

DEVELOPMENT OF CANCER TRAP FOR CAPTURING CANCER CELLS
THAT METASTASIZE IN THE PERITONEAL CAVITY:

A PRELIMINARY STUDY

by

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Presented to the Faculty of the Graduate School of
The University of Texas at Arlington in Partial Fulfillment
of the Requirements
for the Degree of

MASTER OF SCIENCE IN BIOENGINEERING

THE UNIVERSITY OF TEXAS AT ARLINGTON

MAY 2017

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Acknowledgements

I wish to express my profound gratitude to my project supervisor, Dr. Liping Tang for his professional supervision throughout the course of this study. I would like to also thank Dr. Hsieh and Dr. Nair for sparing their time to review this work and provide help when I needed it. My immense gratitude also goes to my parents; Prof. and Dr. Mrs. Okpokwasili, siblings, colleagues, and friends met throughout my journey at University of Texas at Arlington. I also want to thank my fellow researchers in the engineering teaching lab for always being there when I needed one form of help or the other.

April 21, 2017

Abstract

DEVELOPMENT OF CANCER TRAP FOR CAPTURING CANCER CELLS IN
PERITONEAL CAVITY: A PRELIMINARY STUDY

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The University of Texas at Arlington, 2017

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Our laboratory has recently developed an implantable device - cancer trap that can recruit and trap circulating cancer cells inside the bloodstream. Since several types of cancer cells can metastasize inside the peritoneal cavity, this study explores the possibility of using cancer traps to recruit esophageal and prostate cancer cells inside the peritoneum.

Using prostate cancer and esophageal cancer cells as model cancers, this thesis shows that cancer traps can capture cancer cells inside the peritoneal space. EPO releasing traps was also shown to reduce the spread of prostate cancer as well as esophageal cancer compared to controls. Using multiple cancer traps was found to continuously recruit cancer cells with histology results showing that cells are more concentrated on the periphery of the implant with good penetration to the center of the implant.

The results of this work lend strong support to the development of cancer traps for capturing cancer cells inside the peritoneum. However, further studies are needed with larger number of animal and improved cell quantification methods. In conclusion, this thesis has identified several critical conditions and directions for the development of peritoneal cancer traps.

April 5, 2017

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CHAPTER 1

INTRODUCTION

1.1 Spread of cancer through the peritoneal cavity

The transcoelomic spread means spread of cancer across a body cavity with the peritoneal cavity being one of them (Epenetos et al., 1987). According to Griffin et al., cancers that can spread through this route include ovarian cancer with peritoneal dissemination being the most common form of spread to the liver, diaphragm, greater momentum and bowel serosa. Primary lung cancers and mesothelioma can also spread through the pleural space (Jones et al., 2012). This can happen during surgical manipulation. There is transcoelomic spread of colorectal carcinoma if the tumor perforates the peritoneal membrane and gastric cancers are known to spread to the ovary through this route (Krukenberg tumors). Renal cell carcinoma also metastasizes towards the underside of the diaphragm and liver through this route (Griffin et al., 2011). Lower esophageal cancers have a predilection to spread in the peritoneal cavity. Serosal involvement of a tumor has been shown to make it easier for a tumor to gain access into the peritoneum to spread (Ludeman et al., 2007).

1.2 The pathogenesis of metastasis

Abnormal growth of tissues contains phenotypically and genotypically different populations of cancer cells with the capacity to take part in the process of metastasis. Different parts of an abnormal tissue growth have varying behaviors in motility, angiogenesis, invasion, cohesion and proliferation (Simone et. al., 1998). Metastasis is the dissemination of cells from the initial neoplasm to organs far away from it, and is often very difficult to treat. The interaction between factors contributing to cell stability and cancer cells contributes to cancer pathogenesis and

metastasis. Also, each organ microenvironment can reshape the feedback metastatic cancer cells have towards systemic treatment (Fidler, 2003).

Tumors go through a series of stages that prepare them for metastasis. Early tumor growth and cellular transformations are fueled by nutrients available through simple diffusion. This is followed by synthesis and secretion of angiogenic factors that lead to development of capillary networks and thereby extensive vascularization of the tumor. Different mechanisms occurring in parallel causes local invasion of tumor cells into the stromal tissue. The most frequent route for tumor cell passage into the body circulation are thin-walled venules like lymphatic channels which present narrow opposition to infiltration by tumor cells. After this, aggregates or single tumor cells detach and embolize with most of the tumor cells in the circulation getting destroyed rapidly. The ones that survive get trapped in capillary beds in organs far away from the primary tumor by either getting attached to the exposed sub-endothelial basement membrane or capillary endothelial cells. Next, extravasation occurs, leading to proliferation of cancer cells within the parenchyma of the affected organ. The micrometastasis which are cancer cells too tiny to detect must escape extermination by the host defenses and develop a vascular network to keep on growing. This makes it easy for the cells to invade the blood vessels, get into the circulation and continue to generate supplementary metastases (Fidler, 2003). Varying growth factors and cell-surface receptors are expressed by vascular endothelial cells of different organs and these affect the variation of metastases which develop (Simone et. el., 1998).

1.3 Mechanism of transcoelomic spread

According to a previous study (Lengyel 2010), ovarian cancer cells metastasize passively by peritoneal fluid movement to peritoneum and omentum. Here, just before they develop the capacity to invade, they go through an epithelial-to-mesenchymal transition (EMT) by losing E-cadherin (necessary for cell-cell adhesion) and up regulating other cadherins. By a physiological peritoneal fluid movement, they spread to the peritoneum and attach to the secondary site due to up-regulation of fibronectin receptors after losing E-cadherin. Finally, they undergo mesenchymal-to-epithelial transition to an epithelial phenotype to grow fast and respond to paracrine growth factors.

1.3.1 Regulation of metastasis

The cancer metastasis process consists of a lengthy process and series of consecutive interdependent steps when one step fails or is not sufficient the entire process may fail. The host cell response and the innate property of the tumor cell determines the outcome of the process (Fidler, 2002). The properties of the tumor cell which facilitate metastasis are production of growth factors and their receptors, angiogenic factors, cell motility, specific cell surface receptors and adhesion molecules regulate metastasis while antigenicity, angiogenesis inhibitors and tissue inhibitors of proteolytic enzymes inhibits metastasis. The properties of the host cell which facilitate metastasis are paracrine and endocrine growth factors, neovascularization, platelets, platelet products and immune cells while the inhibitors of the host cell metastasis are tissue barriers, anti-proliferative factors and inhibitors of angiogenesis (Fidler, 2003). Each organ microenvironment can reshape the feedback a metastatic cancer cell has towards systemic

treatment and neoplastic progression is peculiar to cancer and is the acquirement of long-lasting, permanent subjective transformation in a neoplasm.

As metastasis is the major cause of fatalities due to cancer, it is plausible that arresting this spread can improve survival outcomes. A few therapies have focused on this aspect. Anti-angiogenic drugs are possible forms of anti-cancer therapy and these include bevacizumab which is a monoclonal antibody which acts against vascular endothelial growth factor (VEGF) (Sato, 2010). The problems with these forms of treatment (discovered in the preclinical and clinical environment) are that the benefits wear off and then the tumor progression and growth is restored (Bergers et. al., 2008). A newer approach to deal with arresting the spread of cancer needs to be developed either separately or in combination with well-established therapy to combat this.

1.4 Cancer traps

The concept of trapping cancer cells involves the implantation of a scaffold into an animal to capture spreading cancer cells, disrupting its spread and helping in their early detection. In an earlier study by our group (Ko et al. 2012), chemokine (erythropoietin (EPO)) releasing particle-based scaffolds was found to recruit far more melanoma cells than controls. This was one of the earlier studies that focused on the concept of trapping cancer cells to prolong the life-span of cancer afflicted animals. The animal model used here was initially implanting subcutaneous biomaterial microspheres into C57BL/6J mice which produced a localized inflammatory response and then followed by injecting the cancer cells into the blood circulation or peritoneal cavity. The metastatic cancer cells used were B16F10 melanoma cells, human prostate adenocarcinoma (PC-3), Lewis Lung carcinoma (LLC) cells, human breast cancer cell line (MDA- MB-231) and rat prostate cancer cell line (JHU-31). The results showed that the

EPO releasing scaffolds, and not the stromal derived growth factors-1 α -releasing (SDF-1 α) scaffolds, accumulated more melanoma cells than controls and in the process, increase the life span of the mice by 30%. Subsequently, a microporous poly(ϵ -caprolactone) scaffold developed by Rao et al. has been used to capture early metastatic breast cancer cells injected into the mammary fat pad *in vivo*, 1-month post implantation of scaffold. Surgical extraction of the primary tumor in this study was shown to improve survival significantly due to a reduction of CD11b⁺Gr1^{hi}Ly6C⁻ cells (Rao et al., 2016). Poly(lactide-co-glycolide) (PLG) microporous scaffold implants have also been used to recruit orthotopically implanted luciferase-expressing MDA-MB-231BR breast cancer cells with the implant put in a week after tumor inoculation (Azarin et al., 2015). These studies were shown to reduce the tumor burden.

1.4.1 Intra-peritoneal cancer trap development

In the previous study carried out by Ko et al., the traps were implanted into the subcutaneous space. This study used IV cells, the trap was particle-based which could scatter when implanted and cannot be used inside the peritoneum. With this the possibility of the cancer traps being as effective in the subcutaneous space as the peritoneum wasn't investigated. For that, a new cancer trap design is required.

CHAPTER 2

OVERALL HYPOTHESIS

There is no cancer trap for intraperitoneal cancer. It would be desirable to develop implantable biomaterial cancer traps to recruit and capture cancer cells that spread intraperitoneally. This would thereby reduce the tumor burden and increase longevity. Our recent studies have accidentally discovered that biomaterial implantation prompted the recruitment of cancer cells. Based on results obtained by others and from our studies, chemokines like EPO are known to recruit cancer cells. Infusing EPO into our trap would help increase the number of possible cancer cells recruited and keep them near the trap. In the long run, the trap should be able to distract metastasizing prostate cancer cells from spreading to healthy organs into the trap that would be irradiated or removed surgically. This research was designed to test a general hypothesis that “Cancer traps can be developed to implant in the peritoneal cavity for capturing cancer cells which spread through the peritoneum”.

CHAPTER 3

CANCER TRAPS INFLUENCE CANCER CELL RECRUITMENT

3.1 Rationale

Erythropoietin (EPO) is a hematopoietic cytokine which has role in the regulation and production of red blood cells (Krantz, 1991). According to Szenajch et al., (2010), EPO applies its cellular effects by connecting to its special transmembrane receptor which is part of the Type I cytokine receptor superfamily which is known to not have an innate tyrosine kinase enzymatic activity. In animal models for cancer research, interruption of the EPO to EPOR signaling pathways in tumors was correlated with anti-tumor results in xenografts of melanoma and human genital tract cancers in female (Arcasoy et. al., 2005). EPO affects other cell types apart from hematopoietic cells and has been found to increase lymphangiogenesis and lymph node tumor metastasis increasing migration (Lee et. al., 2011).

In the clinical environment, the extent of controlling the behavior of cancer spread serves as a break to regulate the metastasis progress and converts a systemic to a localized disease where other curative pathways such as radiation, chemotherapy and surgery can prolong life expectancy in cancer patients (De la Fuente et. al., 2015). The goal of this research was to assess possibility of using a chemokine releasing trap to reduce the spread of cancer through the peritoneum.

3.2 Materials and Methods

3.2.1 Growth and maintenance of cancer cell lines

KYSE-30 (gifts from Dr. Zui Pan) esophageal cancer cell line was used for this study. Human prostate cancer cell line DAB2IP-KD PC3 (PC-3 with the DAB2IP gene knocked out) (gifts from Dr. Jer-Tsong Hsieh) was also used for this study. Trypsin was purchased from (Sigma, St. Louis, MO) and dissolved in phosphate-buffered saline (PBS). Culture media 50% RPMI (Roswell Park Memorial Institute) with 50% F12 (Sigma-Aldrich), 5% FBS and 1% penicillin was used in culturing KYSE-30 cells with 1.5×10^6 cells seeded into T75 flask. RPMI Media 1640 alongside 10% fetal bovine serum and 1% penicillin at a concentration of $800 \mu\text{g/ml}$ was used in culturing DAB2IP-KD cells. 3×10^6 cells were then seeded in 75 cm^2 culture dishes and incubated at a temperature of 37°C . The cells were sub-cultured a total of three times per week.

