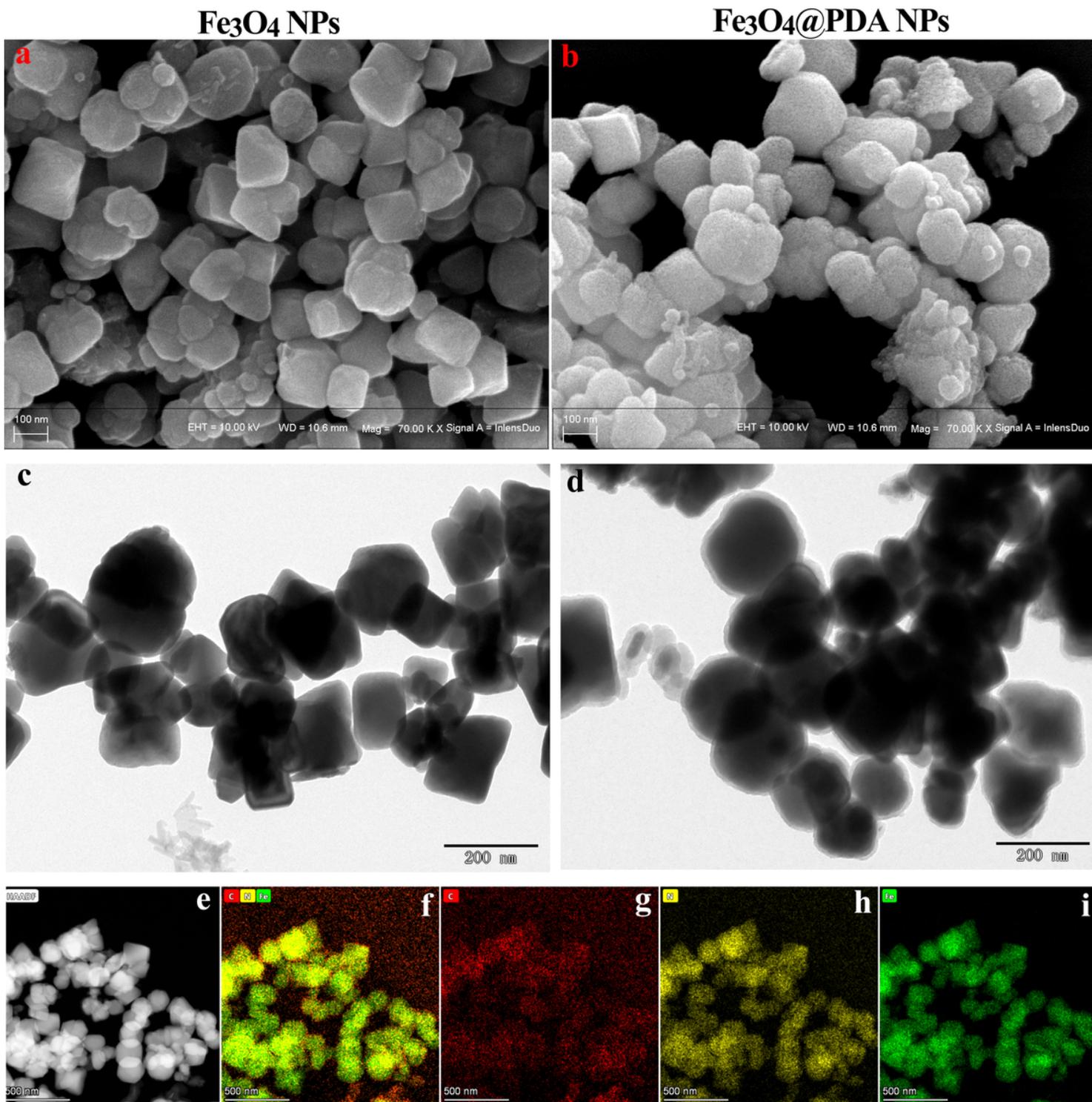


Figure 1

SEM images of (a) Fe_3O_4 NPs and (b) $\text{Fe}_3\text{O}_4@PDA$ NPs. TEM images of (c) Fe_3O_4 NPs and (d) $\text{Fe}_3\text{O}_4@PDA$ NPs. (e) HAADF-STEM images of $\text{Fe}_3\text{O}_4@PDA$ NPs. Elemental mapping of $\text{Fe}_3\text{O}_4@PDA$ NPs: (f) merged image, (g) C, (h) N and (i) Fe.

