A model system for directing blood vessel formation

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Vertebrates depend on a complex vascular system for supply of oxygen and nutrients and for removal of waste products. Blood is transported via a network of blood vessels. The thinnest blood vessels, the capillaries, are only composed of a single layer of endothelial cells which are covered by a certain type of stabilizing cells, called pericytes, and surrounded by a basement membrane. The development of the first vascular structures (vasculogenesis) takes place during embryogenesis and continues throughout the whole life whenever new blood vessels or the remodelling of the vascular system (angiogenesis) is required, for example, for revascularization during wound healing or to supply enough oxygen for growing muscles. Angiogenesis is also implicated in a lot of different diseases, such as tumour growth and metastasis or diabetic retinopathy (damages of small blood vessels in the retina of the eye, untreated it eventually results in blindness). One mechanism of formation of blood vessels from pre-existing vasculature is sprouting angiogenesis. Sprouting angiogenesis describes the outgrowth of endothelial cell sprouts from a mother vessel in response to angiogenic factors. Eventually, this new sprout will fuse with other vessels or vascular sprouts to form a new vessel and allow blood flow through this newly formed vessel (fig. 1).

To study blood vessel formation *in vitro* embryoid bodies (EBs) were chosen as a model system. EBs are spheroids, “clumps”, of differentiating embryonic stem cells. Since those stem cells can give rise to essentially all cell types, interactions between endothelial cells and non-endothelial cells can occur in a similar way as it does in the body. To model sprouting angiogenesis, EBs are embedded in gel of collagen, the major component of the extracellular matrix (non-cellular, supportive area between the different cells) and stimulated with angiogenic factors such as vascular endothelial growth factors (VEGFs) or fibroblast growth factors (FGFs). In my thesis I found that sprouting was initiated most effectively when EBs had been cultured in suspension in the presence of high concentrations of VEGF and FGF thereby stimulating formation of a first capillary network from where angiogenesis occurs soon after embedding the EBs in collagen gel.

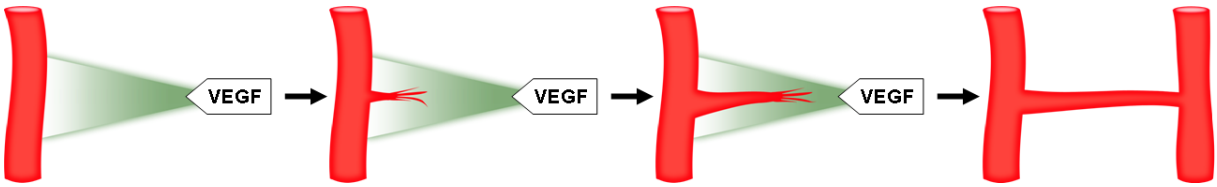

Figure 1: Steps of sprouting angiogenesis. In response to a gradient of angiogenic factors (e.g. VEGF) an endothelial cell sprout will grow out of a vessel and fuse to another vessel.

In the body vascular sprouts are directed by gradients of angiogenic factors, especially VEGF plays an essential role. Stable gradients can be generated by a recently developed microfluidic device. Under guidance of VEGF gradients (0-20 ng/ml) about 73% of sprouting EBs showed a directed outgrowth of vascular sprouts. It is largely unknown how vascular sprouts grow through the extracellular matrix in response to a gradient of angiogenic factors. My data provide the first hints that the gradient might be sensed by branching of the sprout’s tip and selection of certain branches over others.

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