3.2.2 Cell labeling with imaging agents

For the labelling of the KYSE-30 esophageal cancer cells, DID vibrant cell labeling solution was used. 1ml of media was mixed with $5\mu\text{L}$ DID solution. Serine free culture media was used for the labelling protocol and each 1ml gotten was added to 3ml of complete culture media and washed twice. For the fluorescence labelling of the PC3KD cells, we prepared a quantity of 10 nM labeling solution, a volume of $1\mu\text{L}$ each of pre-mixed Component A and Component B Qtracker® were transferred into a 1.5 mL micro centrifuge tube. This was then incubated for a period of 5 minutes at room temperature. 0.2 mL of fresh complete growth medium was then combined with the mixed solution in the tube and vortexed for a period of 30 seconds. 1×10^6 cells from a cell suspension at about 1×10^7 cells/mL were finally transferred to the tube containing the labeling solution and incubated at a temperature of 37°C for 60 minutes. After incubation, the cells were washed twice using complete growth medium. They were loaded on a

well plate and analyzed with Kodak In-Vivo Imaging System FX Pro (Carestream Health Inc, New Haven, CT) (configured for 470nm excitation, 790nm emission, 30s exposure) for the PC3KD cells and 470nm excitation, 790nm emission, 10 seconds exposure for the KYSE-30 cells. A hemocytometer was used to calculate the total cell number.

3.2.3 Production of cancer trap

To prepare the cancer trap, a transparent polyurethane tube was cut to 1.5 centimeter in length and filled with 10 milligrams of cotton. Four equal sized and spaced holes were made using a pair of scissors on the wall of the tube and put in autoclavable bags, sealed and the autoclaved for a period of 15 minutes at a temperature of 121°C before use.

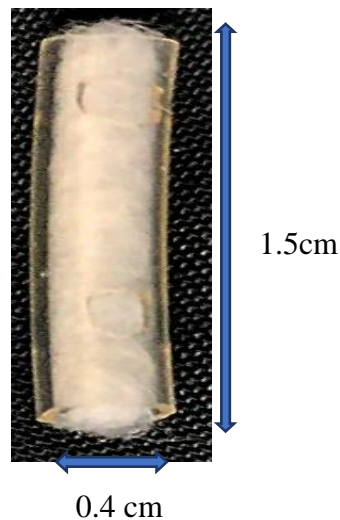


Fig. 3-1. Cancer trap showing the Polyurethane tube with cotton inserted in it. Holes punched into it are 4 per trap.

3.2.4 Mouse model for surgical intra-peritoneal trap implantation

Every experimental design used in this animal study was approved by the University of Texas at Arlington Animal Care and Use Committee (IACUC) closely following the National Institutes of Health guidelines for the use of laboratory animals. The mice were housed and maintained in the

animal care facility of the University of Texas at Arlington under strict pathogen-free circumstances and used following the approved guidelines of the Use Committee for Animal Care. Before the surgical procedure, the female Balb/C mice (20–25g, purchased from Taconic Farms, Germantown, NY) aged between eight to twelve weeks were shaved, depilated with a hair-removal lotion, and then sterilized with 70% Ethyl alcohol. They were then injected with 5 million Qtracker®800 expressing PC3KD cells in 100 μ L media or DID expressing KYSE-30 esophageal cancer cells intraperitoneally using an 18-gauge needle under inhalational general anesthesia with isoflurane (Vedco Inc., Saint Joseph Missouri). The cancer trap was infused with (5 μ L) EPO cytokine. The cancer trap was then implanted intraperitoneally through a 0.5mm inferior abdominal midline incision and then sutured with VICRYL® suture (Johnson & Johnson, New Brunswick, NJ) and cleaned. The animals were sacrificed in a CO2 chamber and by cervical dislocation on the 3rd day for implant extraction and biodistribution studies.

3.2.5 Cancer cell cytotoxic imaging

A baseline fluorescence imaging was carried out on the mice under anesthesia with a Kodak In-Vivo Imaging System FX Pro (Carestream Health Inc, New Haven, CT). *In vivo* cell migration of KYSE-30 cells was tracked using DID vibrant cell label, at an excitation wavelength of 630 nm and an emission wavelength of 700 nm, with an exposure time 10 seconds. *In vivo* cell migration of PC3KD cells was tracked using Qtracker®800, at an excitation wavelength of 470 nm and an emission wavelength of 790 nm, with an exposure time 30 seconds. A field of view (FOV) of 120 mm and an f-stop of 2.5 was used for both. This imaging was repeated at time intervals of 5 and 24 hours. A biodistribution study was done using the same imaging technique but with the abdominal organs removed. To get the best image and results, the same imaging procedure was used for all mice. To reduce the background, an image of a healthy mouse was

taken and the results saved as background that was subtracted from the fluorescence intensity in experimental animals. The images were processed using Carestream MI SE software to get a similar excitation and emission range for the mice we had to compare.

To estimate the number of cells which correspond to the fluorescence intensity results, fluorescence labeled prostate cancer cells numbering 4000, 20000, and 100000 cells were injected into 3 different mice and imaged. The results were plotted against the cell number to produce the standard curve and the slope then calculated.

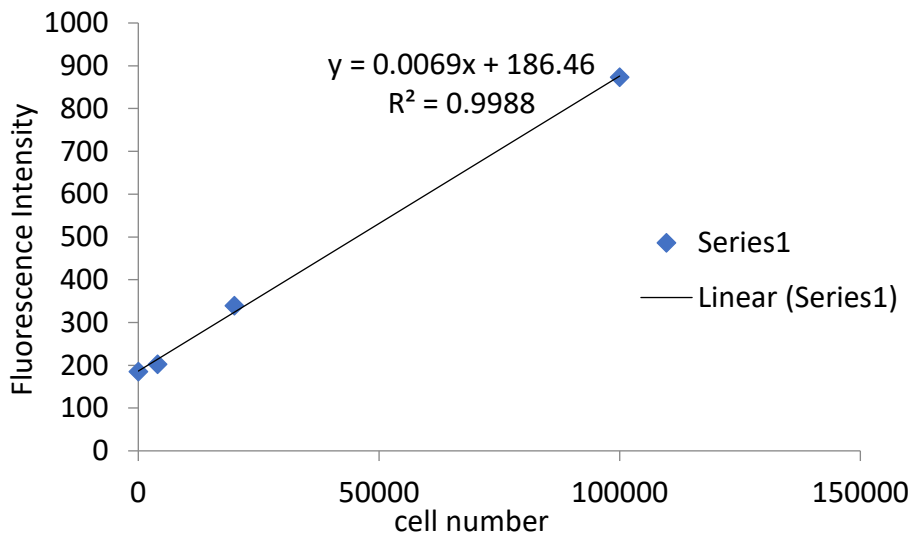


Fig. 3-2. Standard curve comparing the fluorescence intensity with the cell number. The estimated cell number is calculated by solving for “x” in the above equation.

3.2.6 Histology analysis

The freshly collected tissue samples and implant were immediately immersed in plastic molds containing optimal cutting temperature compound (OCT) and frozen in the -20 °C freezer for a day. Tissue sections (10µm thick) were cut using a Leica Cryostat (CM1850, Leica

Microsystem, Wetzlar, Germany) and mounted on glass slides. Fluorescence microscopy was then carried out for the DID expressing cancer cells to localize the cell distribution in the trap.

3.2.7 Statistical analysis

Experiments were repeated at least twice with the data shown as a mean with a 95% confidence interval. Student t-test was used to compare the difference between the groups and a P value of <0.05 was interpreted as statistically significant.

3.3 Results

3.3.1 EPO and foreign body reactions trigger cancer cell migration.

To find out the influence of inflammatory and chemotaxis signals in cancer cell migration, a mouse was injected with 0.5ml of 5 million labelled prostate cancer cells intraperitoneally and then implanted with two intraperitoneal implants with only one infused with EPO while an empty trap was used as control. Fluorescence images were taken 5hrs after the surgical procedure and the next after 24hrs. It was observed that the trap+EPO had higher fluorescence signal which translates in to a higher number of prostate cancer cells. The location surrounding the other trap had a lower signal on the first day that reduced by the second day (Figure 3-3).

These results support that trap+EPO have a higher tendency to recruit prostate cancer cells inside the peritoneum. We also observed that the trap-associated fluorescent intensities reduced with time. This suggests that some of the injected cancer cells left the trap implantation sites.

The fluorescent images at the trap sites were quantified for fluorescent intensities at different time points (24 hours vs. 48 hours). The fluorescence signal is higher in the EPO-releasing trap and reduces the next day by about 24% (from 358-231) while the empty trap had signals that

reduced from 138 to 34 (more than 76%) the next day (Figure 3-4). These results revealed that cancer cells may have left the implant sites and EPO-releasing trap delayed the escape of more cancer cells than trap controls.

The biodistribution of prostate cancer cells was then quantified (Figure 3-5).

Biodistribution results show that the traps had a higher fluorescence intensity when compared to the intra-abdominal organs (Figure 3-6). The results show that cancer traps had excellent ability to capture prostate cancer cells inside peritonea, although trap control can also recruit significant number of prostate cancer. It is also possible that the implantation of both trap+EPO and the trap not infused with any chemokine into the same animal may influence their individual responses.

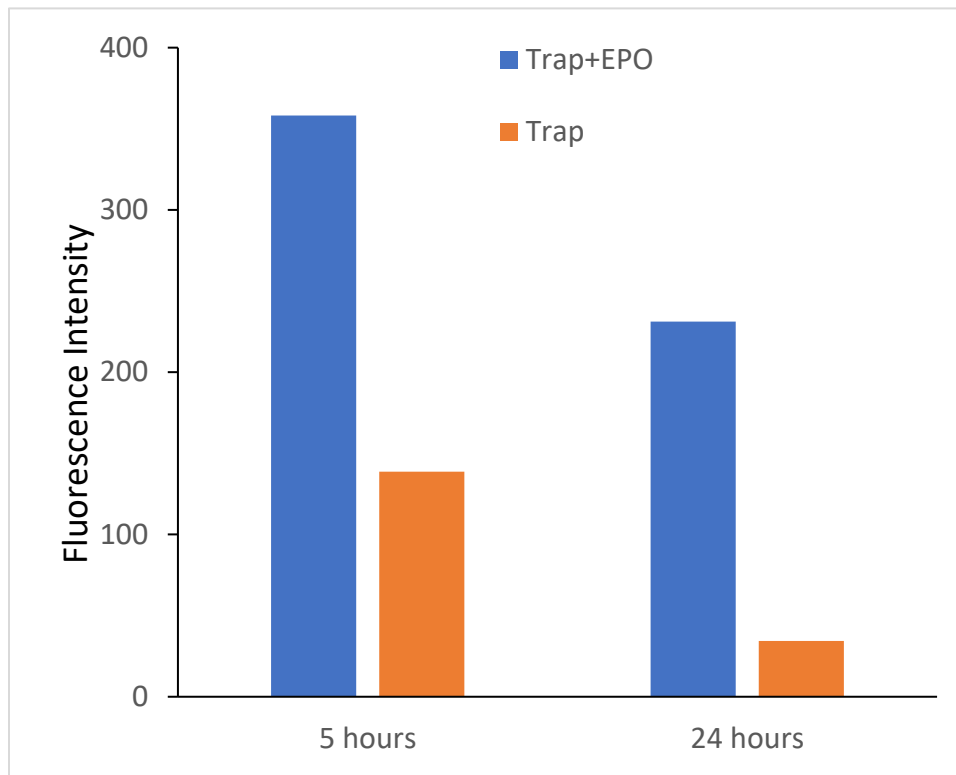


Fig. 3-4. The area of implantation of the trap+EPO the other trap with the fluorescence intensity in these regions recorded and plotted 5 and 24 hours later.

