# IN OVO PET IMAGING OF A HUMAN COLORECTAL CARCINOMA MODEL IN CHICKEN CHORIOALLANTOIC MEMBRANE

PW003

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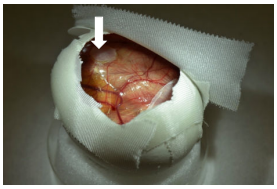
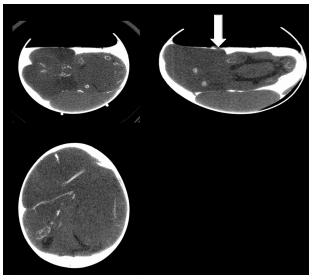
## Aim

- The initial objective of this study was to assess the feasibility of in vivo PET/CT imaging in a novel human colorectal carcinoma model being developed in chicken chorioallantoic membrane (CAM).
- In an initial pilot study a cell line modeling colon cancer was selected and imaged using [18F]fluorodeoxyglucose (FDG).

## Materials and methods – Colorectal carcinoma

- A window was made in the shell of fertilized chicken eggs at day 3 post-fertilization and $3x10^6$ SW1222 human colorectal carcinoma cells were implanted (1:1 medium:Matrigel) at day 11 post-fertilization.
- On day 17 the shell window was enlarged to allow direct injection of FDG (12.2 ± 4.5 MBq/egg) into a CAM blood vessel.
- A mixture of ketamine/medetomidine (50 :1 mg/ml, 0.2 ml/egg) was injected into the albumin in selected eggs to assess the effect of anesthesia.
- After FDG injection the egg was returned to the incubator for a 45 min uptake period before imaging.
- Imaging was performed on a Siemens Focus 120 microPET with structural CT on a General Electric eXplore CT120. A Minerve cell system allowed reproducible positioning between modalities. PET data was acquired as a static 10 min frame and reconstructed using a 3D maximum a posteriori (MAP) method with all corrections except scatter. A standard 100 µm (theoretical) resolution protocol was used to obtain structural CT data. Image coregistration was performed in PMOD version 3.3.
- Additional contrast on the CT data was achieved by adding iodinated contrast agent (Iobitridol 35 mgI/ml) to the albumin.





Figures 1 (left) & 2 (right): Left - Photograph of colorectal carcinoma cell layer (arrow) on chorioallantoic membrane. Shell window enlarged for access to inject tracer. Right – Planar images from structural CT scan with albumin contrast to aid identification of chicken embryo, yolk sac and tumor (arrow).

## Results – Colorectal carcinoma

- FDG uptake was clear in chick and tumor, with notably high uptake at the major joints.
- Tumors were identified by localization of FDG uptake on the surface of the CAM.
- A lack of soft tissue contrast between tumor, CAM and albumin made precise structural identification of the tumor difficult. Anesthesia was crucial to image quality in both PET and CT. CT contrast between the soft tissues of the chick and surrounding albumin/structures was improved by addition of contrast agent.

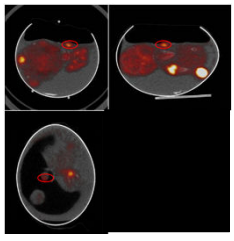


Figure 3: Overlaid PET (hot red color scale) and CT (grey scale) images from 10 min static scan of FDG uptake after 45 min. Accurate coregistration was achieved using fiducial markers – glass capillary tubes containing FDG. High FDG uptake is evident in the embryo with hot spots at major joints. FDG uptake in the tumor localized on the CAM was readily identified (circled in red).

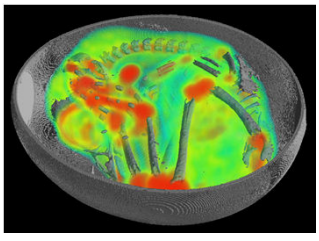


Figure 4: 3-dimensional visualization of overlaid PET (color)/CT (solid grey scale) data. The 3D visualization was of particular use in identifying sites of tracer uptake in the embryo.

