Do extravillous trophoblastic cells promote vascular smooth muscle cell invasion in human spiral artery remodelling?

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Introduction

- Remodelling of the uterine spiral arteries is essential for human pregnancy and its failure may lead to late miscarriage, pre-term birth, pre-eclampsia and fetal growth restriction.
- During spiral artery remodelling these blood vessels lose their vascular smooth muscle cells (VSMCs) which are then replaced by fibrinoid material and intramural extravillous trophoblast cells (EVT).
- Eventually this makes the blood vessels increase in diameter and lose their vasoactive control.
- This enables them to deliver large volumes of maternal blood to the fetal-placental unit in a non-pulsatile manner.
- The exact physiology of the invasion and loss of VSMC from the wall during spiral artery remodelling is not clearly understood.
- Recent studies have shown that the VSMCs migrate away from the spiral arteries into the surrounding decidual stroma, a process that appears to be associated with the presence of interstitial EVT.
- Once in the decidual stroma, the migrated VSMCs appear to then undergo apoptosis and be phagocytosed by uterine macrophages.

Aim

To develop an invasion assay using aortic VSMC and human trophoblast-like (HTR-8/SVneo) cells to represent spiral artery VSMCs and interstitial EVT cells respectively

Methods

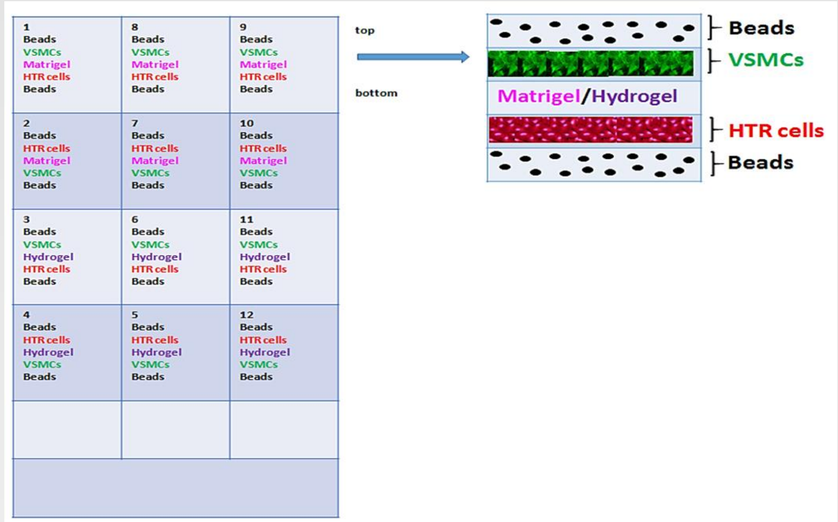
Novel Invasion Assay

- Human aortic VSMC and human trophoblast-like (HTR-8/SVneo) cells lines were used as models for spiral artery VSMCs and EVT respectively.
- Each cell type was tagged with a fluorescent live cell tracking dye; VSMC-green, HTR-8/SVneo-red.
- Fluorescent beads were placed into each well of a 15-well chamber slide before adding the cells (as shown in the diagram).
- Either VSMC or HTR-8/SVneo cells were placed on top of the beads and left to adhere overnight.
- Matrigel or Hydrogel were added on top of the cells before adding another layer of VSMC or HTR-8/SVneo cells.
- The wells was observed for any invasion under a confocal fluorescent microscope at the time of T=0h, T=24h, T=48h and T=72h.

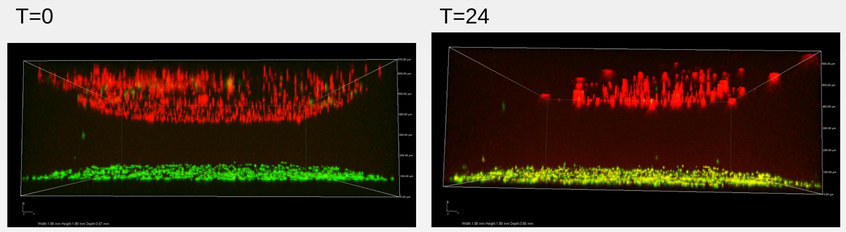
Classic Invasion Assay

- Both fluoresescently stained and non-stained HTR-8/SVneo cells were used.
- Matrigel was placed on a cold filter and allowed to spread evenly.
- Either stained and non-stained HTR-8/SVneo cells were placed on top of the matrigel and the cells were left to invade through the filters for T=24h, T=48h and T=72h.
- In another experiment either VSMCs or HTR-8/SVneo cells were grown in the bottom of a 24 well plate and the transwell filter coated with matrigel with either HTR-8/SVneo cells or VSMCs as appropriate placed above for T=48h (see diagram).
- The filters were then stained with haematoxylin and eosin (H&E) and the number of cells invaded through the filter was counted using a light microscope.