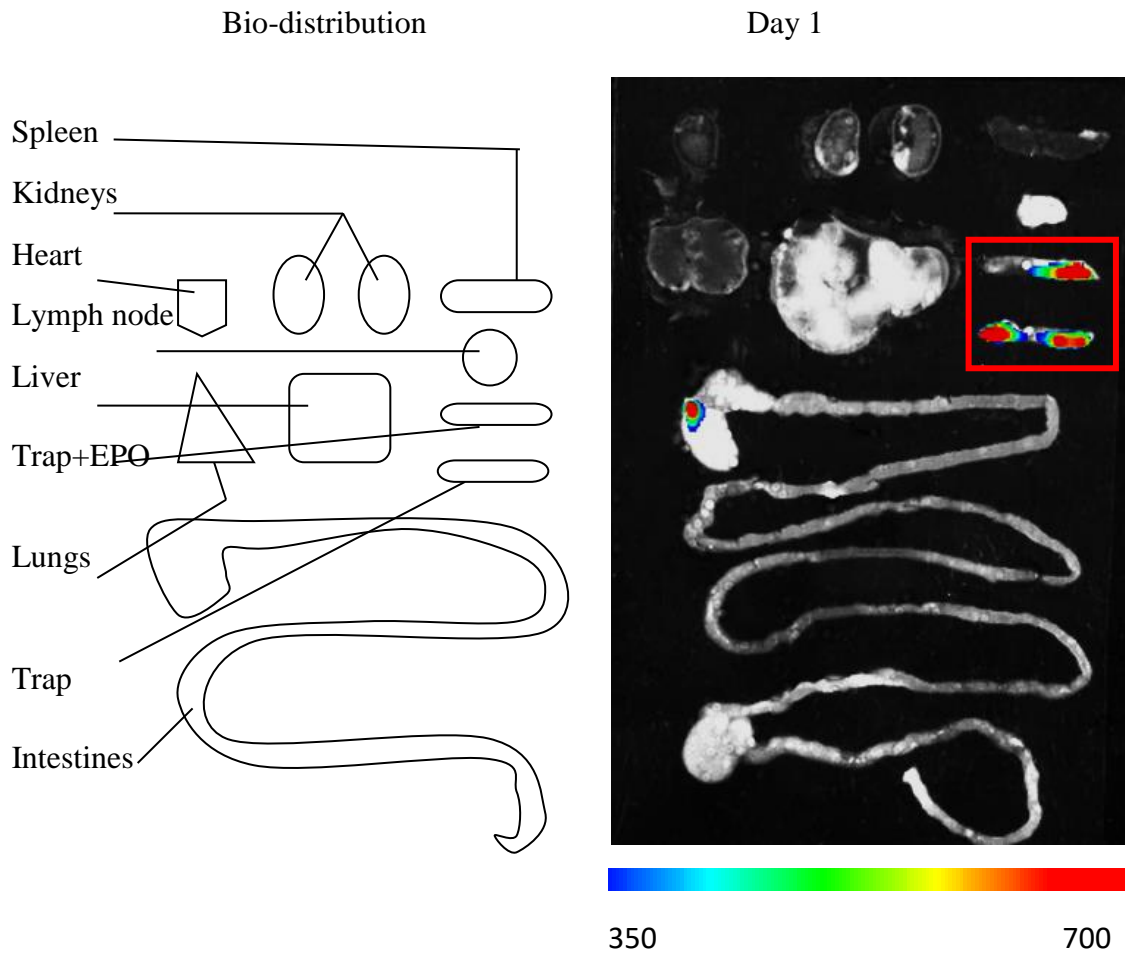


Fig. 3-5. The biodistribution study visibly shows more signal in the trap+EPO than the other trap. Some signal can be visualized on a portion of the stomach.

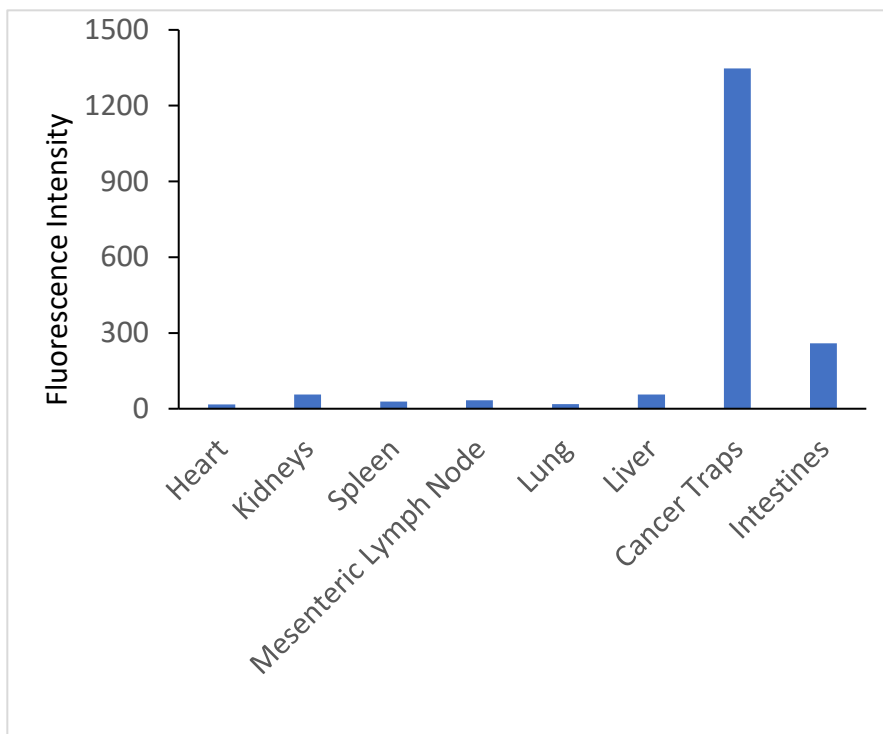


Fig. 3-6. The biodistribution study showing the relationship between the fluorescence intensity in the combined traps and the rest of the thoraco-abdominopelvic organs.

3.3.2 EPO-releasing cancer traps reduces cancer spread

Previously, both trap and trap+ EPO were implanted in the same animal. To study the individual trap's ability to recruit cancer cells, the experiment was repeated with three groups of animals with either trap+EPO, trap, and control mouse (with just cancer cells injected) (Figure 3-7). Briefly, 3 mice were injected with 5 million/1.5ml esophageal or prostate cancer cells intraperitoneally. Next, the first mouse's abdomen was incised and sutured and used as control, the second mouse was implanted with trap (empty), while the 3rd was implanted with trap+EPO the same day.

With the DID labelled esophageal cancer cells injected, the mice had its abdominal contents removed for biodistribution studies 24 hours post OP and the empty abdomen washed with saline. The biodistribution studies show that more cells are recruited by the trap+EPO with less in the abdominal organs (Figure 3-8)

Biodistribution studies show more cells to be recruited by the trap+EPO is higher than that found in the plain trap. In the control mouse without an implant, the greater percentage of cells are in the abdominal fat and mesentery (14221±5941) followed by the abdominal wall (10687±1198), liver (6049±2494) and intestines (2570±897). The mice with the plain trap had more cancer cells in the trap (26467±1903), abdominal fat and mesentery (10807±279) followed by the abdominal wall (12188±619), and liver (7104±494). Lastly, the trap+EPO has the greatest fluorescence signal (36813±3105) and this was followed by the abdominal fat and mesentery (14328±3412) and abdominal wall (13131±3484) (Figure 3-9).

When comparing the three mice, percentage biodistribution studies show more cells to be recruited by the trap+EPO and is about 45% and higher than that found in the empty one (38%).

In the mouse without an implant (control), the greater percentage of cells are in the abdominal fat and mesentery (27%) followed by the abdominal wall (23%), liver (12%) and intestines (6%). The mice with the plain trap had more cancer cells in the trap (38%), abdominal fat and mesentery (17%) followed by the abdominal wall (17%), and liver (11%). Lastly, the trap+EPO in the third set of mice has the greatest percentage (45%) and this was followed by the abdominal fat and mesentery (19%) and abdominal wall (21%) (Figure 3-10).

A different set of mice were injected with Qtracker labelled prostate cancer cells next, fluorescence images taken 24hrs post OP concurrently with biodistribution studies. The mice with its abdomen contents removed was washed with saline and the control mice had a wider spread of fluorescence signal (640) but the mice with traps implanted (both the plain trap and trap+EPO) have a noticeable reduced number (reflected by fluorescent intensities, 242 and 225). This shows that the trap+EPO reduces the level of cancer spread and this relationship is also seen with the empty implant(Figure 3-11).

The biodistribution studies show that more cells are recruited by the trap+EPO with less in the abdominal organs. Some signals were also found on the plain trap, may be due to cancer cells being attracted to the foreign body or fibrin deposition trapping cancer cells. This may also explain some of the signal that is seen on the intra-abdominal organs (Figure 3-12).

When comparing the three mice, biodistribution studies showed that more cells were recruited by the trap+EPO and was about 50% higher than that found in the plain one. Similar numbers were recorded for the liver of the control mouse, the plain trap and intestines of the mouse with the plain trap. The liver, intestines and mesenteric lymph node of the control mice show signals

which are lower than that gotten in the other mice and may be due to the high fluorescence in the peritoneal wall indicating the invasion of cancer cells to peritoneal walls and associated lymphatic system (Figure 3-13).

The results thus far suggest that cancer trap implantation can capture prostate cancer cells inside the peritoneum. However, some of the recruited cancer cells may only stay at the cancer traps for a short period of time. New strategies are needed to improve the numbers of cancer cells captured by the cancer traps.

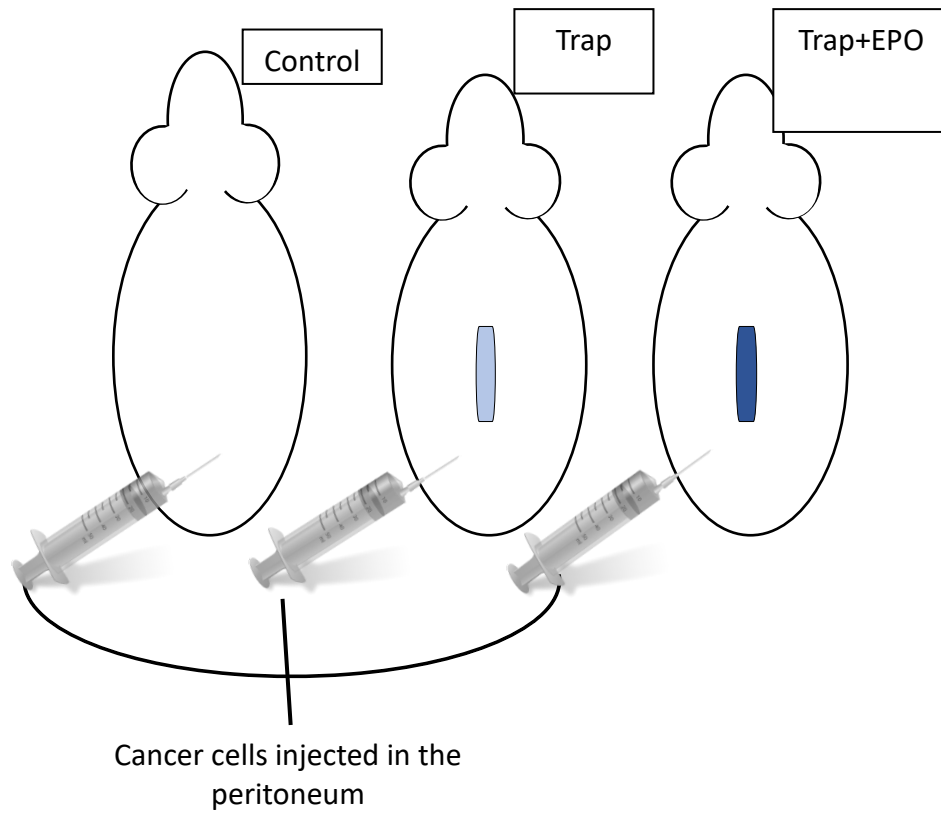
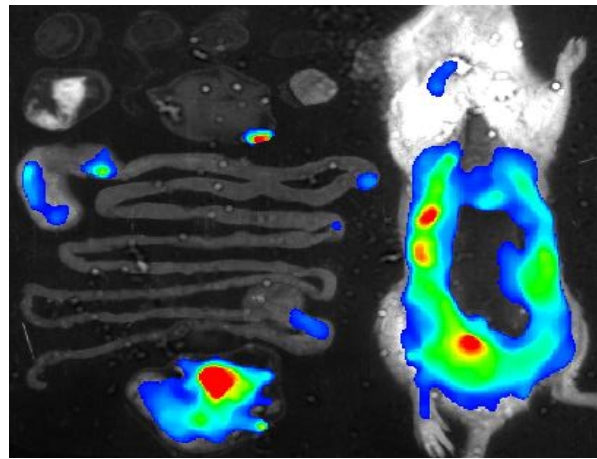
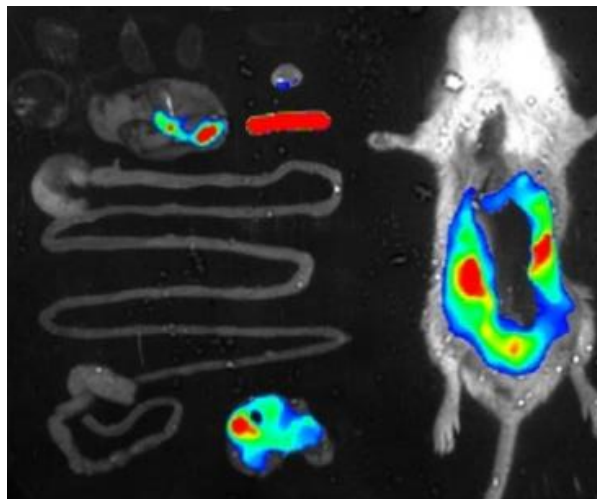


Fig. 3-7. Figure showing the procedure of cancer cell injection and trap implantation in the peritoneal cavity.

