



***In vivo* targeting of xenografted human cancer cells by functionalized fluorescent silica nanoparticles in zebrafish**

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Abstract

Developing nanoparticles capable of detecting, targeting, and destroying cancer cells is of great interest in the field of nanomedicine. *In vivo* animal models are required for bridging the nanotechnology to its biomedical application. The mouse represents the traditional animal model for preclinical testing; however, mice are relatively expensive and have long experimental cycles due to limited progeny from each mother. The zebrafish has emerged as a powerful model system for developmental and biomedical research, including cancer research. In particular, due to its optical transparency and rapid development, zebrafish embryos are well suited for real-time *in vivo* monitoring of the behavior of cancer cells and their interactions with their microenvironment. Here, we developed a method that sequentially introduces human cancer cells and functionalized nanoparticles in transparent *Casper* zebrafish embryos, and monitors *in vivo* recognition and targeting of these cancer cells by nanoparticles in real-time. Our optimized protocol shows that fluorescently labeled nanoparticles, which are functionalized with folate groups, can specifically recognize and target metastatic human cervical epithelial cancer cells that are labeled with a different fluorochrome. The recognition and targeting process can occur as early as 30 min post-injection of the nanoparticles tested. We also demonstrate that the whole experiment only requires the breeding of a few pairs of adult fish and takes less than four days to complete. Moreover, zebrafish embryos lack a functional adaptive immune system, allowing the engraftment of a wide range of human cancer cells. Hence, the utility of the protocol described here enables the testing of nanoparticles on various types of human cancer cells, facilitating the selection of optimal nanoparticles in each specific cancer context for future testing in mammals and the clinic.

SUMMARY:

Here we describe a method about how to utilize zebrafish embryos to study the ability of functionalized nanoparticles to target human cancer cells *in vivo*. This method allows for the

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evaluation and selection of optimal nanoparticles for future testing in large animals and in clinical trials.

Keywords

Zebrafish; *Casper*; transplantation; in vivo targeting; nanoparticles; cancer

INTRODUCTION:

The development of nanoparticles that are capable of detecting, targeting, and destroying cancer cells is of great interest for both physicists and biomedical researchers. The emergence of nanomedicine led to the development of several nanoparticles, such as those conjugated with targeting ligands and/or chemotherapeutic drugs^{1,2,3}. The added properties of nanoparticles enable their interaction with the biological system, sensing and monitoring biological events with high efficiency and accuracy along with the therapeutic application. Gold and iron oxide nanoparticles are primarily used in computed tomography and magnetic resonance imaging applications, respectively. While the enzymatic activities of gold and iron oxide nanoparticles allow the detection of cancer cells through colorimetric assays, fluorescent nanoparticles are well suited for *in vivo* imaging applications⁴. Among them, ultrabright fluorescent nanoparticles are particularly beneficial, due to their ability to detect cancers early with fewer particles and reduced toxicities⁵.

Despite these advantages, nanoparticles require experimentations using *in vivo* animal models for selection of the suitable nanomaterials and optimization of the synthesis process. Additionally, just like drugs, before their clinical testing, nanoparticles rely on animal models for preclinical testing to determine their efficacy and toxicities. The most widely used preclinical model is the mouse, which is a mammal with a relatively high cost. For cancer studies, either genetically engineered mice or xenografted mice are typically used^{6,7}. The length of these experiments often spans from weeks to months. In particular, for cancer metastasis studies, cancer cells are directly injected into the circulatory system of the mice at locations such as tail veins and spleens^{8–10}. These models only represent the end stages of metastasis when tumor cells extravasate and colonize in distant organs. Moreover, due to visibility issues, it is particularly challenging to monitor tumor cell migration and the targeting of tumor cells by nanoparticles in mice.

The zebrafish (*Danio rerio*) has become a powerful vertebrate system for cancer research, due to its high fecundity, low cost, rapid development, optical transparency, and genetic conservations^{11,12}. Another advantage of the zebrafish over the mouse model is the fertilization of their eggs *ex utero*. The embryos can be monitored throughout their development. In particular, embryonic development is rapid in zebrafish, and within 24 h post-fertilization (hpf), its vertebrate body plane has already formed¹³. By 72 hpf, eggs are hatched from the chorion, transitioning from the embryonic to the fry stage. The transparency of the zebrafish, the *Casper* strain in particular¹⁴, provides a unique opportunity to visualize the migration of cancer cells and their recognition and targeting by nanoparticles in a living animal. Finally, zebrafish develop their innate immune system by

48 hpf, with the adaptive immune system lagging behind and only becoming functional at 28 days post-fertilization¹⁵. This time gap is ideal for the transplantation of various types of human cancer cells into zebrafish embryos without experiencing immune rejections.