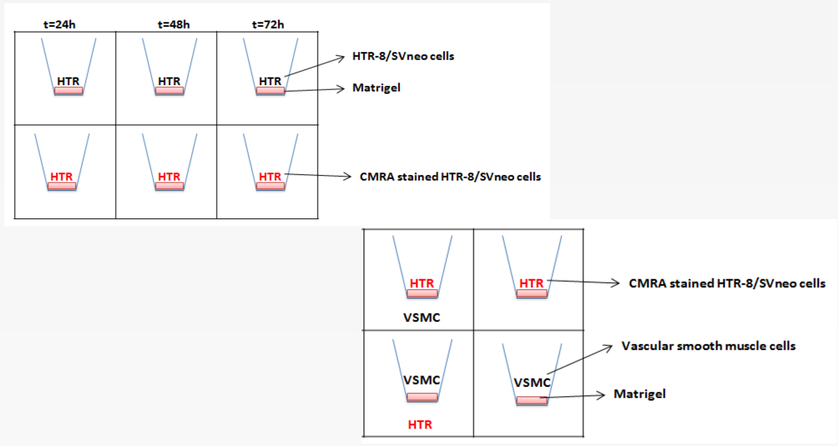
Layout of Novel Invasion Assay



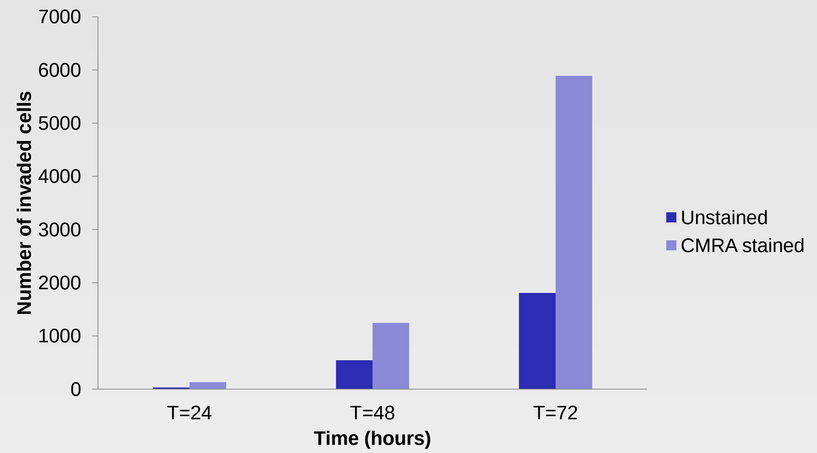
Matrigel expands during the first 24 hours of the invasion assay making assessment difficult



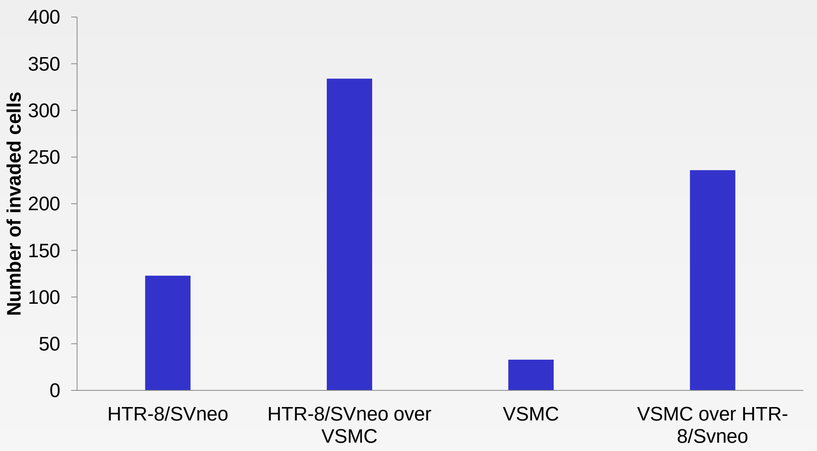
Layout of Classic Invasion Assays



CMRA stained HTR-8/SVneo cells appear to be invasive than unstained cells



Both VSMCs and HTR-8/SVneo cells appear to influence the invasive capacity of the other cell type



Summary

- No invasion of either VSMCs or HTR-8/SVneo trophoblast-like cells into matrigel or hydrogel was observed in the novel invasion assay.
- This may be due to the size of the wells not allowing delivery of sufficient oxygen and nutrients to the cells during the time span of the assay as both cell types were able to invade through matrigel in a classic invasion assay.
- Classic invasion assay have proved that the CMRA dye do not inhibit the invasion of human trophoblast-like (HTR-8/SVneo) cells through the Matrigel but may promote them.
- There may be a reciprocal attraction between VSMCs and trophoblast cells in the process of spiral artery remodelling although further experiments are required.