matrix in a serum-free medium. A 3-day culture shows microvessels, same as normal blood vessels, sprouted interacting with other cells mimicking normal angiogenesis.

12.5 IN VIVO ASSAYS

Angiogenesis can be no doubt better understood, with in vivo assays. Assays for the study of proangiogenic and antiangiogenic molecules have been developed, viz, corneal angiogenic assay, chick chorioallantoic membrane (CAM) assay, Matrigel plug assay.

Cornea when embedded, with proangiogenic molecules, new blood vessels are formed. Cornea being avascular helps these observations to be noted clearly.

Membranes under the shell of chick eggs, the Chorioallantoic Membrane (CAM) is used, as they are highly vascular, multiplication of samples are easier, consequent regular measurements, visualization, quantification and the same CAM model can be used for control and experimental samples (Auerbach et al., 2000, 2003; Staton et al., 2009; Zijlstra et al., 2006)

The method of choice of in vivo assay is Matrigel (Auerbach et al., 2003; Akhtar et al., 2002). This is a protein mixture, containing growth factors and is similar to the extracellular matrix. It is delivered into the body subcutaneously, which solidifies in 30 min. Imaging of the Matrigel plug after few days shows blood vessel formation. It is an important assay for testing antiangiogenic molecules in the system.

Metabolic factors which regulate blood vessel formation are the following:

- Increased muscular activity increases vascularity (Wagner, 2001; Adolfsson et al., 1981; Andersen and Henriksson, 1977; Holloszy and Coyle, 1984; Ingjer, 1979; Terjung et al., 2002).
- Decreased vascularity and rarefaction are associated with muscular disuse (Desaki et al., 2000; Oki et al., 1999; Jozsa et al., 1980).
- Chronic increase in blood pressure causes vascular rarefaction (Prewitt et al., 1982; Hutchins and Darnell, 1974; Harper and Bohlen, 1984).
- With oxygen being the key indicator and regulator of vascularity.

The process of angiogenesis is stimulated not only by growth hormones and mechanical factors such as shear stress, shape of endothelial cells which facilitate angiogenesis, but also by mechanisms which are mechanosensory. ENaC (epithelial sodium channel) seems potentially to facilitate the mechanosensory actions. It is basically, a protein (Rivilis et al., 2002; Drummond et al., 2008; Benos and Stanton, 1999; Kellenberger and Schild, 2002; Garty and Palmer, 1997) seen in many cell types of mammals (Ugawa et al., 1998; Price et al., 2000, 2001) adhered to the cytoskeleton and the extracellular matrix allowing exchange between the exterior and the interior cell membrane by action of actin (Jovov et al., 1999; Copeland et al., 2001). Studies have shown that blood vessel growth is affected by ENAC (Adair and Drummond, 2009, 2010).