Here we describe a method that takes advantage of the transparency and rapid development of the zebrafish to demonstrate the recognition and targeting of human cancer cells by fluorescent nanoparticles *in vivo*. In this assay, human cervical cancer cells (HeLa) cells that were genetically engineered to express a red fluorescent protein were injected into the vascularized area in the perivitelline cavity of 48 hpf embryos. After 20–24 h, HeLa cells had already spread throughout the embryos through the fish circulatory system. Embryos with apparent metastasis were microinjected with ~0.5 nL nanoparticle solution directly behind the eye where the rich capillary bed is located. By applying this technique, we demonstrate that the ultra-bright fluorescent silica nanoparticles can target HeLa cells as short as 20–30 min post-injection. Due to its simplicity and effectiveness, the zebrafish represents a robust *in vivo* model to test a variety of nanoparticles for their ability to target specific cancer cells.

PROTOCOL:

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at Boston University School of Medicine, under the protocol #: PROTO201800543.

1. Generation of *Casper* zebrafish embryos

- 1.1. Choose adult *Casper* fish that are at least 3 months of age for natural breeding to generate transparent *Casper* zebrafish embryos.
- 1.2. Fill two-chamber mating tanks with fish water in the evening, separate the upper tanks using dividers, place one male fish into one side of the chamber and one or two female fish into the other side of the chamber, and leave the fish separated overnight by dividers.
- 1.3. Pull out the dividers the next morning at 8 am when the lights are on. Add artificial enrichment plants and tilt the top chamber slightly to create a shallow area of water. Allow the fish to breed for 3–4 h.
- 1.4. Lift the top chambers of the mating tanks that contain the fish and return them to their original tanks.
- 1.5. Collect eggs located in the bottom chambers by pouring the water through a mesh net. Transfer eggs to a sterile petri dish at a density no greater than 200 eggs per dish. Remove any dead or unfertilized eggs and fill the dish 2/3 full with fresh fish water.

Note: Fertilized and healthy eggs should be translucent and round. Any eggs that are cloudy, white, or disfigured should be removed. The fish water is obtained from fish tanks in the fish facility.

- 1.6. Incubate embryos in the incubator at 28.5 °C overnight.

1.7. Bleach the embryos the next morning using the standard protocol as described in *The Zebrafish Book*¹⁶, and put the embryos back to the incubator. (optional step)

1.8. Take the 24 hpf embryos out of the incubator in the afternoon, and dechorionate the embryos using pronase.

1.8.1. Remove as much fish water as possible from the embryos in the petri dish and add a few drops of the pronase solution (1 mg/mL in fish water) to the dish. Gently swirl the petri dish. Once the chorions show signs of disintegration, pipette the embryos up and down a few times to break down chorions to release embryos.

1.8.2. Add fresh fish water immediately into the petri dish to terminate the process once a majority of the embryos are out of the chorions. Rinse the embryos 3 more times using fish water to remove the floating chorions. Return the embryos to the incubator.

2. Preparation of human cancer cells for transplantation

2.1. Set the incubator temperature to 35.5 °C exactly. Monitor the incubator to ensure a consistent and stable temperature using a thermometer inside the incubator.

2.2. Autoclave 1 L fish water in a glass bottle. Make a 3% agarose solution by adding 3 g of electrophoresis grade agarose to 100 mL of autoclaved fish water and microwaving until the agarose is completely dissolved.

2.2.1. Pour hot agarose solution into a petri dish until it is $\frac{3}{4}$ full. Place the microinjection mold on the agarose. Ensure that the mold is not in contact with the bottom of the petri dish and no bubbles form underneath.

2.2.2. Allow the solution to solidify and carefully remove the mold from the plate. Fill the plate with autoclaved fish water and store the plate at 4 °C. Pre-warm the agarose plate and fish water in the 35.5 °C incubator before harvesting HeLa cells.

2.3. Pull borosilicate glass capillaries that are 1.0 mm O.D. $\times$ 0.78 mm on a pipette puller using the following settings: pressure at 500, heat at 560, pull at 100, velocity at 100, and time/delay at 200. Store needles on putty in a large petri dish that has been wiped with an ethanol towel.

CAUTION: Pulled needles are very sharp and fragile. Use caution when handling.

2.4. Prepare a stock solution of tricaine methanesulfonate (MS222, 4 mg/mL) by dissolving MS222 into autoclaved fish water. Vortex well before use. Dilute MS222 stock solution 1:100 in fish water (add 200 μ L MS222 stock solution to 20 mL of fish water, final concentration 40 μ g/mL) to anesthetize embryos in the following procedures.

2.5. Label HeLa cells by transducing them with PLenti6.2_miRFP670 lentivirus using the protocol as described¹⁷. Harvest RFP+ HeLa cells 30 min to 1 h before transplantation. Human HeLa cells have been cultured in a tissue culture incubator up

to 70% confluency in complete growth medium (DMEM medium with 10% FBS) at 37 °C supplemented with 5% CO₂.

2.5.1. Remove the medium of HeLa cells by aspiration in a tissue culture hood. Briefly rinse the cell layer with sterile PBS to remove all traces of the serum.

2.5.2. Add 3.0 mL of sterile Trypsin-EDTA solution to a T-75 flask and place the cells back into the 37 °C tissue culture incubator for 3–5 min to facilitate enzymatic digestion. Observe the flask under the microscope until ~80% of the cells become suspended.