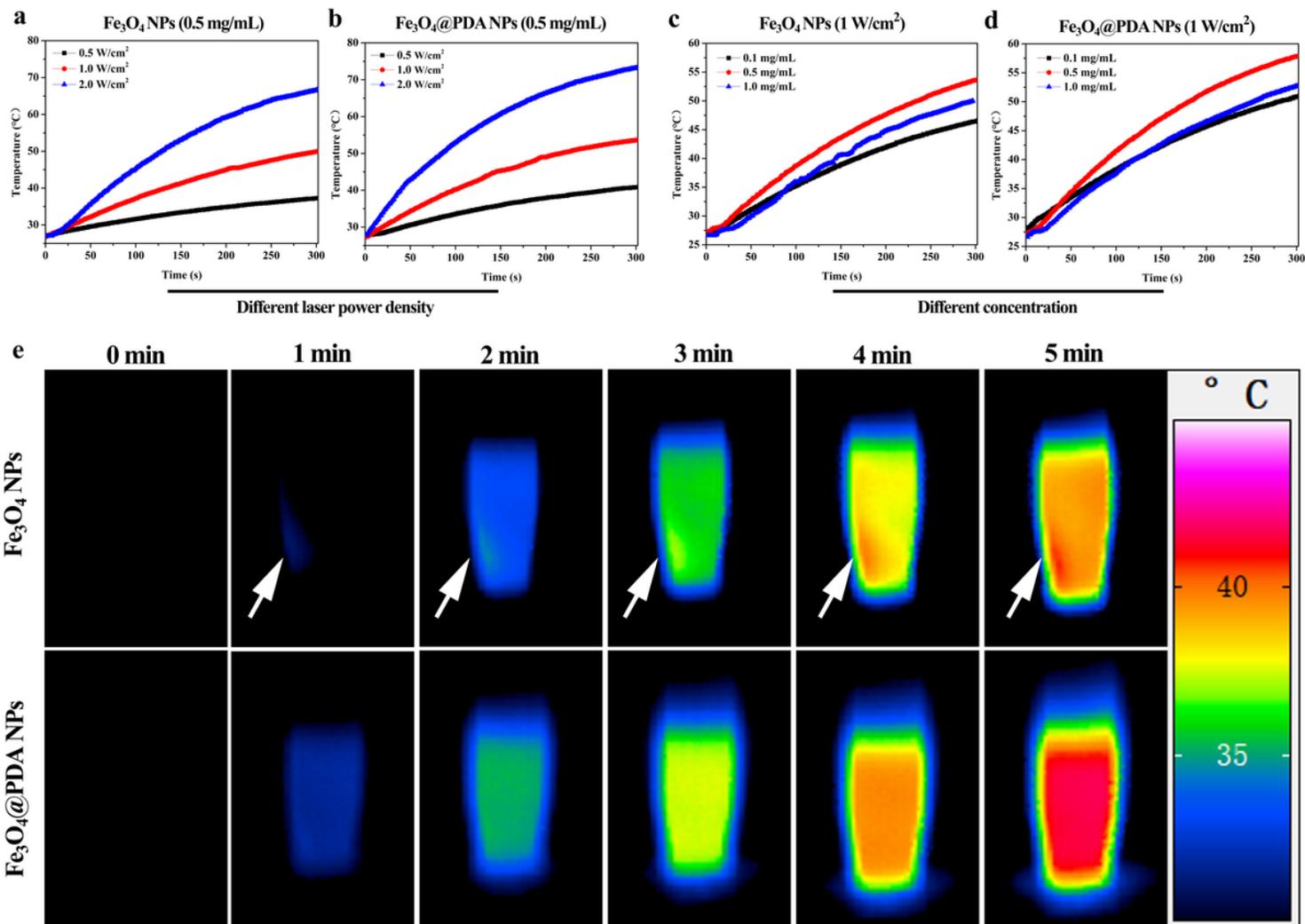


Figure 2

Temperature vs. time plots for (a) Fe_3O_4 NP and (b) Fe_3O_4 @PDA NP suspensions with a laser power density of 0.5 mg/mL. Temperature vs. time plots for (c) Fe_3O_4 NP and (d) Fe_3O_4 @PDA NP suspensions at different concentrations with a laser power density of 1 W/cm^2 for 5 min. (e) Photothermal images for Fe_3O_4 NP and Fe_3O_4 @PDA NP suspensions (0.5 mg/mL) under continuous NIR laser irradiation (1 W/cm^2) for 5 min. The white arrows indicate aggregation.