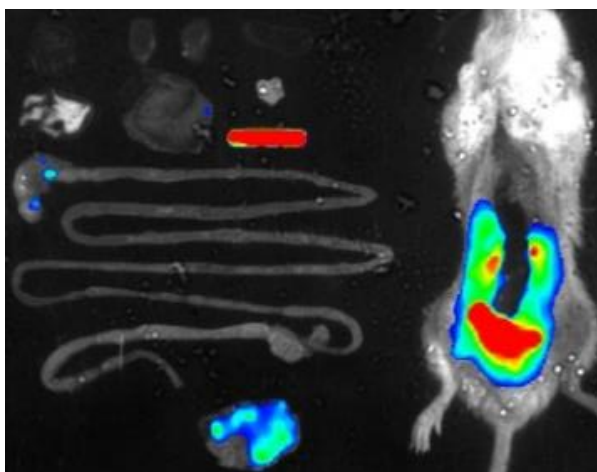
Control



Trap



Trap+EPO



10000



30000

Fig. 3-8. In-vivo bio-distribution images of the contents of the thoracic and abdominal cavity of the three mice showing the organ and cancer trap locations. This was taken 24 hours after the implantation surgery.

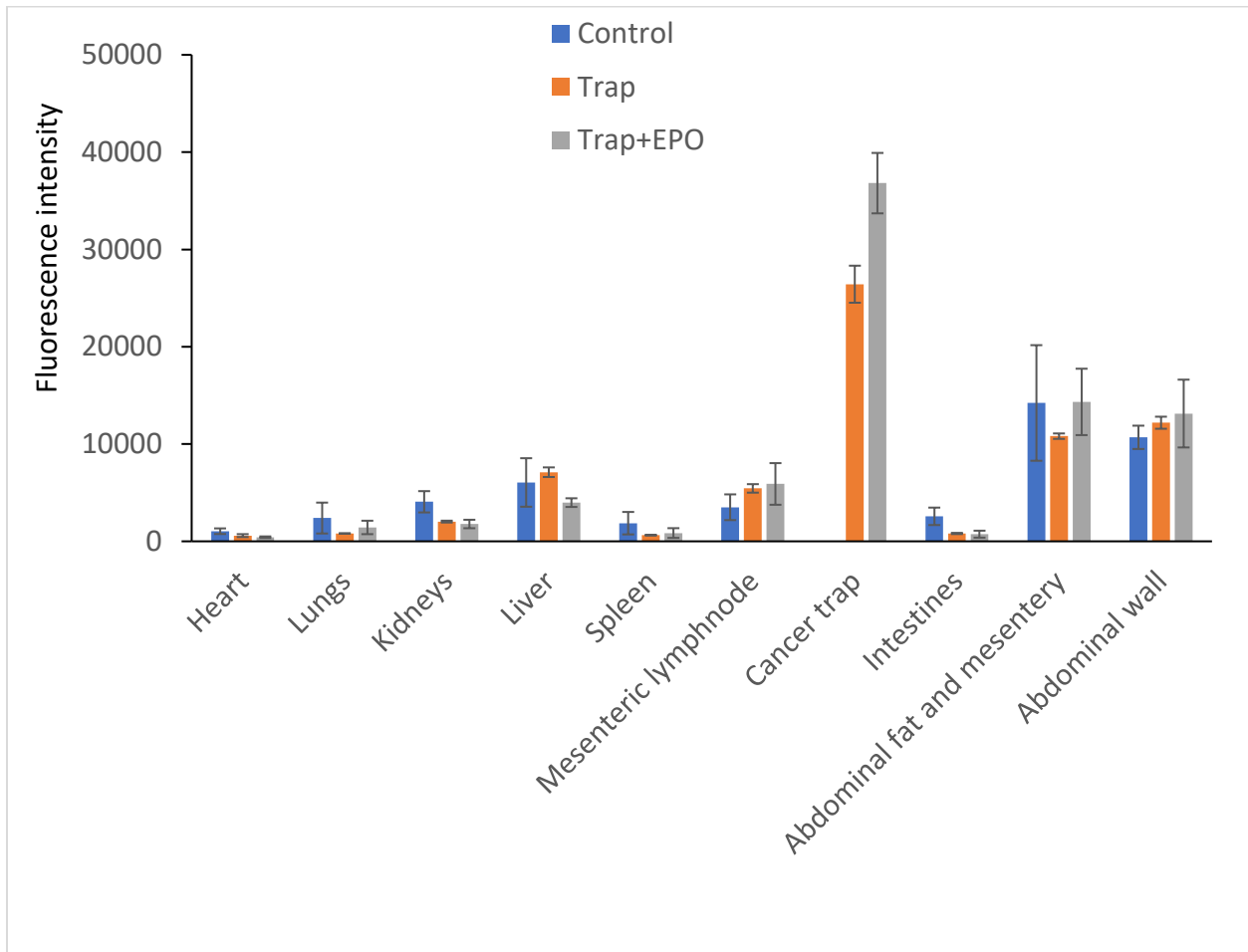


Fig. 3-9. The fluorescence intensity biodistribution study of the three mice showing the relationship between the mouse with just esophageal cancer cells injected (control), trap, the trap+EPO and the rest of the thoraco-abdominopelvic organs.

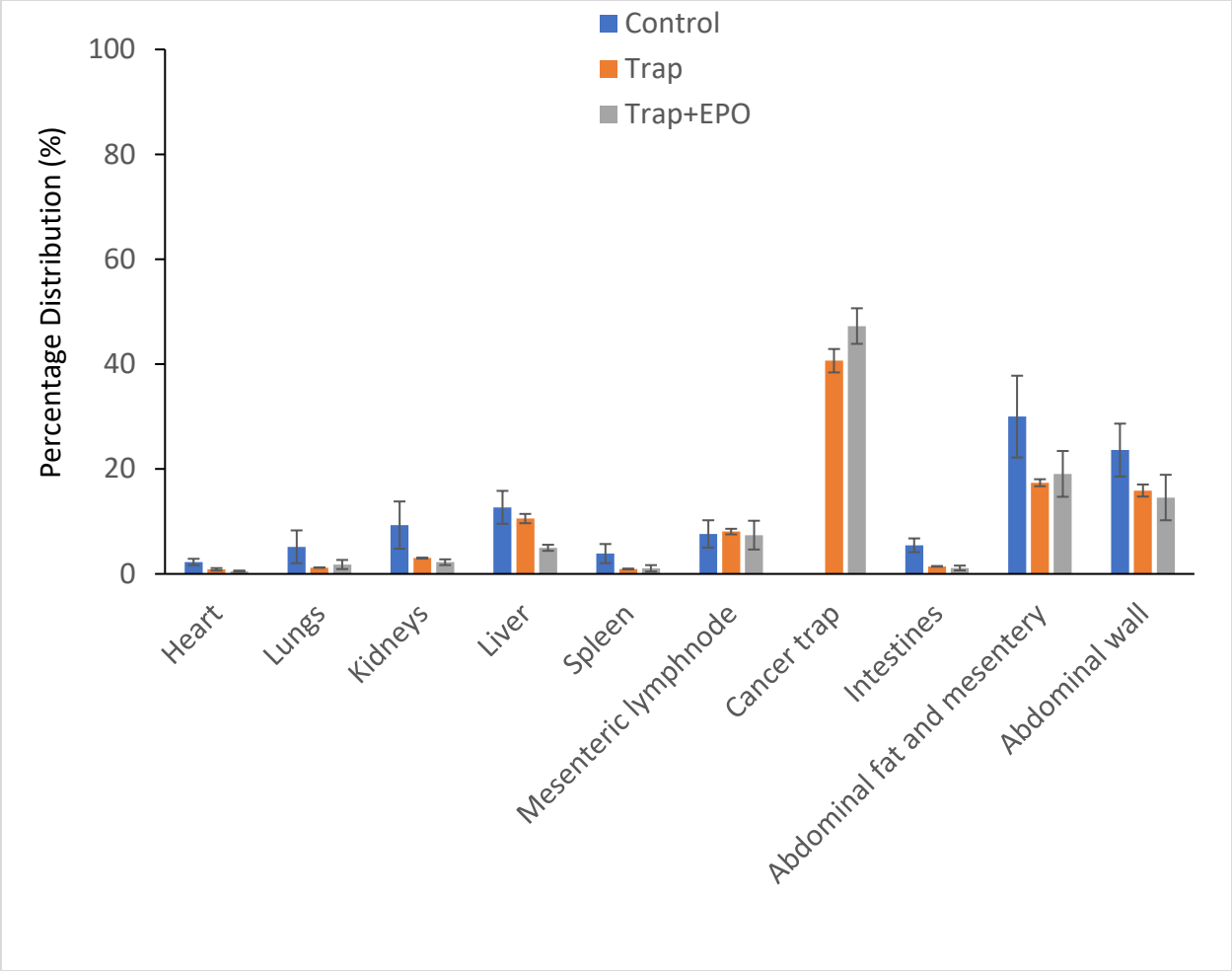


Fig. 3-10. The percentage biodistribution study of the three mice showing the relationship between the mouse with just esophageal cancer cells injected (control), trap, the trap+EPO and the rest of the thoraco-abdominopelvic organs.

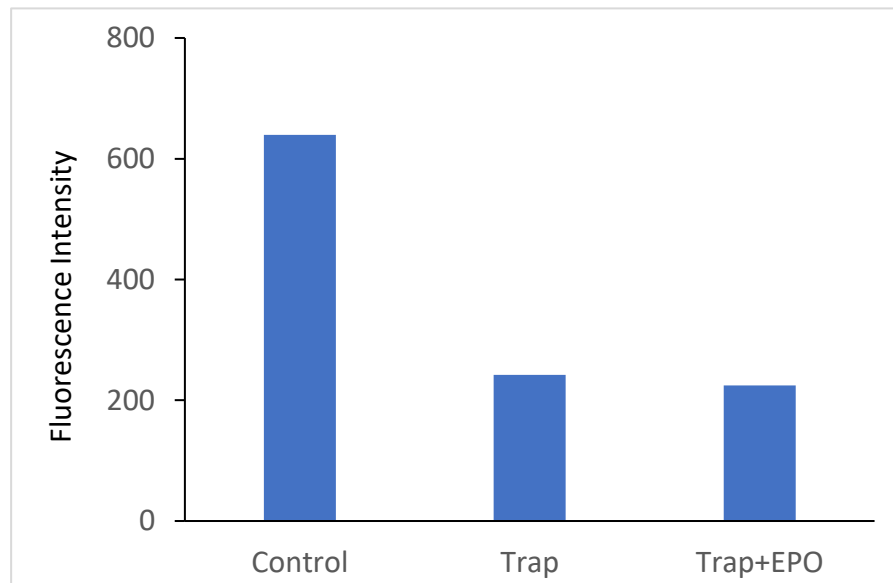
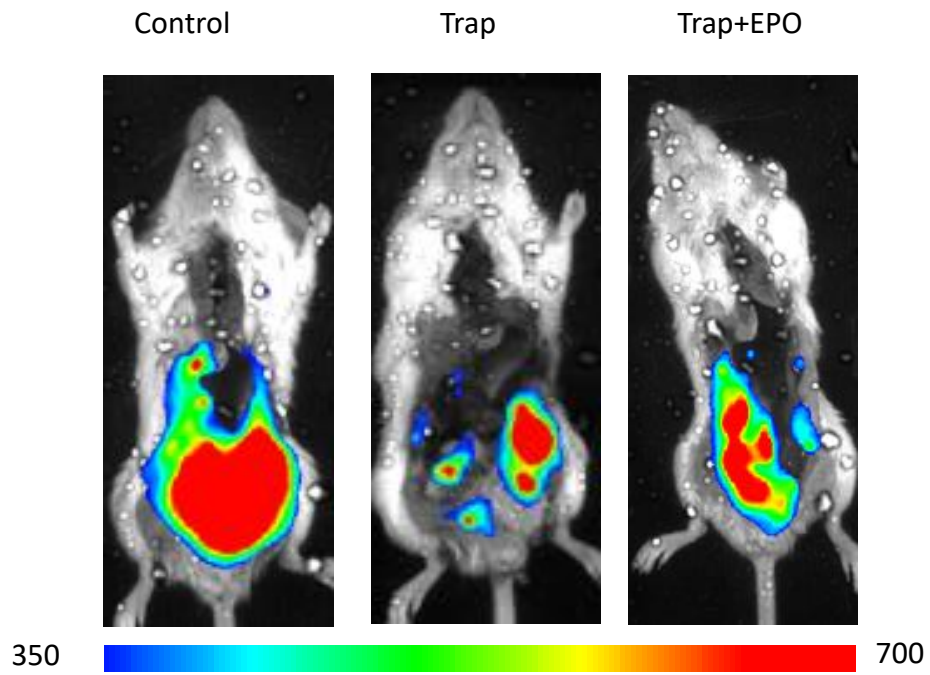


Fig. 3-11. In-vivo imaging of the mice abdomen with all its contents removed and washed with saline and a histogram showing the relationship between them.