2.5.3. Add 6 to 8 mL of complete growth medium into the flask. Collect the cells into a 15-mL sterile tube by gently pipetting. Centrifuge at 135 ref for 5 min.

2.5.4. Aspirate the supernatant and resuspend HeLa cells in 3 mL complete growth medium. Repeat the above washing step twice. Resuspend the cells in 1 mL complete growth medium and count the cells under the microscope using a hemocytometer. Spin down the cells again, remove supernatant, and resuspend the cells in a 1.5-mL microcentrifuge tube at a concentration of 5×10^7 cells/mL. Keep the cells warm by holding the tube in hand when transporting to the fish facility.

Note: Keep cells warm at all times by storing the cells inside the 35.5 °C incubator before or when injecting the embryos.

3. Transplantation of human cancer cells

3.1. Clean up the work area using ethanol towels before transplantation (*e.g.*, scissors, tweezers, plastic pipettes, razor blades).

3.2. Align embryos within the grooves of the agarose plate using a plastic pipette. Lay embryos on the side with the anterior facing forward.

Note: Make sure that the fish water covers the embryos. Set some embryos aside without injecting cancer cells as controls.

3.3. Turn on the air source and microinjector. Take HeLa cells out of the incubator and pipette the cells up and down 20–30 times using a P200 tip. Load 3 µL of cell mixture immediately into a needle using a gel loading tip that has the end cut. Carefully insert the tip toward the sharp bottom end of the needle. If needed, shake the needle to ensure the cell mixture moves down the needle to fill up the sharp end.

3.3.1. Insert the needle into the needle holder. Use a pair of tweezers to carefully break open the tip of the needle. Adjust the pressure and duration of time on the microinjector to push out all of the air bubbles inside the needle tip. Reduce the pressure and injection duration time till the size of injection droplets is ~1 nL.

3.3.2. Place the injection plate under the microscope in an appropriate position to have the yolk side of embryos facing the needle. Anesthetize the embryos by adding five drops of the diluted MS222 solution (40 µg/mL).

3.3.3. Position the injector and allow the needle to touch the perivitelline cavity of each embryo.

3.4. Inject the cell mixture into the embryos at the vascularized area under the perivitelline cavity by pressing the foot pedal.

3.5. Use the left hand to move the injection plate to the next embryo. Use the right hand to extend and retract the injector while pressing the foot pedal simultaneously to continue the injection.

3.6. Pipette a few drops of sterile fish water to embryos that have been injected. Once the injection of all embryos on the plate is completed, wash the embryos off with sterile fish water to a sterile petri dish and immediately move them to the 35.5 °C incubator.

3.7. After 3 h, examine the injected embryos and remove the dead ones.

3.8. Return the live embryos to the 35.5 °C incubator and incubate them for 20 to 24 h to allow HeLa cells to spread from the injection site to other parts of the body.

Note: Embryos are kept in 35.5 °C incubator to allow the survival and migration of human cancer cells because they do not do well at 28.5 °C, the temperature fish embryos are normally incubated.

4. Injection of nanoparticles or vehicle

4.1. Anesthetize the transplanted embryos the next morning with five drops of diluted MS222 solution (take care not to add too much MS222 as this will kill the embryos). Under a fluorescent microscope, carefully pick up embryos with tail metastasis of RFP+ HeLa cells and place them into a new petri dish with sterile fish water.

4.2. Make the injection needles as previously described in section 2 using the following setting: pressure at 500, heat at 645, pull at 60, velocity at 50, and time/delay at 100.

4.3. Follow the procedures as described in 3.1–3.3 to align embryos and load vehicle (H₂O) or the nanoparticle solution into the needle.

4.4. Inject 0.5 nL of 1 mg/mL nanoparticle solution behind the eye and continue the injection following procedures as described in 3.5–3.6 (Figure 1B). This location behind the eyes is enriched with capillaries allowing the nanoparticles to enter circulation.

4.5. Follow a similar procedure, inject the vehicle (H₂O) that was used to resuspend nanoparticles into embryos with or without HeLa cells transplanted as controls (Figure 1A,C).

4.6. Incubate all injected embryos in the 35.5 °C incubator.

5. Imaging and tracking of nanoparticles and cancer cells

5.1. Examine injected embryos under a fluorescent microscope at 0, 30, 60, 90, 120, 180, and 210 min post-injection of nanoparticles to monitor the distribution of

nanoparticles in circulation and the degree of targeting of cancer cells. The targeting of cancer cells by nanoparticles can be observed as early as 30 min post-injection depending on the type of nanoparticle tested.

5.2. Pipet 2–3 embryos into a petri dish and immobilize them by adding five drops of diluted MS222 solution (40 µg/mL). Once the embryos stop swimming, remove a majority of the water to allow the embryos to lie on their sides.

5.3. Use a pipet with a thin soft brush attached at the end to align the embryos so they lie on their sideways with the anterior facing forward and only one eye visible.

5.4. Image the embryos in red, blue, and brightfield channels at low magnification (2x) to capture the whole embryo and repeat at higher magnification (6.4x) to capture the tail area. Focus the embryo under the red channel to avoid the bleeding of nanoparticles.

