# In vivo monitoring of angiogenesis within Matrigel chambers using MRI

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## Introduction

Angiogenesis is a critical process in tumour development and presents an important target for the development of a range of anti-cancer agents[1,2]. To assess the in vivo efficacy of these 'angiotherapeutics', a simple and reproducible in vivo model would be of significant value. Here we show that a gel containing pro-angiogenic agents held within a chamber may be positioned sub-cutaneously in mice and the angiogenic process followed using MR methods. This approach allows an individual animal to be followed with time in order to study the initial development of vasculature within the chambers.

## Methods

Perspex chambers containing Matrigel, bFGF (1000 ng/ml, Sigma, Denmark) and VEGF (500 ng/ml, Sigma, Denmark) were assembled as previously described[3] (figure 1). Following radiation sterilization, the chambers were implanted subcutaneously on both flanks of 24 male NMRI nu/nu mice. Magnetic resonance imaging was performed using a 4.7T Varian system two weeks after implantation under hypnorm/dormicum anesthesia. Contrast agent was administered via a cannulated tail vein (0.4mg Endorem, n=12, 0.3ml Magnevist, n=12).

Following MR examination, the chambers were excised and imaged using a digital camera.

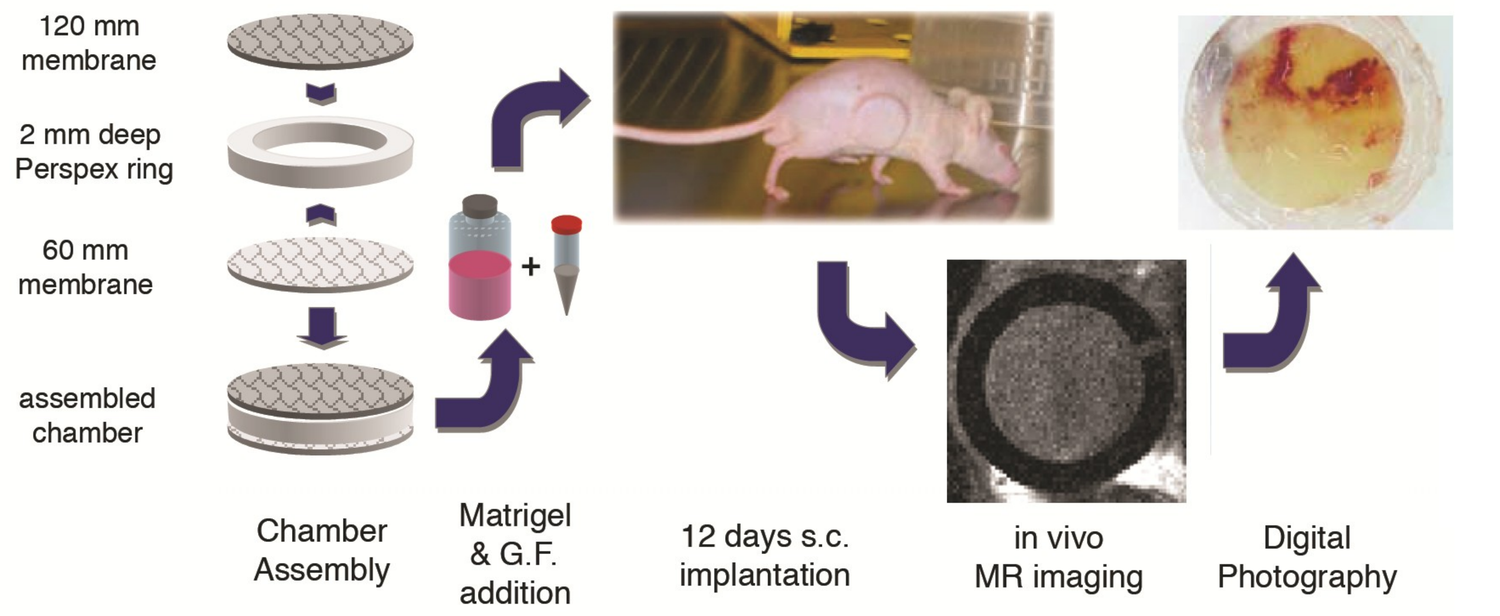


**Figure 1:** Schematic diagram of the assembly and imaging of the chamber

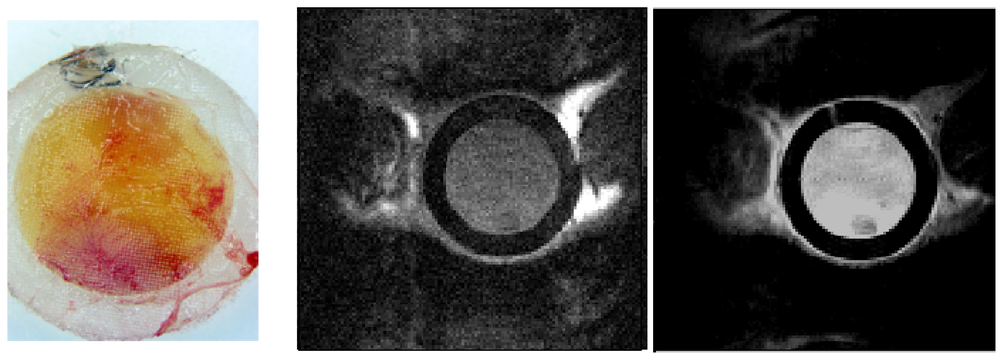

**Figure 2:** Ex vivo optical image **(left)** together with in vivo pre- **(middle)** and post- **(right)** contrast (Magnevist, 0.3 ml) T1W spin echo images (TR/TE 300/3 ms) of the same Matrigel implant.




**Figure 3: Left:** Quantitative T2 image post contrast (multiple readout spin echo sequence, TR=3s, TE=15-180ms in steps of 15ms, T2 values obtained by least squares fitting). **Middle:** dMRI difference image (gradient echo FLASH sequence, TR/TE=20/5.2 ms, Fl= 20°, nt=150). The image shown is the difference between the mean of the 25 first and last images. **Right:** Time curves for the ROIs shown in the dMRI difference map (middle) are shown in the plot.

