

Studies on angiogenesis took a major leap after the identification of pro- and antiangiogenic molecules. In vivo tests, though accurate, are time consuming and not quantifiable easily, as the in vitro tests. But though in vitro tests are quicker and quantifiable, they need to be established and correlated with in vivo findings.

## 12.4 IN VITRO ASSAYS

The heterogeneity (Goodwin, 2007; Auerbach et al., 2000, 2003; Staton et al., 2009; Femke et al., 2006) of endothelial cells and the ease of their harvesting from larger blood vessels make them invaluable in in vitro assays. Human umbilical vein endothelial cells and bovine aortic endothelial cells are commonly used for the assays. Though in in vitro setting, 2D and 3D scaffolds of collagen, fibrin, and Matrigel are used to simulate, the complete hemodynamics, in vivo shear stress, and oxygen tension cannot be simulated. The assays can be used as a steering force. The various in vitro assays are the:

- Endothelial cell proliferation assay
- Endothelial cell migration assay
- Endothelial tube formation assay
- Rat and mouse aortic ring assay

Direct cell counts, DNA synthesis, or metabolic activity (Goodwin, 2007; Auerbach et al., 2000, 2003; Staton et al., 2009; Lee, 2006) will help recognize the effects of proangiogenic and antiangiogenic molecules in proliferation of the endothelial cells in developing capillaries. The proliferation rate should be reduced in the media to be able to test the action of proangiogenic molecules. Antiangiogenic molecules can be checked on cells that have increased proliferation rate. The most reliable method to assess proliferation is counting of individual cells. The counting should be at 24 h intervals using a hemocytometer and light microscope or electronic Coulter counter.

Endothelial cells migrate degrading the basement membrane in sprouting angiogenesis along the set gradient of growth factors. This cell migration is gauged by transfilter assay, which is the most responsive even with reduced chemotactic factors. Other cell migration assays would be Teflon fence assay, under-agarose assay, phagokinetic track assay, and wound-healing assay (Goodwin, 2007; Auerbach et al., 2000, 2003; Staton et al., 2009; Polytarchou et al., 2006).

Subsequent to proliferation and migration of endothelial cells toward the stimulus, lumens are formed to carry blood. Differentiation of endothelial cells leads to the formation of tubes (Goodwin, 2007; Smith and Staton, 2006).

Along with 2D Matrigel assay, 3D fibrin gel bead assays are the most useful tube formation assays.

In rat and mouse aortic ring assay, 1 mm sections of aorta (Nicosia and Ottinetti, 1990; Aplin et al., 2008; Nicosia, 2009) are implanted in the collagen \fibrin