Note: The embryos must not move during imaging. Any movement will lead to blurry images and the inability to overlap images from different channels.

5.5. Add fresh fish water to embryos immediately after imaging and return them to the incubator. Repeat step 5.2–5.4 to image the embryos at different time points.

REPRESENTATIVE RESULTS:

The protocol schematic in Figure 1 illustrates the overall procedures for this study. Transparent *Casper* male and female adult fish were bred to generate embryos (Protocol Section 1). RFP+ HeLa cells were injected into the vascularized area under the perivitelline cavity of zebrafish embryos at 48 hpf, with uninjected embryos as controls (Protocol Section 3). For individuals who are experienced in microinjection, the survival rate of embryos is often high, with at least 50% of embryos transplanted with cancer cells surviving in the 35.5 °C incubator, a temperature suboptimal for zebrafish embryos but required for the survival and migration of human cancer cells. HeLa cells are highly invasive and can intravasate and spread to the tail region of the embryos as early as 8 h post-injection. By 20–24 h post-transplantation, ~50% of embryos transplanted show signs of metastatic spread of HeLa cells. We selected the embryos with tail metastasis of cancer cells for the downstream experiments. At 72 hpf, these embryos were subsequently injected with blue fluorescent nanoparticles behind the eyes (Protocol Section 4 and Figure 1B), using embryos injected with the vehicle as controls (Figure 1A). Meanwhile, we also injected nanoparticles into age-matched embryos without cancer cell transplantation as the second group of controls (Figure 1C). Please see our recent publication for the detailed information for nanoparticle synthesis, preparation, and characterization¹⁸.

At 0, 30, 60, 90, 120, 180, 210 min post-injection of nanoparticles, we monitored the injected embryos by imaging to determine the interaction of nanoparticles with RFP+ HeLa cells, using the vehicle injected embryos as controls. Specifically, we imaged the zebrafish tail areas where RFP+ HeLa cells had spread to at red, blue, and brightfield using a fluorescent microscope (Protocol Section 5). The detailed characterization for the ability of our ultra-bright nanoparticle to target xenografted cancer cells in zebrafish over time is

shown in Figure 5 of our paper recently published in *Nanoscale*¹⁸. The red dots in the tail of embryos were metastatic human cervical cancer cells that were visible in both vehicle and nanoparticle injected embryos (Figure 2A,D; Figure 3A,D). As expected, we failed to detect any specific blue fluorescent signals in embryos with the vehicle injection (Figure 2B,E). Additionally, when we merged the images captured in the red and blue channels, we only observed red cancer cells in the tail region without any blue signals (Figure 2C,F). However, in embryos that were injected with ultra-bright fluorescent silica nanoparticles, there were blue dots in the tails, concentrated near and around the cancer cells at 3.5 h (Figure 3B,E). In the overlaid images captured from both red and blue channels, we also observed the co-localization of red HeLa cells and blue nanoparticles, indicated as pink dots (Figure 3C,F). We also injected nanoparticles into the embryos that were not transplanted with HeLa cells. Instead of concentrating into particular cells or areas, the blue fluorescent particles in these embryos distributed relatively evenly into the circulatory system of embryos, highlighting blood vessels (Figure 4B,E). As expected, we could not detect any specific red fluorescent signals in these embryos despite some weak background fluorescent signals (Figure 4A,C,D,F).

We subsequently applied this protocol to test different types of nanoparticles^{18–20}. Depending on the properties of the nanoparticle tested, we observed co-localization of cancer cells with certain types of nanoparticles as early as 30 min post-injection. By 120 min, there was >80% targeting of cancer cells by these nanoparticles in the tail region of the fish. However, for other nanoparticles, minimal targeting of cancer cells was observed, consistent with their lack of cancer-specific ligand. The detailed results and analysis are included in our recent publication in *Nanoscale* (see Figures 3–4, supplementary Figures S12–S16, and supplementary Table S6)¹⁸. These results demonstrated the differential targeting of nanoparticles to xenografted HeLa cells in zebrafish. Thus, using this protocol, one should be able to efficiently select nanoparticles based on their ability to recognize and target metastatic human cancer cells *in vivo*.

DISCUSSION:

The protocol described here utilizes the zebrafish as an *in vivo* system to test the ability of nanoparticles to recognize and target metastatic human cancer cells. Several factors can impact the successful execution of the experiments. First, embryos need to be fully developed at 48 hpf. The correct developmental stage of the embryos enables them to endure and survive the transplantation of human cancer cells. We found that embryos younger than 48 hpf have a significantly lower survival rate compared to older and more developed embryos. Second, cancer cells should be as healthy as possible by ensuring that cells are: a) in the exponential growth phase²¹, b) freshly harvested 30 min to 1 h right before transplantation, and c) kept warm at all times. Third, the needle must not be clogged. Pipet the HeLa cells up and down at least 20 times before loading the cell mixture into the needle. Fourth, different types of needles are used for transplantation of human cancer cells and the injection of nanoparticles. The needle for human cell transplantation is relatively wide with an angle to avoid cell clogging, whereas the needle for nanoparticle injection is sharp and thin. Fifth, the location of the injection differs. The location for transplantation of human cells is the perivitelline cavity, but for nanoparticle injection, the needle should be inserted

behind the eye where there are enriched capillaries. Finally, the skill of the individual who performs transplantation matters. An experienced individual can accurately inject HeLa cells into the perivitelline cavity space, while an inexperienced person often injects tumor cells into the yolk area where tumor cells barely spread into the fish body. Similarly, the embryos survival rate is much higher for the experienced individual, with at least 50% of embryos transplanted with cancer cells surviving.