## Results & Discussion

Figure 2 shows significant enhancement of the Matrigel chamber following administration of GdDTPA. The region of cellular infiltration apparent in figure 2 (left) becomes hyperintense compared to uninfiltrated Matrigel (right). The high vascular permeability due to VEGF results in diffuse contrast enhancement. The long T2 values of the Matrigel chamber prior to implantation suggested the use of a T2* blood pool agent (Endorem), to provide more vascular specific information. As shown in figure 3 (left), a region of cellular infiltration is seen in the upper left region of the image. What appears to be a vessel may be seen on the dMRI difference map (red ROI, middle). Some correspondence between the post contrast T2 map (left) and dMRI difference (middle) is seen. As shown in the time curves (right), three distinct regions could be identified. The red region appears to be a vessel where washout causes the signal to increase slightly following initial signal reduction following contrast agent administration. The green and blue regions are different parts of the infiltration that might have different vascular or cell densities.

The dMRI difference maps show the initial effects of the contrast agent while the T2 maps show the extent of tissue infiltration and steady state effects. The difference between Figure 3 left and middle may be interpreted as an area corresponding to a vessel (red ROI) where the intravascular contrast agent clears before the post contrast T2 map is acquired.

Whilst a previous study by Pilatus, U. et al.[4] has shown the potential of using Matrigel for studying cell invasion, this study has applied MRI methods to study vascular development in an in vivo model.

## Conclusion

This study has shown that it is possible to monitor angiogenesis using a minimally invasive magnetic resonance method in vivo. Furthermore, a single animal may be followed longitudinally and act as its own control. The method may be developed further to study the effects of agents designed to influence the angiogenic process.

## References

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