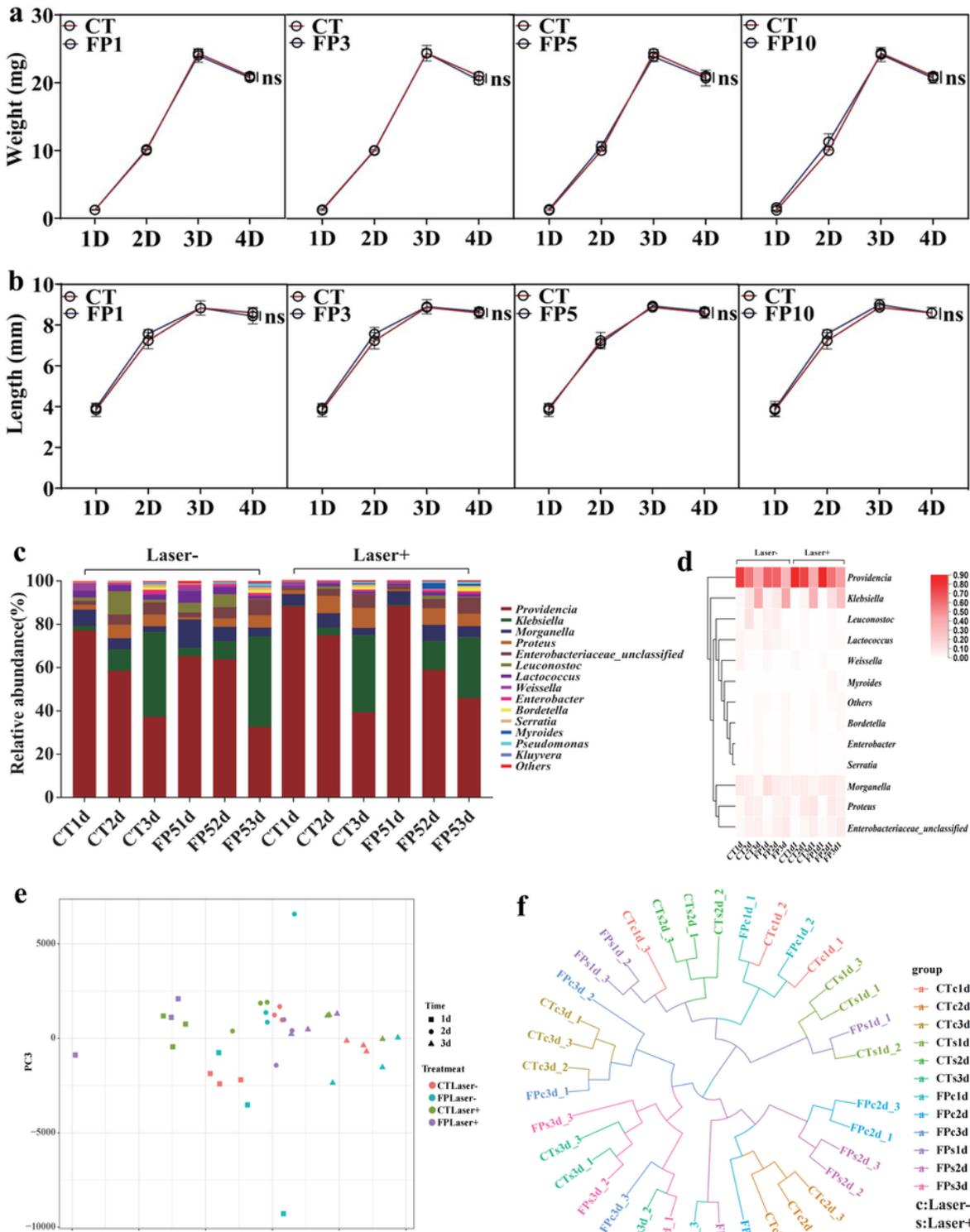


Figure 3

Effects of different $\text{Fe}_3\text{O}_4\text{@PDA}$ NP doses on the growth and development of housefly larvae. (a) Body lengths and weights of housefly larvae; (b) Housefly pupae weights. CT: sterile water. Repeated measures ANOVA followed by Sidak correction was used for multiple comparisons. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Effects of $\text{Fe}_3\text{O}_4\text{@PDA}$ NPs on the gut microbiota of housefly larvae. (c) Relative abundance of bacterial components in the intestines of housefly larvae before and after NIR laser irradiation. Each

treatment included three biological replicates. (d) Heatmaps of relative abundances and distributions of bacterial genera in housefly larvae. Heatmap based on the bacterial genera of different groups and color coded by genus following the legend in the panel. (e) Principal component analysis (PCA) of bacterial community structures for different groups. Each symbol represents one sample of intestinal bacteria. (f) UPGMA evolutionary tree analyses of samples. Laser-: before NIR laser irradiation; Laster+: after NIR laser irradiation. 1 d, 2 d and 3 d represent the development times of housefly larvae.