Although zebrafish embryos are usually incubated at 28.5 °C, human cancer cells require higher temperatures to survive and migrate^{22,23}. To allow the survival of both fish embryos and human cancer cells, we incubate the embryos transplanted with human cancer cells at 35.5 °C instead. Although it may be easier to deliver cancer cells into the yolk sac, they barely spread into the circulatory system. Therefore, it is critical to inject the cancer cells into the vascularized area under the perivitelline cavity to ensure intravasation and spread of cancer cells. Additionally, one must take care not to add too much or too concentrated MS222 when anesthetizing the embryos during injection and imaging. To aid in imaging and visualization of nanoparticles, we chose the *Casper* zebrafish instead of AB fish for our experiments. The *Casper* fish is a double mutant for *Nacre* and *Roy* that lacks melanocytes and iridophores, resulting in increased transparency compared to AB fish¹⁴. The transparency of the *Casper* zebrafish enables us to monitor the spreading of nanoparticles in circulation and the targeting of the nanoparticles to cancer cells. The challenge of this protocol is the relatively high mortality rate of the embryos if an inexperienced individual performs the transplantation of human cancer cells. Interestingly, the injection of nanoparticles behind the eyes barely is rather well tolerated. This is likely due to the use of thinner needles compared to the needles used for tumor cell transplantation. To avoid damaging human cancer cells, one must use needles with wide openings that can lead to damage of embryos if handled inappropriately.

We developed this protocol that utilizes the *Casper* zebrafish to visualize the targeting of metastatic cancer cells with functionalized nanoparticles *in vivo*. A major advantage of this assay is that it allows the researchers to perform real-time imaging over the course of zebrafish development to monitor the interaction of cancer cells with nanoparticles. In fact, due to its high fecundity and rapid development, the zebrafish helps the researcher to obtain results in just a few days^{18–20,24}. Moreover, this assay also allows the elimination of toxic nanoparticles if most embryos die after injection of a particular type of nanoparticle. Although the zebrafish is not a mammal, it facilitates the selection of a large number of nanoparticles in a rapid and economical manner, providing useful information for downstream studies in large animals and clinical testing. Taken together, the zebrafish is making an impact in nanomedicine and nanotechnology by helping select suitable nanoprobe for early detection and potential destruction of cancer cells through cancer-specific targeting.

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DISCLOSURES:

I.S. declares interest in NanoScience Solutions, LLC (recipient of STTR NIH R41AI142890 grant). All other authors declare no conflicts of interest.

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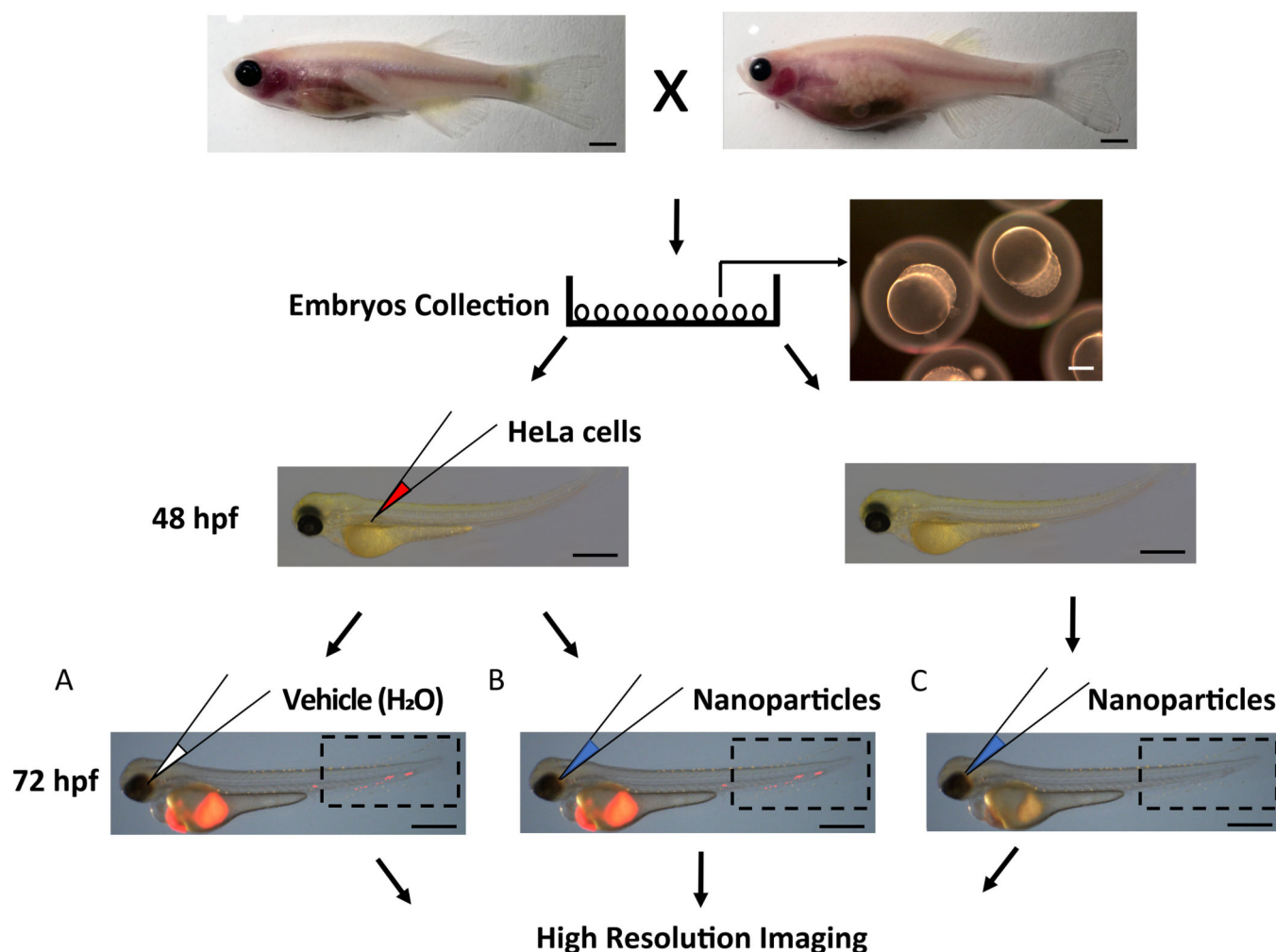


