Software to automatically quantify in vitro angiogenesis

Ryan M. Brown[1], Roy Bicknell[2], Victoria L. Heath[2], and Iain B. Styles[3]

PSIBS, Chemistry West, University of Birmingham, Birmingham B15 2TT.[1] Institute of Biomedical Research, Medical School, Birmingham B15 2TT.[2] School of Computer Science, University of Birmingham, Birmingham B15 2TT.[3]

Introduction and Aims

- A major process in the metastasis of cancers is unregulated angiogenesis, which delivers nutrients and oxygen for the further invasion of the tumour (Fig. 1) [1-2].
- Inhibition of this pathological process could result in a range of new anti-cancer therapies.
- In vitro angiogenesis assays, such as the matrigel assay, are used extensively as 2D scaffolds for forming the network of vessels found in angiogenesis (Fig. 2) [3-4].
- Current methodologies for image analysis are manual, and thus subjective and time-consuming, therefore an automated method that can quantify the networks is needed
- Angiogenesis involves the formation of new blood vessels from the existing vascular network.

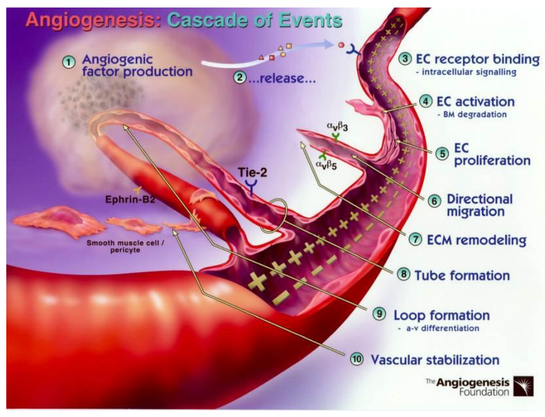


Fig. 1: Events in the angiogenesis of solid tumours[3].

- The aim of this project is to develop software that can automatically quantify network formation in matrigel assays.

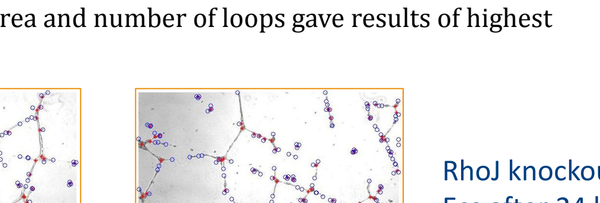


Fig. 2 Matrigel assay of HUVECs showing the network formation as cells migrate. Control experiments give well formed networks (left) and HUVECs with depleted RhoJ levels (right) give highly fragmented networks.

Results

Computational workflow

To reduce the amount of specialised imaging conditions, all computation was performed post-processing. The first challenge is to obtain a representation of the network in its simplest form:

- Network skeletonisation allows greatly simplifies node connectivity allowing rapid quantification of the network.
- The post-processing workflow for how this is achieved is displayed in Fig. 3.
- Software was originally implemented in Matlab, and has been implemented in Java for release of an ImageJ plugin.