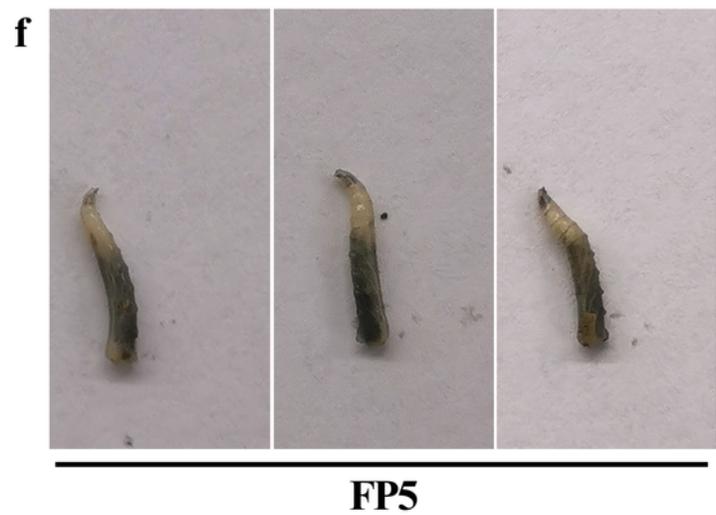
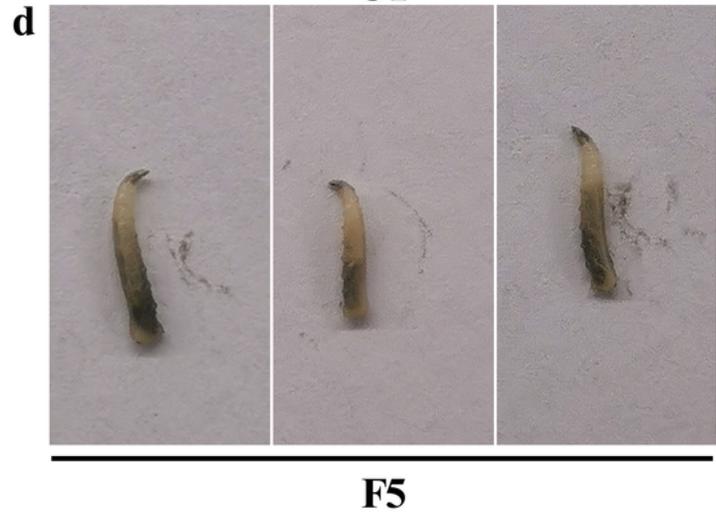
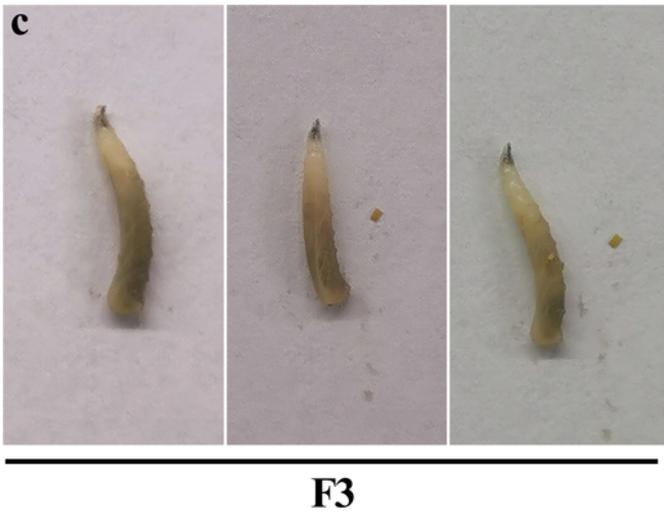
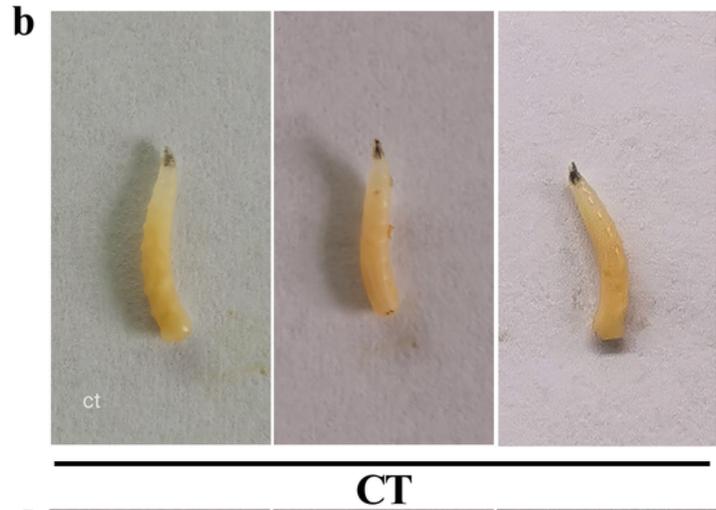
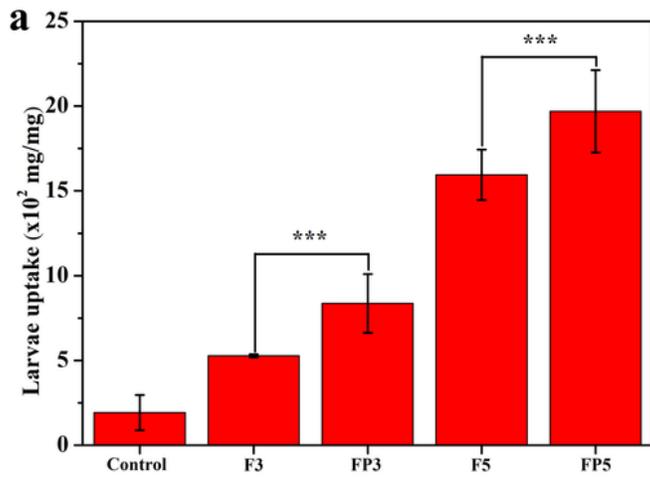


Figure 4

(a) Uptake data for F3 vs. FP3, F5 vs. FP5. Data are reported as the ratio of Fe content in larvae to the body weight. Representative photographic image of (b) control larvae and larvae after feeding with (c) F3, (d) F5, (e) FP3, and (f) FP5 for two days. NP-free feeding materials were used to feed larvae serving as controls.