Figure 1. Protocol schematic for studying the ability of nanoparticles to target human cancer cells.

Transparent *Casper* embryos are generated through breeding male and female adult fish. Fertilized embryos are collected in a petri dish. At 48 hpf, RFP+ HeLa cells are injected into zebrafish embryos at the perivitelline cavity, leaving some age-matched embryos uninjected as controls. At 72 hpf, embryos with metastatic RFP+ HeLa cells are selected and split into two groups: **A**) injected with vehicle (H₂O) as control and **B**) injected with nanoparticles suspended in H₂O. The third group is age-matched embryos that are injected with nanoparticles alone (**C**). All three groups are imaged under a fluorescent microscope. The boxed area is where images will be captured (see Figures 2–4). Scale bars for adult fish = 1 mm and for embryos = 500 μ m.

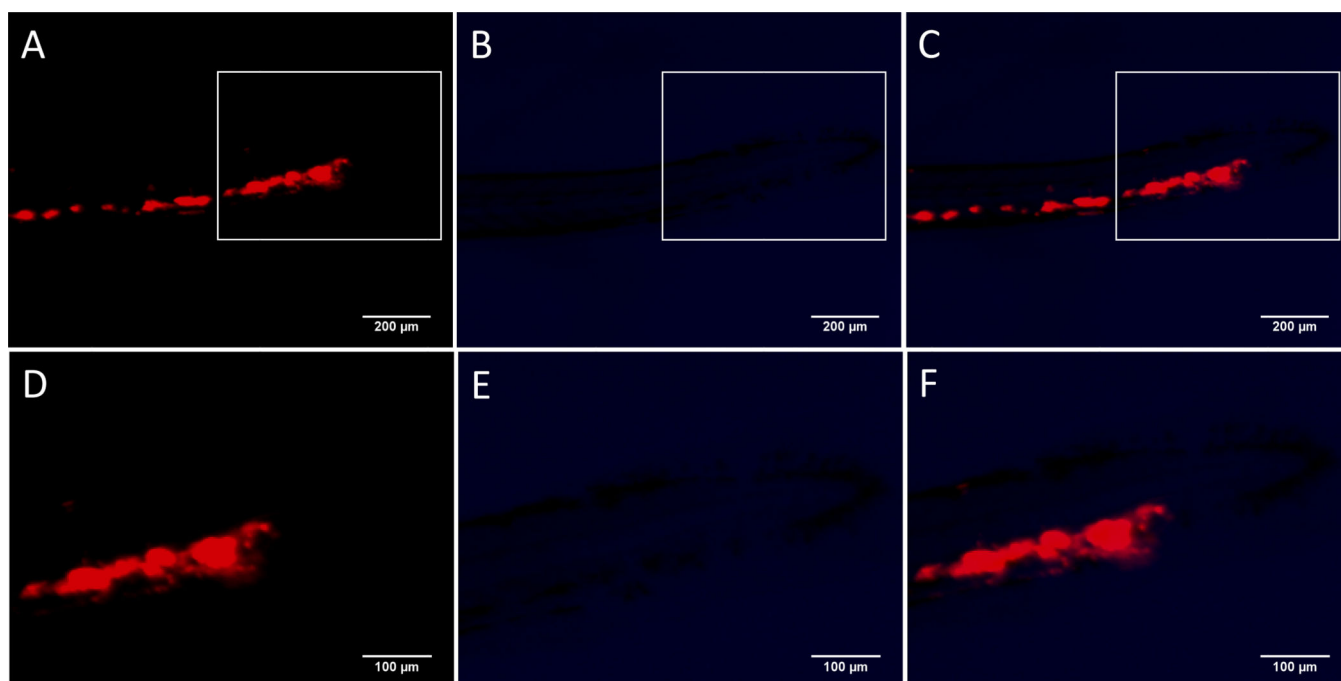


Figure 2. Zebrafish transplanted with metastatic HeLa cells without nanoparticles.

