

Czech Centre for Phenogenomics

## Precise low invasive orthotopic placement of carcinoma cells into the mouse mammary fat pad using transillumination

### **Abstract:**

Standard procedures for the delivery of carcinoma cells into the mouse mammary fat pad comprise of either blind injections through the skin, or surgery where the wound must be sewn (Kocatürk and Versteeg, 2015). In this work, two new methods were developed for carcinoma cell delivery into mouse mammary fat pads. The first method includes a small surgical procedure under general anaesthesia. Two doses of mouse mammary gland carcinoma cells 4T1 labelled with Red-Fluc reporter (Perkin-Elmer) were delivered into the mammary fat pads of NSG mice (5x10<sup>4</sup> cells in 0.02 ml, left and right mammary fat pads). The resulting wounds were less than 2.5 mm in size, therefore sutures were not necessary. The second method is performed with an insulin syringe under a bright light to deliver the human carcinoma cells 4T1 (4T1-Red-FLuc) (5x10<sup>4</sup> cells in 0.02 ml, left and right mammary fat pads) through the skin, and into the mammary fat pads. Lean female mice with lightly-pigmented skin must be used, and any hair removed from the area of interest. Additionally, keeping the animals under an optimal diet is mandatory. Tumor transplant growth was monitored and visualised weekly using luminescence, and at the final step, tumorous and healthy tissue sections were processed with histological staining. All mice developed tumors in their mammary fat pads within 2 weeks. The first method is less time consuming, less expensive and less invasive than standard surgery techniques, and therefore increased animal welfare is achieved. The second method is leften the surgical technique. The use of a strong light to visualise the structures under the skin appeared essential to distinguish mammary gland fat pads from vessels and subcutaneous fat. This allowed the tumor cells to be administered precisely. After applying either of the above methods, mice recovered very quickly without any unexpected side effects. Due to the 100% success rate for both methods, these techniques have the potential to be recognised as standard methods for cancer cell ad

#### Introduction

Currently the delivery of carcinoma cells to the mammary fat pad, is either done by blind injection or by an invasive surgery, which requires wound suturing. The blind injections are poorly controlled, while the standard surgery is too inasive. In the current work, both techniques were optimized in order better control the injection for the direct injection or reduce the burden on the animal by performing a less invasive surgery. Both optimized methods lead to tumor development within 2 weeks after the procedure is performed.

#### **Materials and methods**

For ease of detection of the tumors, 4T1-Red-FLuc carcinoma cells were used. Their red luciferase label was detected and measured through whole body imaging using the In-vivo Extreme from Bruker. Injections were performed with standard 1 ml syringes fitted with a 26G needle.

#### Method 1

As the first method requires surgery, the normal precautions for surgery need to be taken, i.e. the shaving and disinfection of the area and sterilization of the equipment. A small incision is made lateral of the nipple. This incision should not be larger than 3 mm. Forceps are passed through the opening to move and expose the fat pad. The injection of the carcinoma cells (5 x 10<sup>4</sup> cells in 20 µl) is then be performed after which the fat pad can be moved back to its place. As the injection site is moved away from the incision, the risk of leakage of the injected solution outside of the body is reduced to a minimum. As the wound does not exceed 3 mm no wound closure procedure is necessary.

#### Method 2

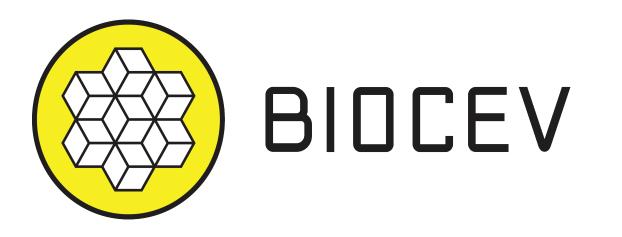
In the second method the cells (5 x 10<sup>4</sup> cells in 20 µl) are delivered through direct injection into the fat pad. As the mammary gland is visualised through the skin, it is essential to use lightly pigmented mice (preferable white) and too shave the area around the nipple well. An area of around 0.5 cm of skin around the nipple is held up using a pair of forceps (figure 2A). With strong lighting from the side the mammary gland and the surrounding blood vessels can be visualized. The injection is preformed a few mm away for the mammary gland and care is taken to avoid injection in a blood vessel. To confirm that the needle is truly in the fat pad and not subcutaneous, one can observe the tip of the needle, which should not appear through the skin (figure 2B). If it does, the needle need to be inserted deeper into the fat pad. When the injection is succesful a bubble is seen at the tip of the needle.

#### **Results and discussion**

Tumor development is observed with both methods within 2 weeks after inoculation (Figure 3), which is comparable to the results obtained with the more invasive surgery described by Kocatürk and Versteeg. However the surgical technique presented here is much milder for the animal and easier to perform. The method for the direct fat pad injection with visualisation of the tissue through transillumination is even less invasive and leads to the same results. However there is 1 major restriction to the use of this method as it can only be performed in very slightly or non-pigmented mice.