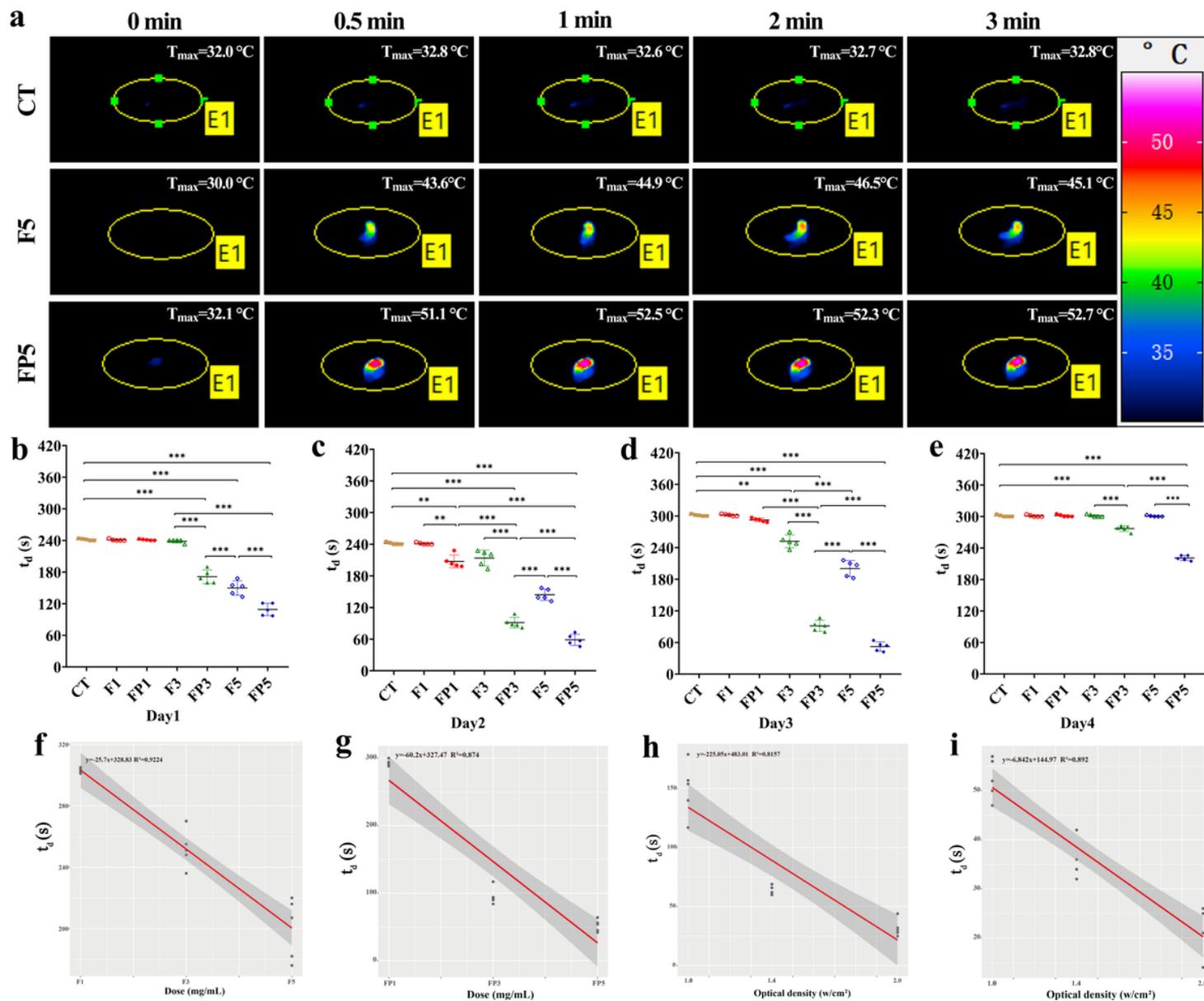


Figure 5

(a) Thermal images of F5 and FP5-fed larvae after exposure to NIR irradiation for 3 min (1 W/cm^2). The time of larval death (t_d) after irradiation by an NIR laser for houseflies fed with different doses of Fe_3O_4 and $\text{Fe}_3\text{O}_4\text{@PDA}$ NPs: (b), (c), (d), (e) represent the development times of the larva. Correlation between the death time of larvae irradiated by the NIR laser and the doses of (f) Fe_3O_4 and (g) $\text{Fe}_3\text{O}_4\text{@PDA}$ NPs; correlation between the death time of larvae fed with (h) Fe_3O_4 and (i) $\text{Fe}_3\text{O}_4\text{@PDA}$ NPs and irradiated by

the NIR laser with different optical densities. CT: sterile water. The data were compared by using one-way ANOVA. Significance analysis was performed with Fisher's LSD test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Each treatment included five biological replicates.