Only red fluorescent HeLa cells are visible in the individual (A,D) or overlaid images of the red channel and blue channel (C,F). No specific blue fluorescent signals are detected in the embryo with vehicle injection control (B,E). Images in (A-C) show the fish tail region as boxed in Figure 1A. Images in (D-F) are enlarged views of the boxed areas in (A-C). Scale bars in (A-C) = 200 μm and in (D-F) = 100 μm .

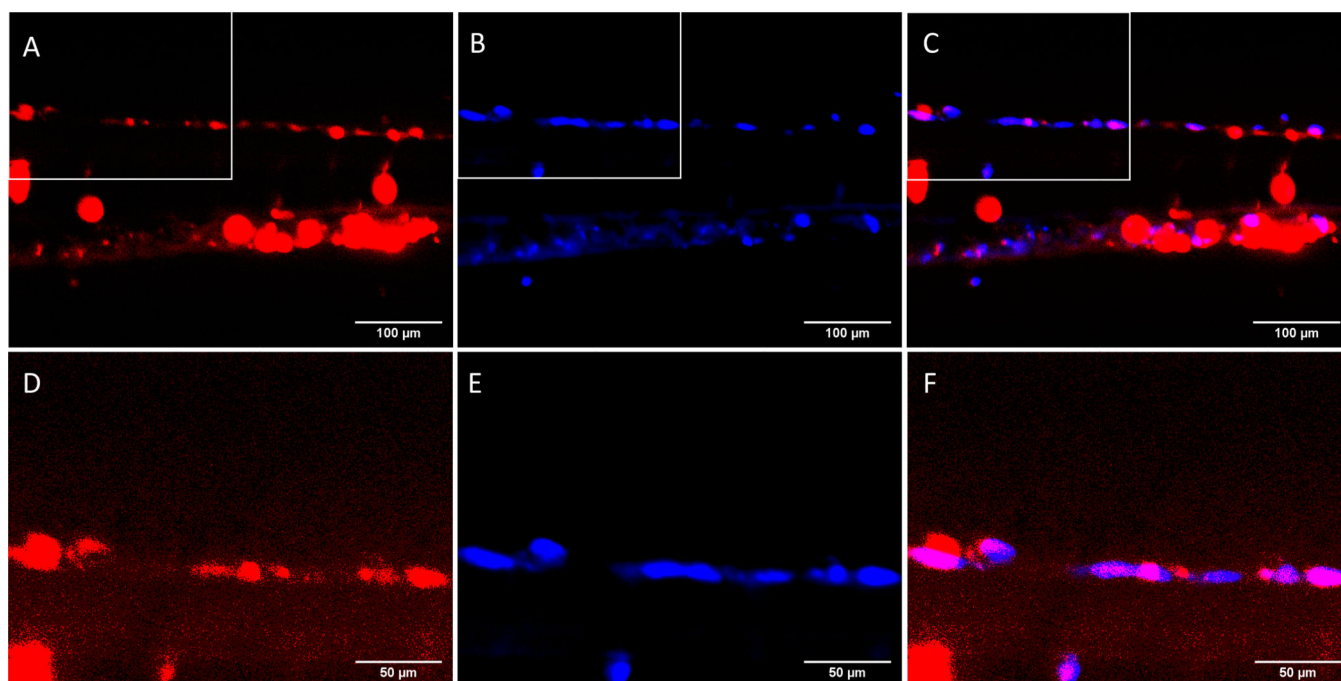


Figure 3. Co-localization of red fluorescent HeLa cells and blue fluorescent nanoparticles in zebrafish.

The zebrafish tails are imaged at both low (**A-C**) and high (**D-F**) magnification in the red and blue channel. Red fluorescent signals reveal metastatic HeLa cells (**A,D**), whereas blue fluorescent signals document the nanoparticles (**B,E**). The overlaid images from both red and blue channels (**C,F**) show co-localization of HeLa cells and nanoparticles. The images are taken after 3.5 h of injection with ultra-bright silica nanoparticles. Images in (**A-C**) show the fish tail region as boxed in Figure 1B. Images in (**D-F**) are enlarged views of the boxed areas in (**A-C**). Scale bars in (**A-C**) = 100 μm and in (**D-F**) = 50 μm .

