Non-invasive in vivo MRI angiogenesis assays

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Introduction: Angiogenesis, the development of blood vessels, is a key element in many normal and pathological processes, such as tumor establishment and metastasis.[1] Therefore, many studies have attempted to develop a reliable and reproducible method to assess the effect of pro- or anti-angiogenic factors. The Matrigel (Basement membrane extract) plug assay, which consists in implanting Matrigel subcutaneously in animals, has become a useful tool to study angiogenesis.[2] However, this assay requires plug recovery and thus prevents follow-up studies. The purpose of this study was to develop a new dynamic contrast-enhanced (DCE)-MRI assay using Gd-DTPA to follow angiogenesis *in vivo* in mice by correlating contrast agent uptake with neovessel formation into Matrigel plugs.

Method: Six Balb/c mice were injected subcutaneously on one hip with Matrigel (350 µl) supplemented with bFGF (500 ng/ml) and heparin (16 U/ml) to promote angiogenesis. Matrigel only was injected on the opposite side as a control where limited angiogenesis is expected. T_1-weighted DCE-MRI experiments were conducted 3 or 4 times on each subject with a few days interval between consecutive scans. All animals were anaesthetized with isoflurane and their body temperature maintained by a warm air blower controlled by a rectal thermistor feedback. They were placed in a 40-mm Millipede™ RF probe inside a Varian 7T scanner equipped with 205/120 mm gradients. From 90 to 120 consecutive sets of gradient-echo images were acquired with the following parameters: TR/TE = 100/2.49 ms, matrix size 128 x 128, FOV 30 x 30 mm^2, 10 slices of 1.5 mm, NA 4, and a 30° flip angle. After the third set, 180 µl of Gd-DTPA (100 mM) was injected i.v. Images were processed after subtraction of a pre-contrast image. After the completion of the series of scans, plugs were harvested, fixed in 10% formalin, embedded in paraffin, sectioned in 4-µm cross-sections, stained with hematoxylin and eosin (H/E) and observed by light microscopy or used for hemoglobin content determination using Drabkin's assay (Sigma, D5941).

Results and Discussion: The variation between the supplemented Matrigel containing pro-angiogenic factors and the control with limited neovascularization was observed with MRI. As shown in Fig. 1 for plugs ten days after implantation, the supplemented plug (right side) generally displays a much higher signal increase and this increase occurs more rapidly after injection of Gd-DTPA than the plug with Matrigel only (left side). No enhancement is detected in the control plug at 1 minute following injection of Gd-DTPA (Fig.1) while a non uniform peripheral entry of contrast agent into the supplemented plug is seen. These favored locations probably correspond to host endothelial cell penetration. No difference can be recorded for DCE-MRI curves and the rise time ratio (Matrigel supplemented/control) is close to 1 for both plugs on day 0 (Fig. 2). The supplemented plug curve rises and drops more rapidly than its counterpart on days 7 and 14, suggesting a more dynamic behavior due to an increase in vascularization. The accuracy of MRI angiogenesis development analyses were compared by hemoglobin quantification in Matrigel plugs. A 15-fold increase was observed in hemoglobin content for Matrigel containing angiogenic compounds in comparison with Matrigel only (Fig. 3). Histological observation showed a significant increase in invasion and organization of infiltrating host cells and a more massive presence of endothelial mature structures (arrows) in the supplemented Matrigel (Fig. 4, B) compared to the control Matrigel (Fig. 4, A).

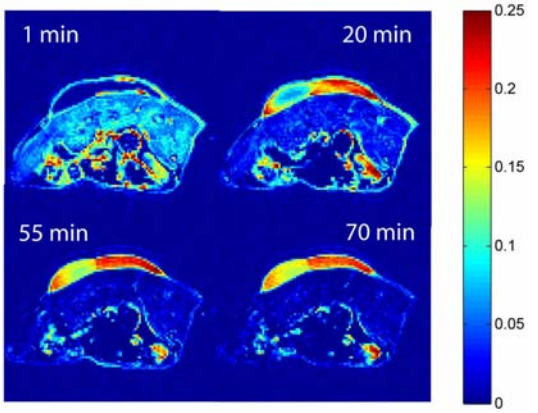

Fig. 1. Contrast–enhanced axial MR images of Matrigel plugs on a mouse on day 10. Matrigel only (left) and Matrigel supplemented with bFGF 500 ng/ml and heparin 16 U/ml (right) at 1, 20, 55 and 70 minutes after an i.v. injection of Gd-DTPA.

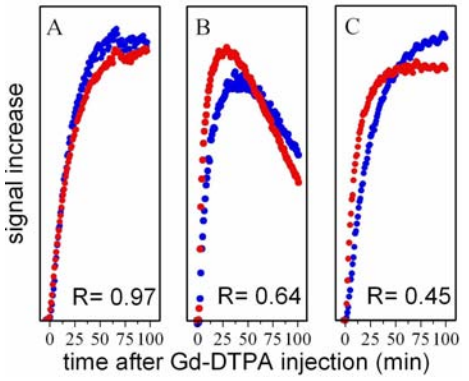

Fig. 2. Time dependency of the MR signal increase in the control (blue) and supplemented (red) plugs on days 0, 7 and 14 (A, B and C) after injection of the contrast agent. R is the rise time ratio (Matrigel supplemented/control).

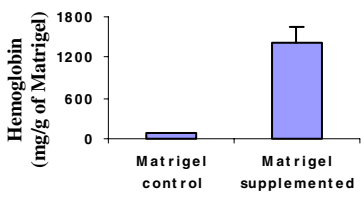

Fig. 3. Quantitative analysis of hemoglobin content in the control Matrigel plug and the plug supplemented with bFGF 500 ng/ml and heparin 16 U/ml on day 14.

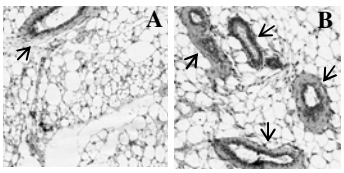

Fig. 4. Histological evaluation with H/E coloration of a control Matrigel plug (A) and a plug supplemented with bFGF 500 ng/ml and heparin 16 U/ml (B) on day 20 (x100 magnification).

Conclusion: We developed an *in vivo* assay to detect the presence and variation of neovascularization with dynamic T_1-weighted Gd-DTPA contrast-enhanced images of Matrigel plugs, confirmed by hemoglobin content and light microscopy observation. These results make our assay a promising tool to test and follow the activity of pro- or anti-angiogenic agents or stimuli *in vivo*.

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