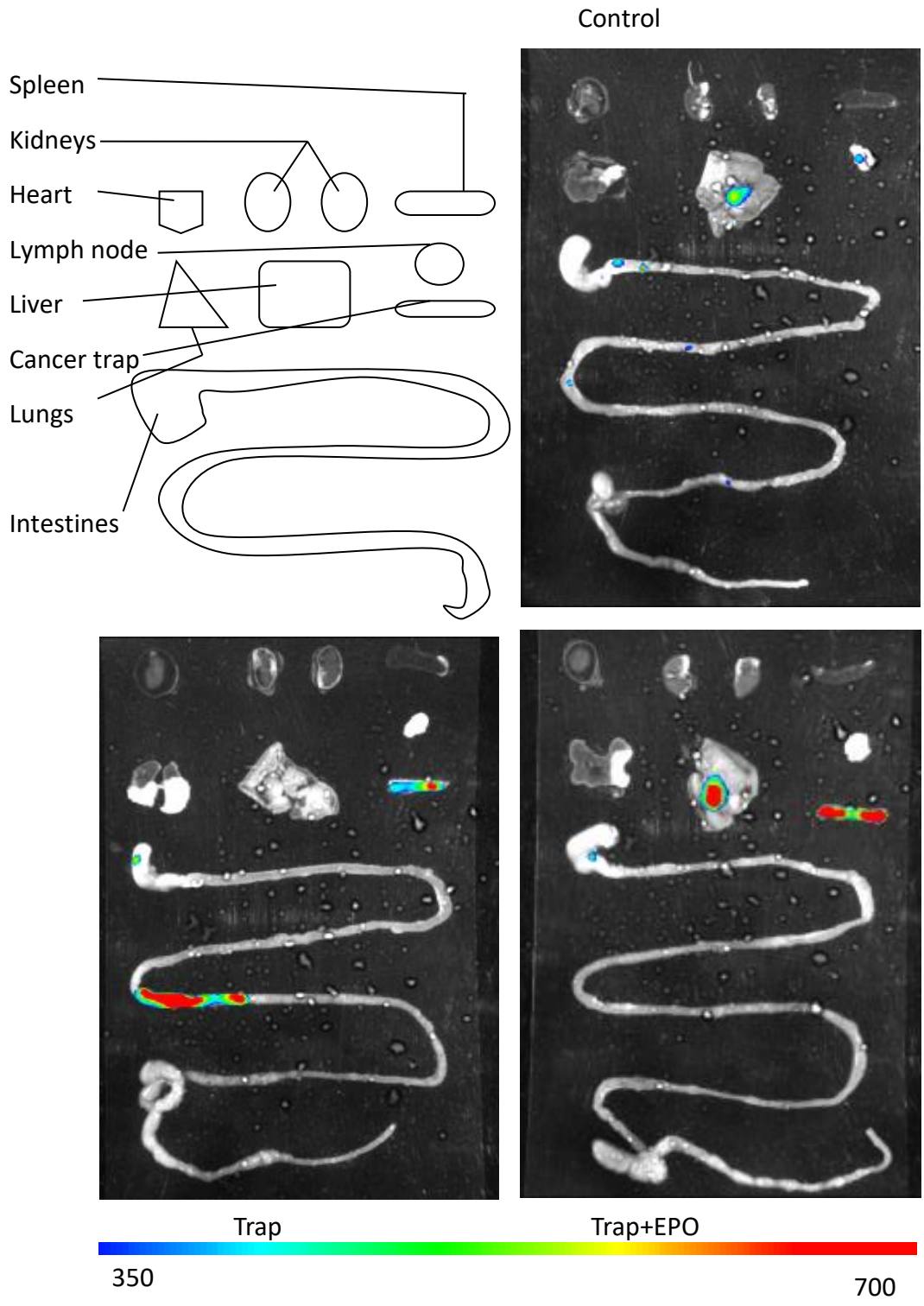


Fig. 3-12. In-vivo bio-distribution images of the contents of the thoracic and abdominal cavity of the three mice with a schematic showing the organ and cancer trap locations. This was taken 24 hours after the implantation surgery.

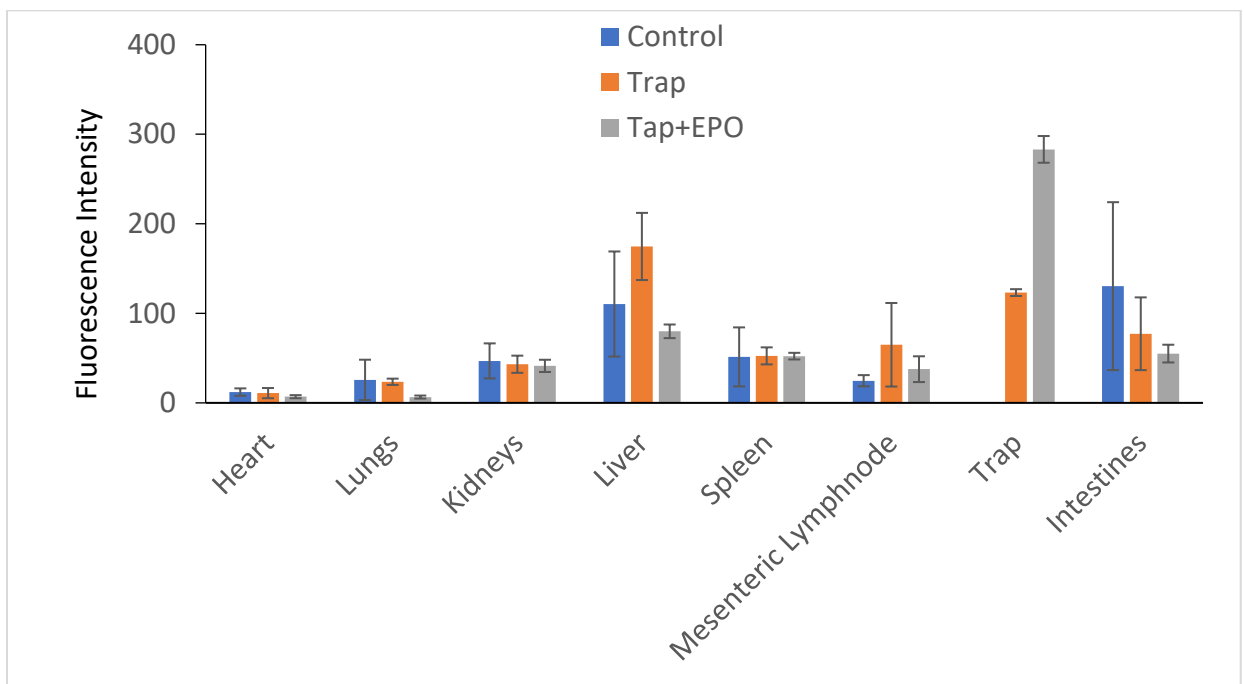


Fig. 3-13. The biodistribution study of the three mice showing the relationship between the mouse with just prostate cancer cells injected (control), trap+EPO, plain trap and the rest of the thoraco-abdominopelvic organs.

3.3.3 Peritoneal cancer cell spread increase with an increase in the number of cancer cells injected

Previously, we found out that EPO reduces prostate cancer spread. We assumed that the more cancer cells injected within the peritoneum, the more the spread to vital organs. To test this, 4 sets of mice were injected with varying number of cancer cells injected (3 million cells, 600,000 cells and 120,000 cells) and a cancer trap was implanted in the peritoneal cavity of each mouse on the first day and sutured. A mouse was also injected with 3,000,000 cells but not implanted with a cancer trap and used as control. A biodistribution imaging study was carried out after 24 hours.

With the DID labelled esophageal cancer cells injected, the biodistribution studies show that more cells are recruited by the EPO-releasing trap with less in the abdominal organs. A similar trend is seen in the mice injected with 120,000 cells (A), 600,000 cells (B) with the differences seen in the fluorescence intensity recorded. The biodistribution studies of the mice with 3,000,000 cells (C) follow the previously discovered trend but more signal recorded this time in the liver, lymph node, abdominal wall, and intestinal fat and mesentery but with an even higher number recorded in the trap. In the mice without a trap (D), this biodistribution is not altered much (Figure 3-14).

The biodistribution study results of the three sets show a greater signal seen in the trap implanted in the mice injected with 3,000,000 cells and a significant number in the intestinal fat and mesentery, and abdominal wall. A smaller amount is seen in the traps implanted in the mice injected with 120,000 and 600,000 cells with an even smaller signal seen in the rest of the organs (Figure 3-15).

When comparing the percentage biodistribution between the three sets of mice, we notice a similar percentage of cells trapped in each mouse with a trap implanted but with more cells trapped in the mice with 3,000,000 cells. The control mouse had more esophageal cancer cells migrating to the abdominal wall, liver, intestinal fat and mesentery (Figure 3-16).

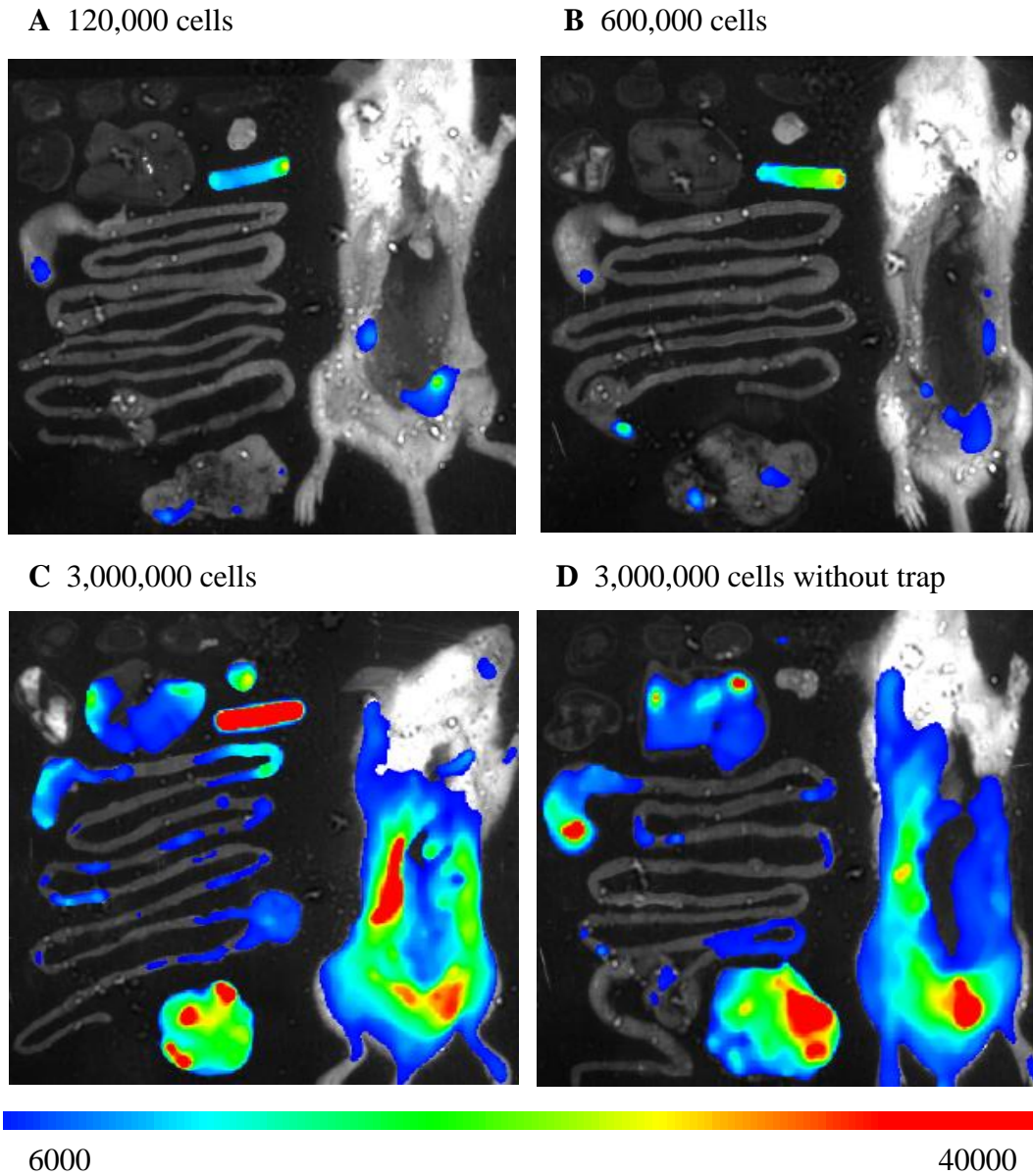


Fig. 3-14. Biodistribution study results. A). First set of mice injected with 120,000 cells with the abdominal organs and cancer trap explanted after 24 hours for fluorescence imaging. B) Second set of mice injected with 600,000 cells. C) Third set of mice injected with 3,000,000 cells. D) Fourth set of mice injected with 3,000,000 cells with the abdominal organs explanted after 24 hours for fluorescence imaging