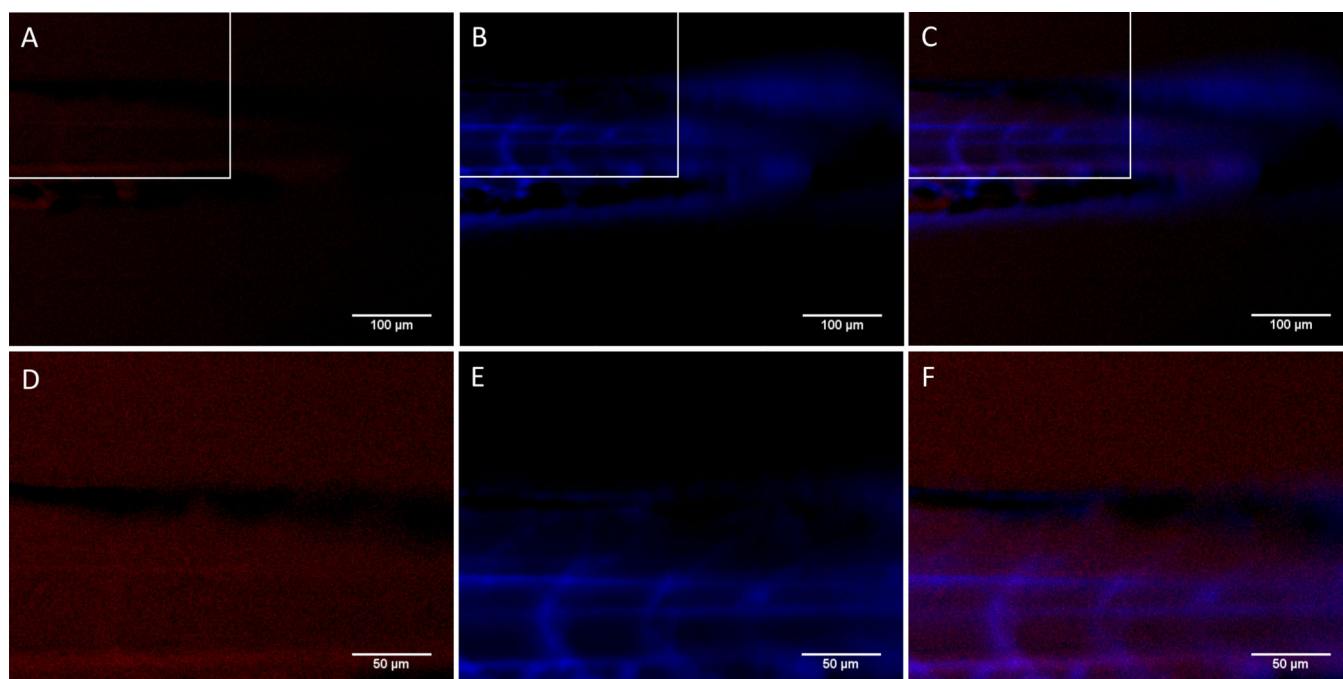


Figure 4. Zebrafish injected with nanoparticles without human HeLa cells.

Blue fluorescent nanoparticles are distributed into the circulatory system of the embryos in the individual (B,E) and overlaid images of the red and blue channel (C,F). No specific red fluorescence is visible at either low or high magnification (A,D) except some background fluorescence common to zebrafish embryos. Images in (A-C) show the fish tail region as boxed in Figure 1C. Images in (D-F) are enlarged views of the boxed areas in (A-C). Scale bars in (A-C) = 100 μm and in (D-F) = 50 μm .

Table of Materials

Name of Material/ Equipment	Company	Catalog Number	Comment/Description
pLenti6.2_miRFP670	Addgene	13726	
Pronase	Roche-Sigma-Fisher	50–100–3275	Roche product made by Sigma- sold by Fisher
Agarose	KSE scientific	BMK-A1705	
Borosilicate glass capillaries	World Precision Instruments	1.0 mm O.D. × 0,78 mm	
PBS (Dulbecco's Phosphate-Buffered Salt Solution 1X)	Corning	21–030-CV	sold by Fisher
Tricaine methanesulfonate	Western Chemicals	NC0872873	sold by Fisher
DMEM (Dulbecco's Modified Eagle's Medium)	Corning	10–013-CV	sold by Fisher
Trypsin-EDTA	Corning	MT25053CI	sold by Fisher
Fetal Bovine Serum	Sigma-Aldrich	F0926	
Microloader tip	Eppendorf	E5242956003	sold by Fisher
Magnetic stand	World Precision Instruments	M10	
Needle Puller	Sutter instruments	P-97	
Micromanipulator	Applied Scientific Instrumentation	MMPI-3	
Pneumatic pico pump	World Precision Instruments	SYSPV820	
Hemocytometer	Fishersci brand	02–671–51B	
Plastic pipette	Fishersci brand	50–998–100	
Petri dish	Corning	SB93102	sold by Fisher
Tweezer	Fishersci brand	12–000–122	
Razor blade	Fishersci brand	12–640	
P200 tip	Fishersci brand	<u>07–200–293</u>	
Olympus MVX-10 fluorescent microscope	Olympus	MVX-10	
SZ51 dissection microscope	Olympus	SZ51	
Fish incubator	VWR	35960–056	
Computer and monitor	ThinkCentre	X000335	