#### Conclusion

Both methods are reliable and less invasive then the original method described by Kocatürk and Versteeg. When white mice or other mice with very slightly pigmented skin are used the method of guided fat pad injection should be preferred as it is the least invasive (Refinement, the 3 Rs). In other cases the surgical method should be used as blind injection in the fat pad is not as reliable.



# Phenotyping module PDX/Cancer models unit

Kralova Viziova P., Indrova M., Piavaux B., Prochazka J., Sedlacek R.

**Contact** Petra Kralova Viziova petra.kralova-viziova@img.cas.cz

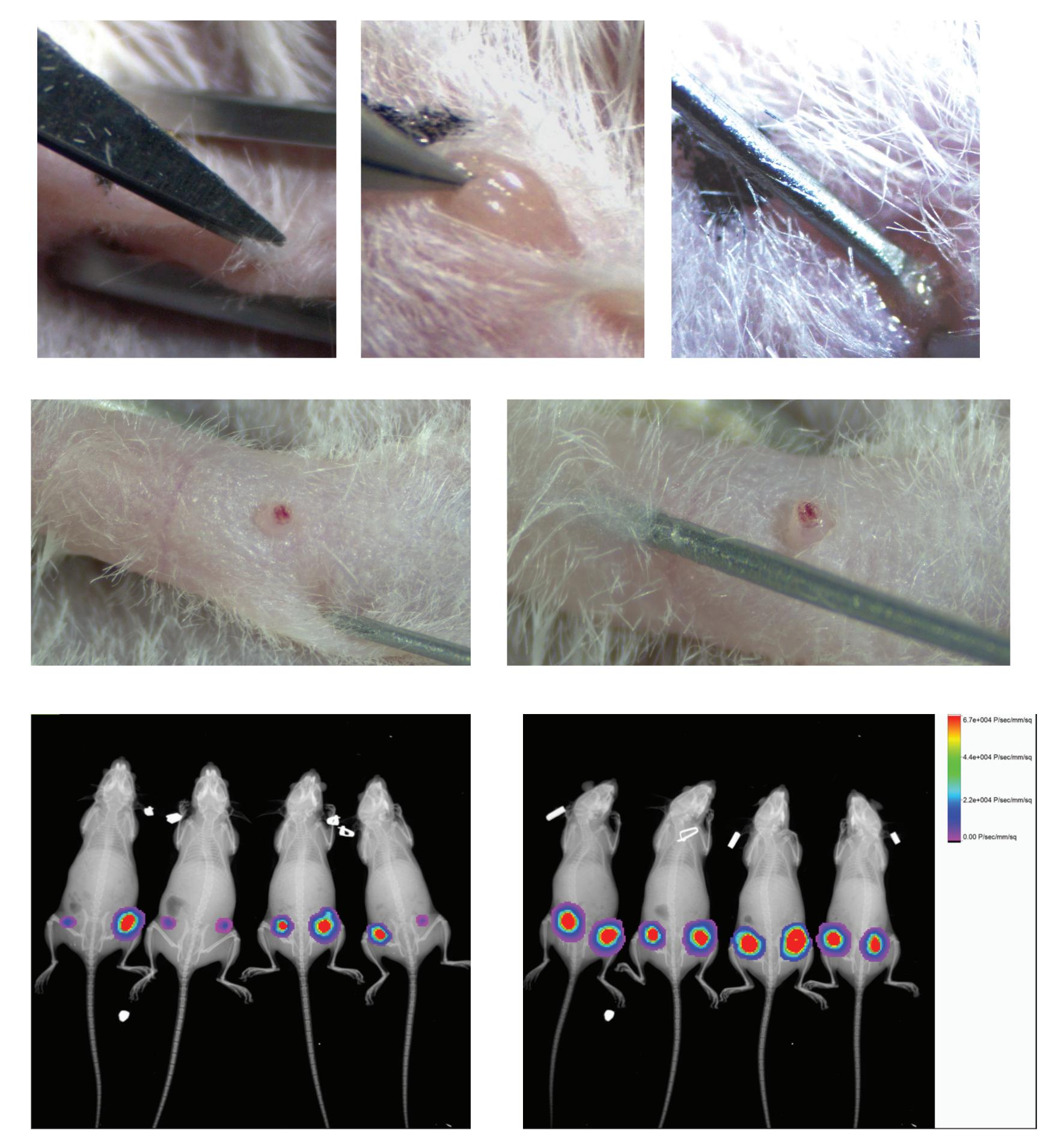


Figure 1: Injection in the mammary fatpadby surgical method : For reference the nipple was stained with a black marker. Panel A: Incision is made a few mm lateral of the nipple (black structure under the scissors);

Panel B: the fatpad is moved to the incision site to expose it; Panel C: The injection is performed.

Figure 2: Injection in the mammary fat pad by non-surgical method:
Panel A: When the skin around the nipple is held upwith forceps, the mammary gland and large superficial blood vessels can be visualised;
Panel B: the actual injection, away from the gland and the blood vessels

Figure 3: Whole body bio-luminescent imaging of tumor development. Panel A: Mice treated by method 1. Panel B: Mice treated by method 2.

References: Kocatürk and Versteeg, J Vis Exp. 2015 Feb 8;(96)