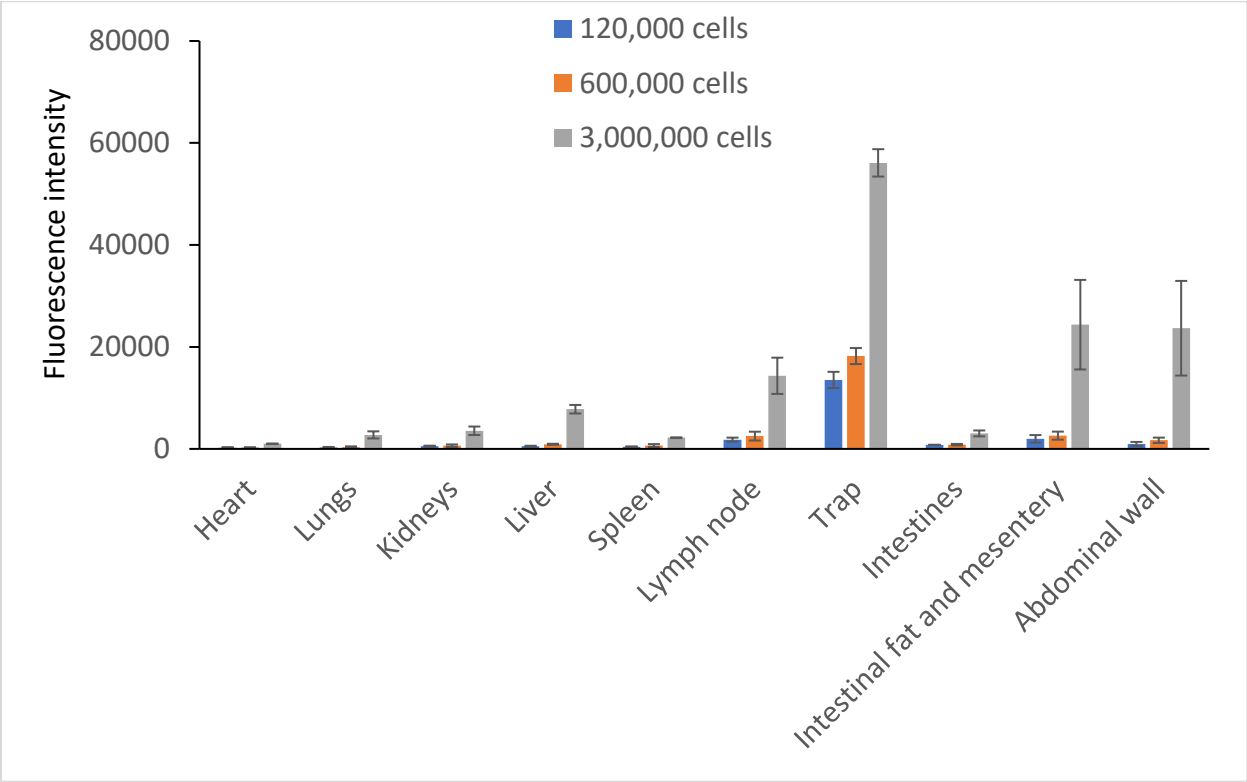


Fig. 3-15. Biodistribution study results of the three sets of mice comparing the fluorescence intensity of the various organs.

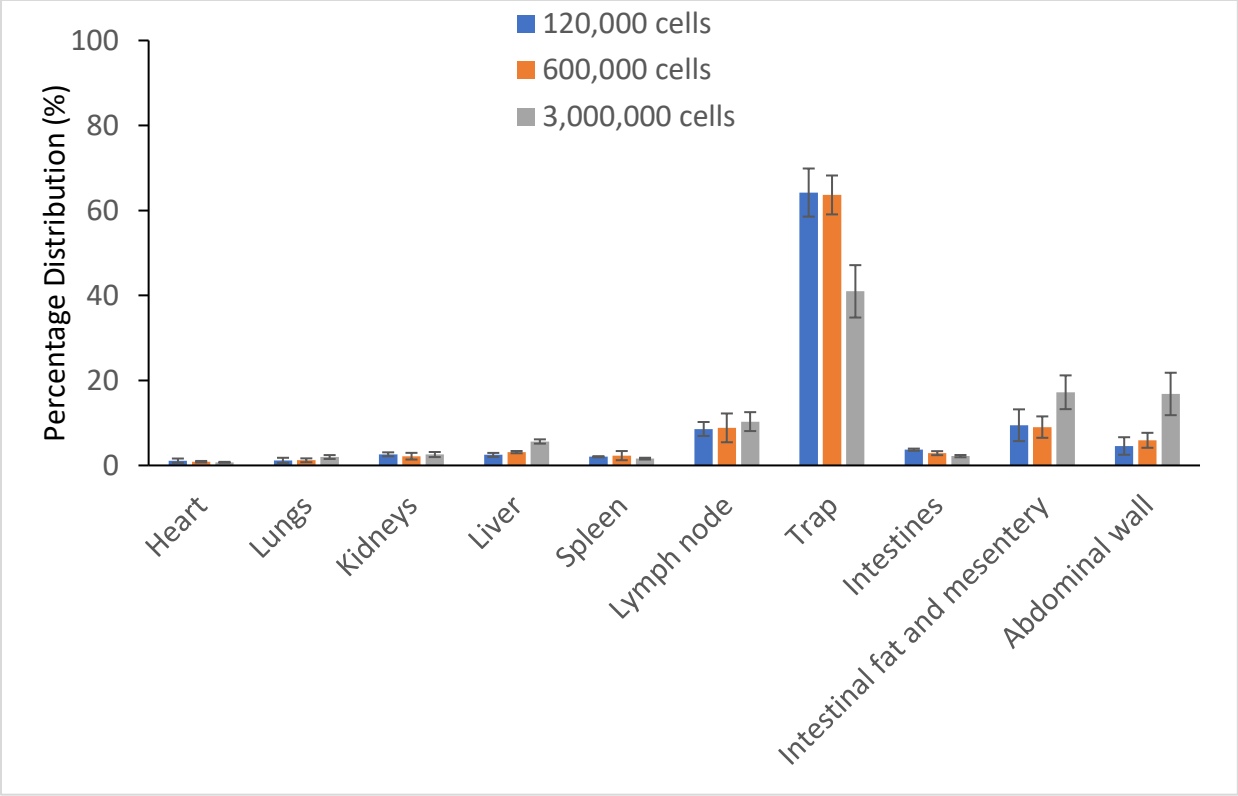


Fig. 3-16. Percentage biodistribution study results of the three sets of mice showing the percentage of cancer cells left over within the abdomen.

3.3.4 Multiple cancer traps increase the number of cancer cells captured

Previously, we found out that EPO reduces prostate cancer spread and that the more cancer cells are injected within the peritoneum, the more the spread to vital organs. We then assumed that the more cancer cells can be removed from the peritonea with multiple replacement of the traps. To test this hypothesis, 3 mice were injected with varying number of cancer cells injected (3 million cells, 600,000 cells and 120,000 cells) and a cancer trap was implanted in the peritoneal cavity of each mouse on the first day and sutured. The trap was extracted the next day, a new one inserted in place, and re-sutured. This procedure was repeated for a total of three times (Figure 3-17).

The extracted traps were imaged daily and showed a greater signal in the mice with the higher number of cancer cells injected. This reduced each day with more cells in the trap inserted in mouse with higher number of cancer cells (Figure 3-18).

Fluorescence images of the extracted cancer traps were taken over 3 days and the results from this study showed that the cancer traps could continuously recruit cancer cells daily. And this is shown by the fluorescence intensity numbers in the traps (mostly in the mice with higher number of cells injected in them) (Figure 3-19).

The cancer trap was washed with Triton x-100 and the resulting solutions fluorescence intensity measured with a well-plate reader. The values gotten for the mice with 3 million cells increased a bit from the first to second day and drastically reduces by the third day, there is a reduction in the values gotten from the mice with 600,000 cells from day 1-3 while the mouse with 120,000 cells had less changes. This shows that our trap can be used as a vacuum for cell trapping with the cancer trapping ability reducing with each day and with more cells trapped in the mice with most

cells. This also shows that the traps have a finite cell loading capacity. This method doesn't account for the exact cell number extracted (Figure 3-20).

In the biodistribution study, there is an increase in the fluorescence signal in the fat and mesentery of the mice with the higher number of cells and the intestines also have some noticeable fluorescence signals. (Figure 3-21).

The percentage biodistribution for the cells left over in the abdomen show a reduction in number compared to that trapped by the cancer trap (Figure 3-22).

The cancer traps from the set of mice injected with the DID stained esophageal cancer cells were then extracted and the cotton from the polyurethane tube taken out. Sectioned and viewed under the microscope. It is seen that the cells are more concentrated on the periphery of the implant with good penetration to the center of the implant showing that the trap can attract cells into the deeper parts of the trap. This relationship is similar with the empty trap. The problem with extracting the cotton fiber is that the polyurethane tube has some cells on the surface also and they wouldn't be accounted for (Figure 3-23). This shows that cells are more on the outer portion of the trap with less penetrating deeper. The EPO-releasing trap has a significant number of cells on the periphery compared to the empty trap.

The cell count show and increase number of cells in the periphery and center of the EPO trap compared to the control trap (Figure 3-24). The cotton used for this experiment isn't standardized. In order to develop a known controllable scaffold to help in absorbing the chemokine used (EPO) that can degrade at a controllable rate and with good biocompatibility, different combinations of polymers would be studied next.

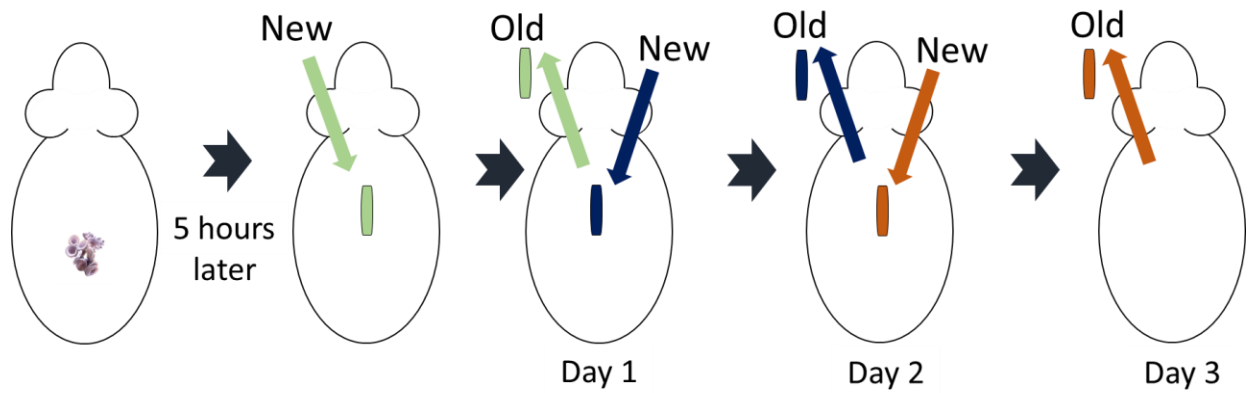


Fig. 3-17. Schematic showing the steps taken to implant multiple traps in a single mouse sequentially. Cancer cells are injected and the trap implanted. This trap was extracted the next day, a new one inserted in place and re-sutured. This procedure was repeated for a total of three times.

