Culture of Vascular Cells In Vitro

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Ross Harrison (1870–1959) was the first to attempt to culture tissues in vitro using culture dishes. In 1907 he had grown tadpole tissue in a coagulated interstitial liquid from frog using the hanging drop technique [104]. This technique is still used today to generate spheroids in vitro and to culture them.

A major figure in the development of cell culture was Alexis Carrel, a French surgeon who made his career in the United States and worked at the time at the famous Rockefeller Institute in New York. Basing his work on the technique developed by Harrison, he succeeded in 1912 for the first time in cultivating tissue explants containing animal cells in vitro. These first cultures were established from chicken heart in a medium developed by Carrel containing coagulated plasma, serum, saline, and a chicken embryo extract [105, 106].

The culture conditions were then greatly improved by the development of defined culture media. Harry Eagle (1905–1992) was the first to define more accurately the nutrient requirements for cells grown in vitro. This resulted in the development of the minimal medium called Eagle's Minimal essential medium (MEM) [107]. Subsequently, Renato Dulbecco succeeded in improving the conditions of culture by developing an optimized medium, now famous as DMEM (Dulbecco's modified Eagle medium), which is still used today by most laboratories in the world. Endothelial cells were isolated from various species, including bovine, rodent, and human sources. If the in vitro culture of bovine endothelial cells was easier, it has been more difficult to establish in vitro cultures of human endothelial cells.

Eric Jaffée at Cornell University in New York, and Michael Gimbrone, then a post-doctoral fellow in the Judah Folkman laboratory in Boston, were able to isolate human endothelial cells from newborn umbilical cord and to define exactly the in vitro culture conditions and propagation of these cells [108, 109] (Fig. 5.1). It was the era of "cord hunting" and many laboratories were embarking on this path. In the laboratory where I worked during my thesis, we were in contact with different maternity hospitals in Paris and there were fixed days to collect the umbilical cords. Beakers were filled with culture medium in which the obstetricians then placed the umbilical cords of newborns, which we then collected the next day.

A. Bikfalvi, *A Brief History of Blood and Lymphatic Vessels*,
https://doi.org/10.1007/978-3-319-74376-9_5

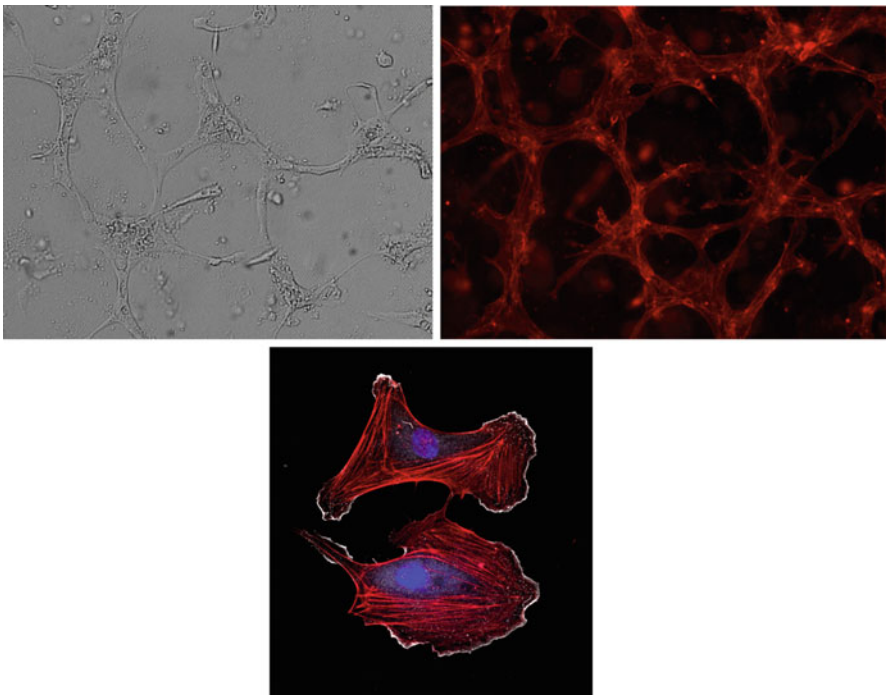

Fig. 5.1 Endothelial cells in culture. Endothelial cells cultured from the umbilical cord are represented in a vitro angiogenesis system (*top*). Phase contrast image (*top left*). Fluorescence image (phallodin in *red*) (*top right*). Endothelial tube formation can be evidenced under these conditions. Microvascular endothelial cells of the lung after immunostaining using an antibody against cortactin (*white*) and actin (*red*) (*bottom*). The nuclei are marked in *blue*. The arrangement of the cytoskeleton and the actin fibers is clearly seen. Images from the author's team (L. Cooley and T. Daubon post-doctoral fellows in the laboratory)

The isolation of human capillary endothelial cells was a more difficult task. Although it was fairly easy to obtain these cells from animal sources (especially from the adrenal cortex in bovine), human capillary endothelial cells were difficult to grow in vitro because their need for nutrient and growth factors was more important. Different sources were then tried, such as the newly circumcised newborn foreskin or omental fat (fat tissue in the abdominal cavity). These difficulties were related to the fact that the culture conditions of these cells were not yet optimized because the factors responsible for vascular growth had still to be identified.