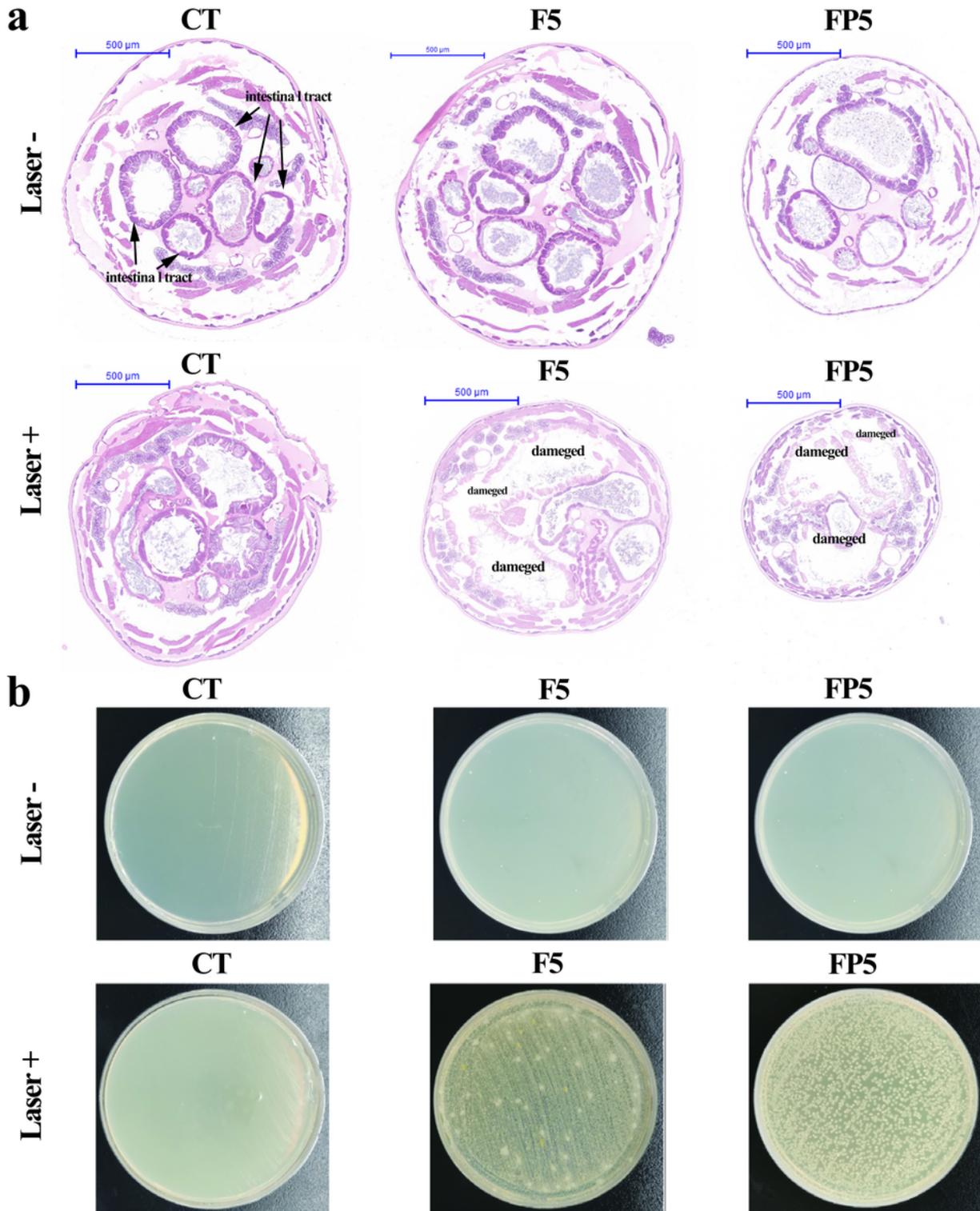


Figure 6

(a) Representative H&E-stained images and (b) intestinal bacterial leakage tests of larvae collected before and after NIR laser irradiation. Each treatment included three biological replicates. CT: sterile water. Laser-: before NIR laser irradiation; Laster+: after NIR laser irradiation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryinformationNC.docx](#)
- [floatimage1.png](#)