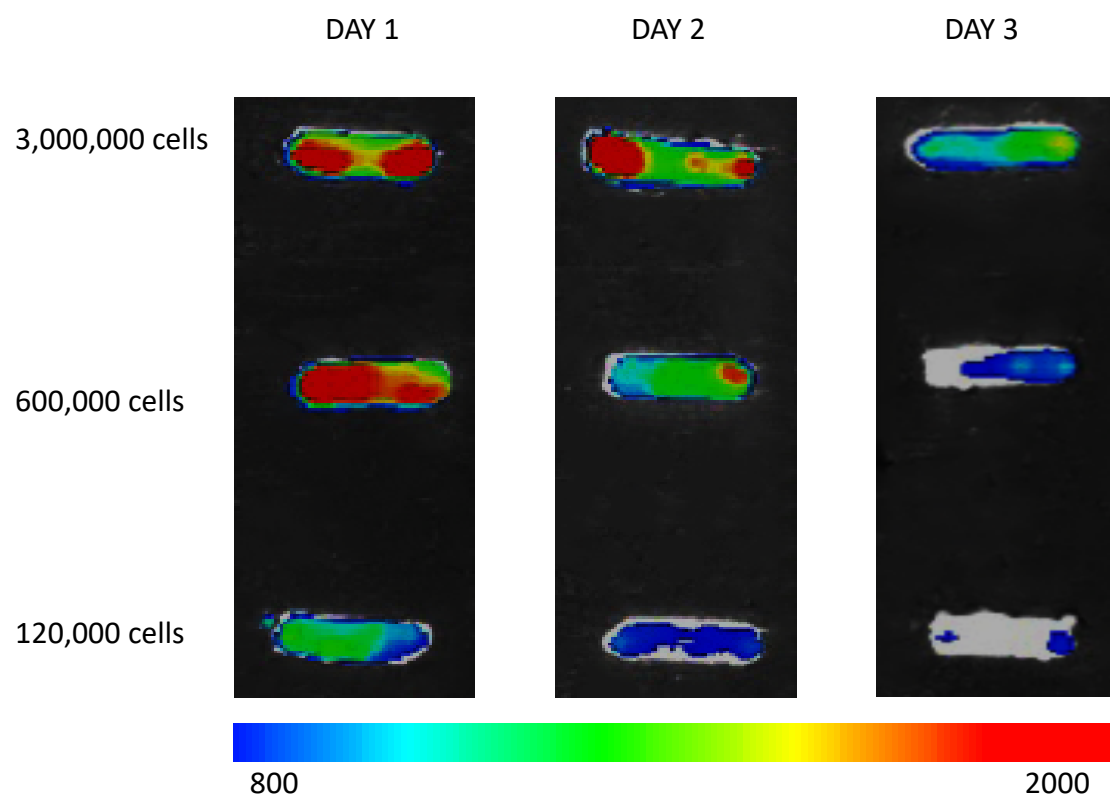


Fig. 3-18. Ex-vivo imaging of the explanted cancer traps showing the fluorescence intensity changes from each tube. The fluorescence intensity reduced each day with more cells in the trap inserted in mouse with higher number of cancer cells.

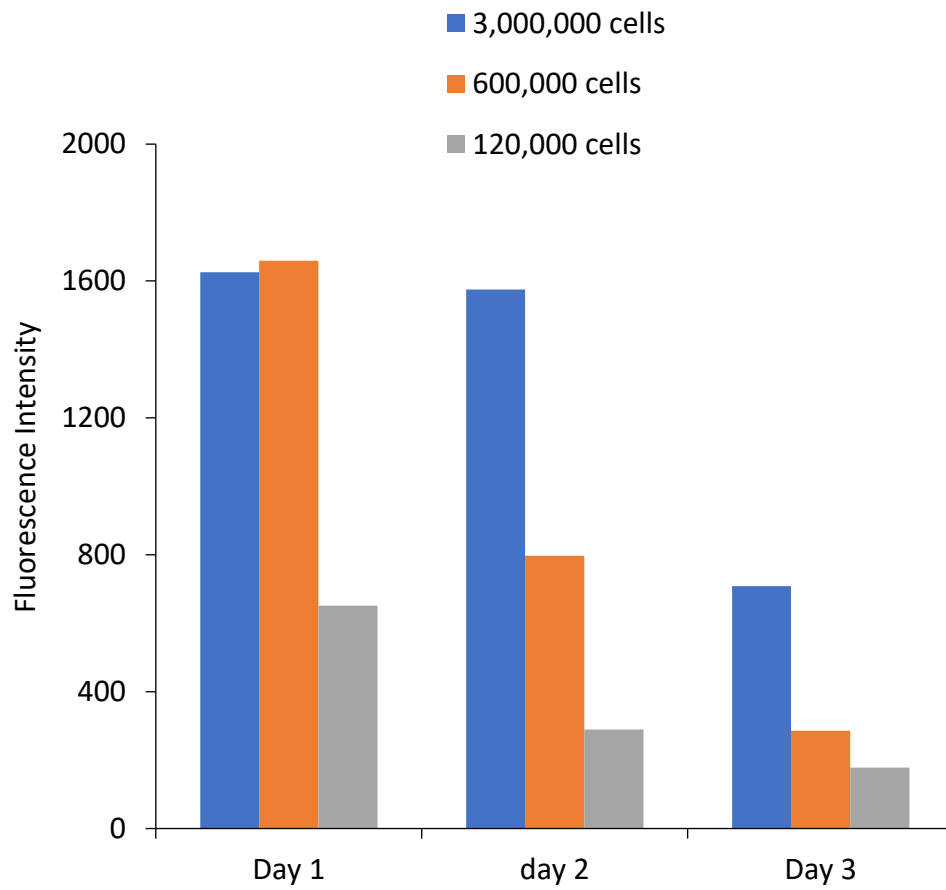


Fig. 3-19. Fluorescence imaging results of the extracted cancer traps over 3 days. The results show that the cancer traps can be used to continuously recruit cancer cells

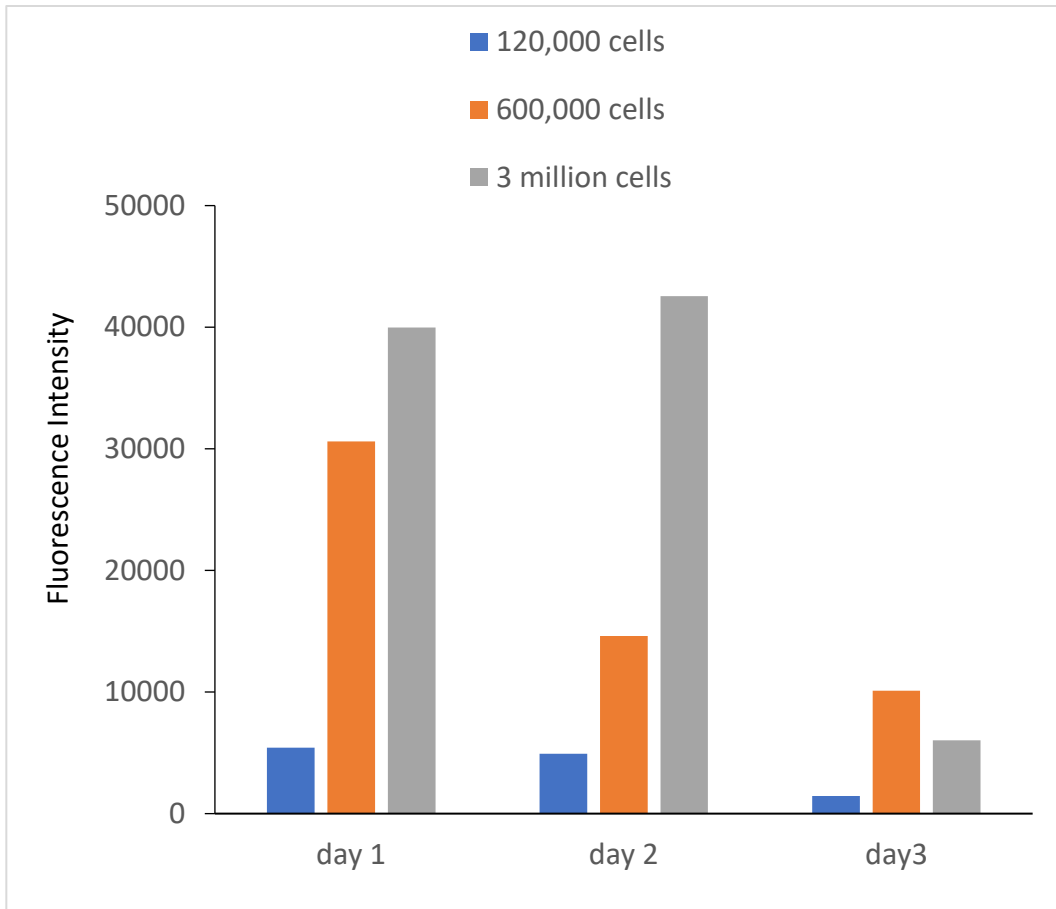


Fig. 3-20. The well-plate reader results of the solution gotten from the Triton x-100 washed cancer traps showing the cancer trapping ability reducing daily.

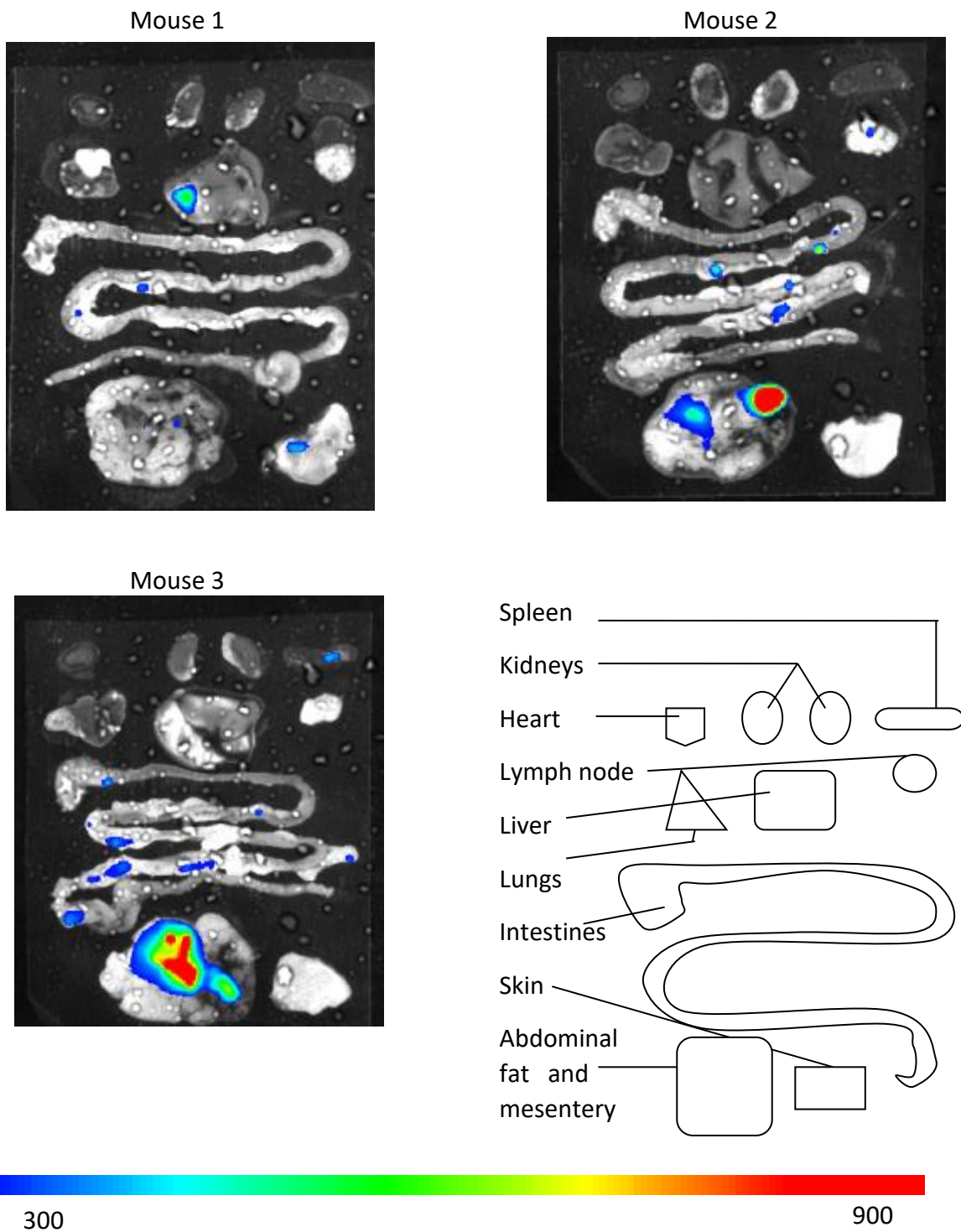


Fig. 3-21. Ex-vivo biodistribution studies of the thoraco-abdominopelvic contents gotten from three mice showing some signal in the intestines, fat, and mesentery.