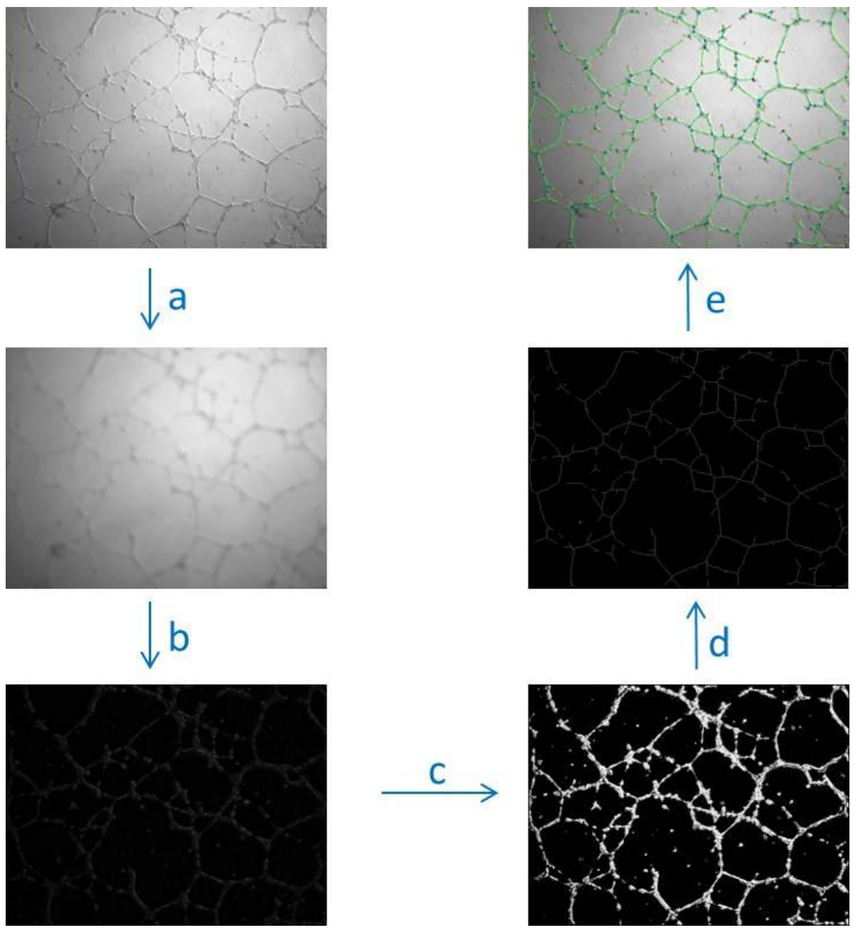
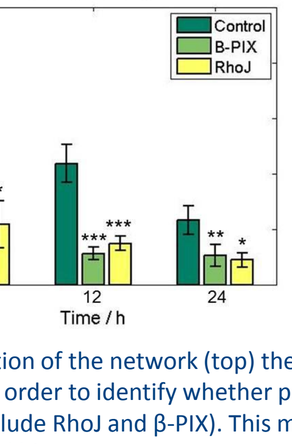


Fig. 3: Computational workflow for the plugin. Flat field correction is applied, beginning with Gaussian blurring (a). Two images blurred by different sized Gaussians are subtracted giving the feature-detected image (b). Edge detection then gives a binary image giving the tubules (c). Dilation followed by skeletonisation gives tubules that are one pixel thick, simplifying network quantification (d). Parameters, such as nodes, are located and quantified; the results are generated and plotted onto the original image (e).

Effects of RhoJ and β-PIX on angiogenesis

- RhoJ and β-PIX are genes involved in cytoskeletal movement, knockdown of these genes inhibits the movement of cells into networks. The knockdown cell lines should give networks that are fragmented and poorly formed.
- Results of the control and RhoJ knockdown Ecs after 24 hours are displayed in Fig. 4.
- Kruskal-Wallis tests showed the area and number of loops gave results of highest significance.

Control ECs after 24 h.



RhoJ knockout Ecs after 24 h.

Fig. 4: Rapid quantitation of the network (top) then allows morphological variables to be compared across experiments in order to identify whether protein downregulation is affecting angiogenesis (bottom), proteins include RhoJ and β-PIX). This method has significantly reduced analysis time and removed observational bias.

Literature cited

1. Auerbach, R. et al. Angiogenesis assays: A critical overview. *Clin. Chem.* **49**, 32–40 (2003).
2. Pandya, N. M., Dhalla, N. S. & Santani, D. D. Angiogenesis—a new target for future therapy. *Vascular Pharmacology*. **44**, 265–274 (2006).
3. Adams, R. H. & Alitalo, K. Molecular regulation of angiogenesis and lymphangiogenesis. *Nature Reviews*. **8**, 464-478 (2007).
4. Heath, V. L. & Bicknell, R. Anticancer strategies involving the vasculature. *Nature Reviews*. **6**, 395-404 (2009).

Acknowledgments

We acknowledge the EPSRC for funding, PSIBS CDT and cohort for advice and support.

EPSRC
Engineering and Physical Sciences Research Council

Conclusion

- Automated methods for quantifying *in vitro* angiogenesis have been successfully achieved.
- Observer bias has been removed and new parameters are available to quantify the network of ECs formed in the matrigel assay.
- This matrigel analysis program is being published in the 3rd edition of Angiogenesis Protocols and will be available for free download as an ImageJ plugin from http://www.birmingham.ac.uk/staff/profiles/iandi/bicknell-roy.aspx