## Further development – Glioblastoma, dynamic PET imaging

- After the successful imaging of glucose metabolism in implanted colorectal carcinomas, a number of methodological developments were investigated using subsequently prepared u87 glioblastomas implanted (without Matrigel) using a similar protocol to that described for SW1222 cells.
- The increased size of the resulting glioblastomas facilitated imaging, and will improve quantification by reducing the influence of partial volume effects.
- Isoflurane (2% in air) was evaluated for improved control of anesthesia[1]. The sealed environment of the Minerve cell proved ideal. Sodium [18F]fluoride was used to evaluate the success of anesthesia. A suitable induction period (min. 10 min) was necessary to ensure no motion during scanning.
- To allow injection of FDG inside the PET scanner for dynamic scanning a PE10 catheter was implanted in a CAM vessel.
- A region-growing algorithm was developed for delineation of the tumor in CT images and subsequent accurate calculation of tumor volume.

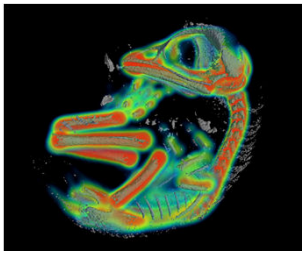


Figure 5: Coregistered NaF PET (color) and CT bone (grey scale) images illustrating the effect of successful anesthesia using isoflurane. The ability to perfectly match uptake of radiotracer to structural information is crucial to delineating uptake in embryo and tumor.

Figure 6: u87 glioblastoma on chorioallantoic membrane with PE10 catheter for injection of radiotracer inside microPET system.

## Results – Dynamic FDG uptake in glioblastoma

- Catheterization of a CAM vessel made dynamic imaging of FDG uptake in tumor and embryo possible. This demonstrates the applicability of the model for assessing the kinetics of novel tracers targeting specific biomarkers.
- Time-activity curves were extracted using an automatic isocountour-based volume of interest seeded in the tumor.
- With the aid of additional albumin contrast the region-growing algorithm accurately delineated tumors in the CT data and yielded reproducible volume measurements. Repeat CT scans during the tumor growth period will allow dynamic tracking of tumor volume. In this way the effect of antitumor treatments could be evaluated.

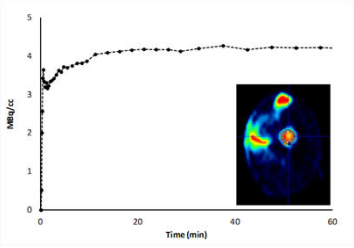



Figure 7: Time-activity curve for FDG uptake in u87 glioblastoma implanted on chorioallantoic membrane. Inset – illustration of volume-of-interest defined on tumor.

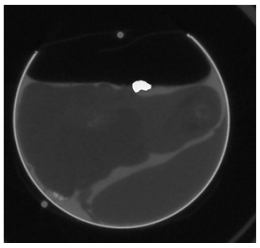


Figure 8: Illustration of tumor delineation using region-growing algorithm on CT data. The tumor volume calculated for this example was 17.2 $mm^3$.

## Conclusion.

- For the first time we demonstrate successful imaging of FDG uptake in human colorectal carcinoma and u87 glioblastoma chicken CAM models in vivo. A number of methodological considerations have been addressed.
- These models could be of great value to PET oncology imaging, reducing costs, lab space requirements and offering an ethically sustainable alternative to mouse models.

REFERENCES

1 – Würbach et al. (2012) Molecular Imaging & Biology DOI: 10.1007/s11307-012-0550-6

ACKNOWLEDGEMENTS & SPONSORS

FB is funded by The Marie Curie Initial Training Network (MC-ITN) "Methods in Neuroimaging" #MC-ITN-238593,
M.A.B is a "collaborateur logistique" for FRS-FNRS Belgium, AP is a senior research associate for FRS-FNRS Belgium

DISCLOSURES

GW consults for PMOD Technologies, Zurich, Switzerland.