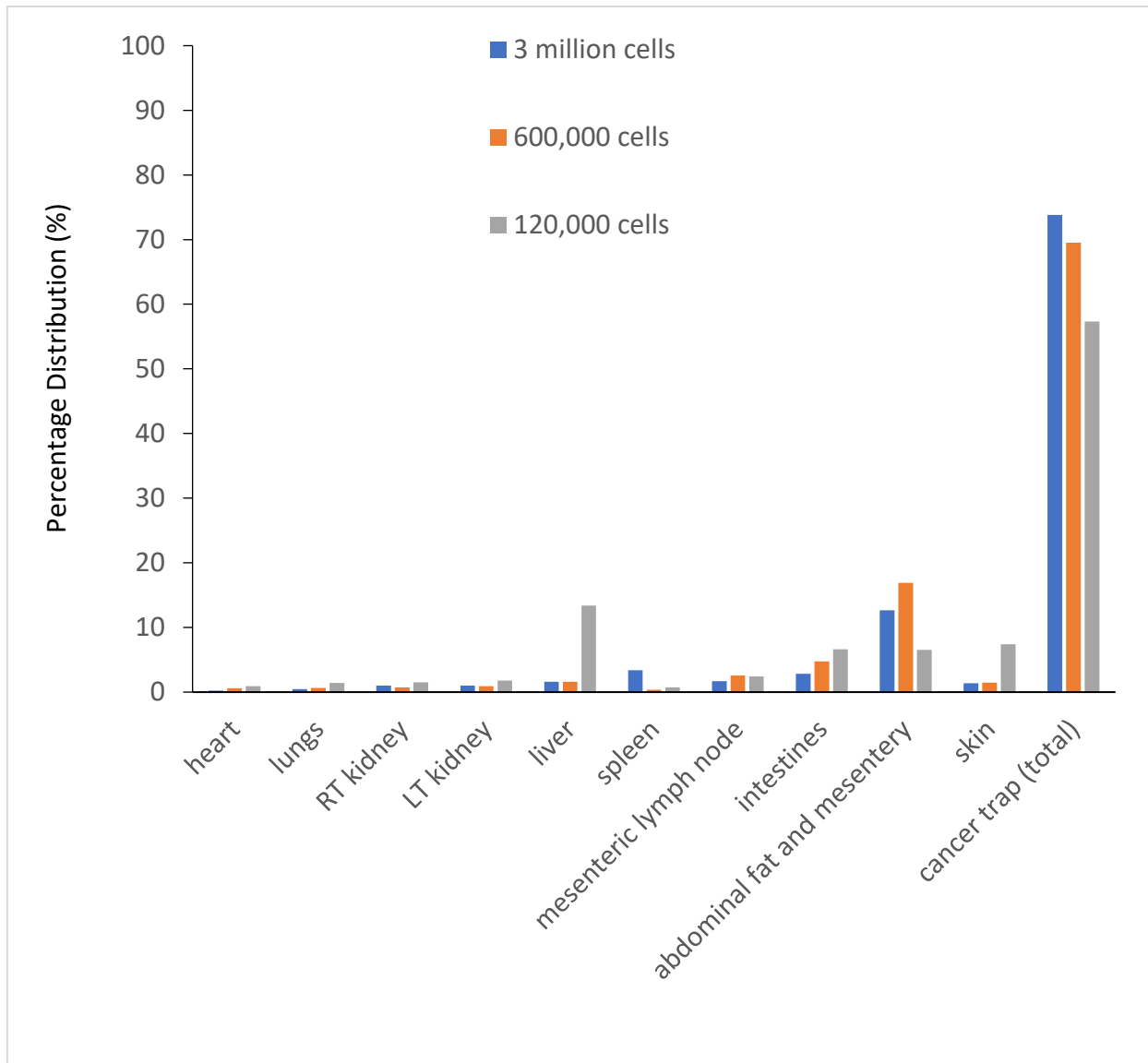


Fig. 3-22. Cell percentage biodistribution for the organs and cancer traps in the thoracic and abdominopelvic cavity. The sum of cell numbers in the cancer trap over three days was used here.

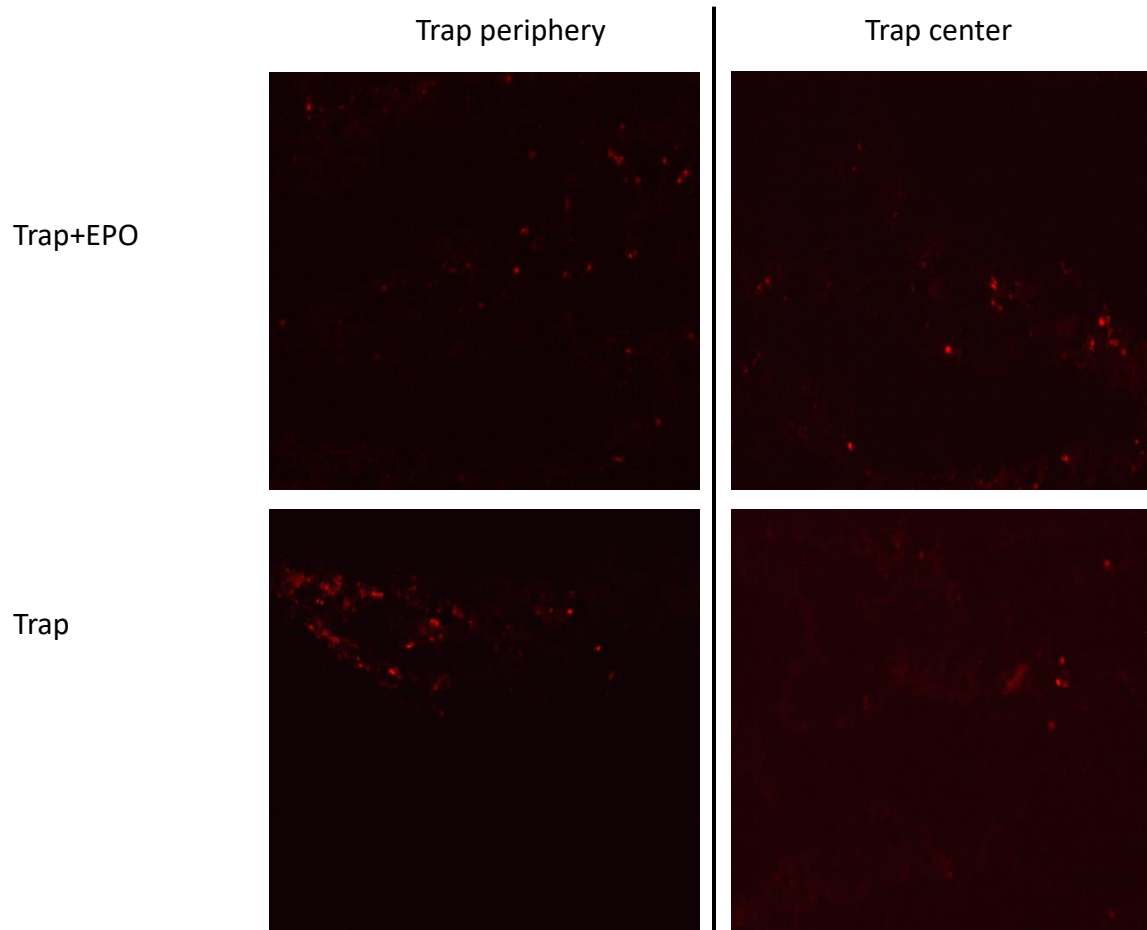


Fig. 3-23. DID staining of the sectioned cancer trap after extraction of the cotton from the polyurethane tube with images taken of the periphery and center.

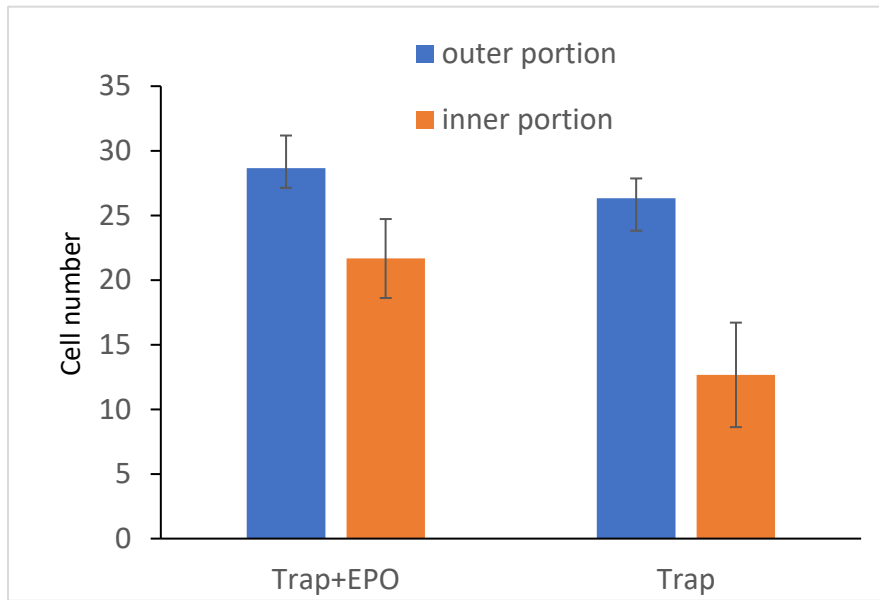


Fig. 3-24. Cell number differences between the outer and inner portions of the trap+EPO, and plain traps.

3.4 Discussion

The results from these studies support the overall hypothesis that cancer traps can be developed to capture cancer cells inside the peritoneal space. This was demonstrated using prostate and esophageal cancer cells as model cancers. The EPO releasing trap influenced the recruitment of esophageal and prostate cancer cells in the peritoneum, reduced cancer spread, could continuously recruit cancer cells, and improved the attachment of cells to the periphery of the implant with good penetration. Despite of promising outcomes, there are many questions that remain to be answered. Some signals were also found on the empty traps which leads us to ask if these are due to cancer cells being attracted to the foreign body, the cotton in the trap soaking the cancer cells injected or if fibrin deposition affects trapping of cancer cells? This may also explain some of the signal that is seen on the intra-abdominal organs

The current cancer trap design is a polyurethane (PU) tube with four holes punched into it and a ball of cotton inserted inside it. This design is wrought with some problems. The insertion of a cotton ball would lead to an uncontrolled fast release profile of EPO and reduce the efficacy of the trap in attracting a higher number of prostate cancer cells over time. Due to the amount and size of the holes in the PU tube, its porosity isn't optimal and would negatively affect the number of recruited cells through the tube into the cotton material. Some solutions to the problem here would be to increase the number of holes in the tube or make the holes larger. The materials used in fabricating the cancer trap are non-degradable and would require a second surgical procedure for removal after use. Using biodegradable materials may alleviate this problem.

We observed increased cancer cell recruitment around the EPO releasing trap on the first day and this reduced on the second day. The reduction of signals the next day, may suggest that some of the injected cancer cells left the trap implantation sites. The cancer trap capacity may be

improved by enhancing the slow release property of EPO. This could also be done by incorporating a locking mechanism within the trap to keep the cells inside the trap after being recruited. Increasing the trap porosity could also be advantageous because this would increase the amount of prostate cancer cells that migrates into the trap, increasing its retention capacity in the long run.

The therapeutic efficacy of the trap could be improved by imitating the roach motel which is a cockroach trap which use some form of bait to lure the insects into them, prevent them from leaving and killing them, the cancer trap could be designed to kill cancer inside the trap. This could be achieved by developing traps incorporated with chemotherapeutic agents. Improving the biocompatibility and biodegradability of the materials used could be beneficial because the traps would be left in the peritoneum and localized radiation used to ablate the trapped prostate cancer cells, eliminating the need for a second surgery to take out the trap.

Future work on the cancer trap would focus on improving the trap design to increase its cell capacity, to improve cell retention, and incorporate therapeutic components to kill cancer cells inside the trap. Also, studies using biodegradable materials would be beneficial in reducing problems associated with it and a better control of the release of cancer cells and studies with slow releasing hydrogels can be investigated. Duplicating the pattern of esophageal or prostate cancer spread by orthotopic implantation of the corresponding cancer cells into the esophageal or prostate gland would be beneficial in imitating real cancer. Figuring out the best location for the implant is also important and this could translate to an increase in the efficiency of the trap against cancer cells.

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Biographical Information

Chuka Okpokwasili was born in Washington DC. He completed his undergraduate education at the University of Port Harcourt College of Health Sciences with an MBBS degree. At the University of Port Harcourt Teaching Hospital where he had his clinical training, he was in contact with various patients and educators during his clinical rotations and got to experience the totality of medicine. It was during this time that he got interested in the creative part of medicine and got interested in biomedical engineering. With these, he started his masters study in August 2015 at the University of Texas at Arlington Biomedical Engineering department in the tissue engineering tract. Being supervised by Dr. Liping Tang exposed him to animal studies in biomaterial and cancer research. He is interested in continuing with research to pursue his interest in this creative process of medicine.