Endothelial cells, similar to any other cell type, are grown in plastic dishes (Petri dishes) in a defined medium containing amino acids, glucose, vitamins, and animal or human serum. The acid or basic character measured by the pH must also be regulated and therefore bicarbonates, which are important for pH stabilization, are required. All this, of course, must be placed in a 37 °C incubator. The tissue extracts are then added to optimize the growth of endothelial cells, in particular brain extracts

Table 5.1 Markers of blood vascular and lymphatic endothelial cells

	Vascular endothelial cells	Lymphatic endothelial cells
CD31	++	+
VE-Cadherin	++	–
PAL-E	++	–
Weibel–Palade bodies	++	–
Willebrand factor	++	–
CD34	++	–
Lyve-1	–	++
Podoplanin	–	++
VEGFR2	++	+–
VEGFR3	+	++

presumed to be rich in growth factors. An extract frequently used and supplied by approved companies was the ECGS or "endothelial cell growth supplement."

Another aspect is the use of proteins called matrix proteins to facilitate adhesion, migration, and growth of endothelial cells. These proteins are also part of what the general public knows as "connective tissue." Collagen, for example, is part of this and is used precisely in its denatured form for the culture of endothelial cells in order to promote the maintenance of these cells in vitro. Endothelial cell culture is now much better defined and extracts such as Endothelial cell growth supplement (ECGS) have been replaced by vascular growth factors.

What about the culture of other vascular cells? There are not only endothelial cells in a vessel but also pericytes in the capillaries and smooth muscle cells in large vessels. These cells can also be isolated and cultured in vitro and various techniques have been developed to isolate both pericytes and smooth muscle cells [110–112].

Nowadays the situation has become much easier (but more expensive!) because many companies offer frozen vascular cells (Lonza, Promocell, ATCC), which can therefore be purchased. This avoids the step of isolation (and characterization), which is time consuming.

What are the morphological and molecular characteristics that allow the identification of vascular wall (Table 5.1). For endothelial cells, because they are in direct contact with blood, the surface must be non-thrombogenic, which means that blood coagulation is inhibited. This is caused by exposure to the surface of certain phospholipids that prevent activation. In addition, endothelial cells harbor special structures called Weibel–Palade bodies, named after their discoverers, Ewald R. Weibel and George Emil Palade in 1964 [113]. These bodies appear under the electron microscope as striated rods and contain an important molecule which intervenes in the interaction with blood platelets called von Willebrand factor. On the surface of endothelial cells, some characteristic markers are present, the best known being Cluster of differentation 31 (CD31), also called Platelet endothelial cell adhesion molecule (PECAM-1). This marker is used in general to identify endothelial cells under a microscope after immunolabeling. Other markers include

Pathologische Anatomie Leiden-Endothelium (PAL-E) and Cluster of differentiation 34 (CD34). The VEGFR2 receptor can also be used as a marker because it is present on the surface of endothelial cells.

For lymphatic endothelial cells, markers are different, the best known being the Lymphatic Vessel Endothelial Receptor 1 (Lyve-1) marker. Lyve-1 is the receptor of a molecule called hyaluronic acid. Hyaluronic acid is a sugar of a particular species called glycosaminoglycan. This sugar is present in the extracellular fluid, plasma, and lymphatic vessels. It is assumed that hyaluronic acid uses Lyve-1 to enter the lymphatic circulation. Another important lymphatic marker is podoplanin, also present in lymphatic vessels.

It should be noted that not all of these markers are absolutely specific. CD31, for example, is detected in vascular endothelial cells and in a fraction of lymph endothelial cells. In addition, monocytes/macrophages also express CD31. These markers are, however, very useful because, if combined with other markers, they are of great help for the identification of the endothelial cells.

Concerning markers of pericytes and smooth muscle cells, these are represented by desmin, actin α2 smooth muscle cells (α2SM actin) or neural/glial antigen-2 (NG2). These molecules can be easily detected using specific antibodies against these molecules.