

Angiogenesis in tissue engineering: from concept to the vascularization of scaffold construct

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Abstract. Angiogenesis, the formation of micro-vascular network from the pre-existing vascular vessels, has been studied in the connection to the normal developmental process as well as numerous diseases. In tissue engineering research, angiogenesis is also essential to promote micro-vascular network inside engineered tissue constructs, mimicking a functional blood vessel in vivo. Micro-vascular network can be used to maintain adequate tissue oxygenation, nutrient transfer and waste removal. One of the problems faced by angiogenesis researchers is to find suitable in vitro assays and methods for assessing the effect of regulators on angiogenesis and micro-vessel formation. The assay would be reliable and repeatable with easily quantifiable with physiologically relevant. This review aims to highlights recent advanced and future challenges in developing and using an in vitro angiogenesis assay for the application on biomedical and tissue engineering research.

1. Introduction

The word of angiogenesis comes from two Greek words, they are: “angêion” which means vessel and “genesis” means birth or emergence. Angiogenesis can also be defined as hemangiogenesis which means the formation of new blood vessels or lymphangiogenesis means the formation of new lymph vessels from the existing ones. In 1971, the “father of angiogenesis”, Judah Folkman hypothesized that angiogenesis was a factor enabling malignant tumour growth in cancer [1].

Recently, there have been growing interests in the development of biomaterial for biomedical, especially in the field of tissue engineering. Tissue engineering has developed as new, interdisciplinary [2] and multidisciplinary research field by which it involves the use of living cells and developing a biological substitute for implantation into the human body. Besides that, biomaterial science and tissue engineering gives a great demand for the replacement of damaged tissue in this growing population [3]. These medical concepts represent a new era in our efforts to treat problems associated with failing tissues and organs.



Further advancements in tissue engineering also have brought significant knowledge on the mechanisms and parameters related to vascularization and angiogenesis development [4-5]. Tissue engineering scientific society relies on the increasing knowledge of vasculo- and angio-genesis within the polymer scaffolds. This review aims to contribute on the current status and future challenges on development of an assay to study and follow-up capillary formation and micro-vessels network promotion. Tissue engineering societies seeks knowledge on angiogenesis assay for further application on the vascularisation of thick engineered scaffold construct.

2. Angiogenesis assay

Over the past decades, 2D system has been converted into 3D system in order to ensure a higher physiological relevance of experiments. However, the limitations of 2D system in cell culture become increasingly evident [6-7]. While 3D culture systems are widely used in tissue engineering and biomedical research, 2D culture system is still entrenched in the pharmaceutical industry. 2D models refer to those in which cells develop a tubular structure on the surface of substrate meanwhile, 3D models refer to the cell that invade the surrounding matrix that consists of biogel such as collagen, fibrin and matrigel in order to form a 3D structures [8-9]. 3D system is much better than 2D system to stimulate in vivo environment because 3D system able to describe more steps involved in the angiogenesis process [10-11]. Furthermore, 3D system can be used to study the effect of gradient diffusion of nutrient and oxygen, accumulation of waste and stimulating factors on the event that occur during angiogenesis. Although 2D system is less expensive compared to 3D system but still 2D system cannot provide a third dimension that will correctly mimic in vivo surrounding [12]. 2D system also fails to express cell to cell communication [12], tissue- specific architecture and their environment lack of mechanical and biochemical signal. Based on this reason, this is why result obtained from 2D system is not very reliable because they are unable to mimic in vivo environment which is very important to study the angiogenesis process.

Angiogenesis is the process of new blood vessel formation from the existing vasculature [7-8]. Angiogenesis play an important role in the physiological condition such as wound healing. However, one of the problems faced by many researchers is to find suitable assays in order to assess the effect of regulators of the angiogenic response. The selection of appropriate assay neither in vivo nor in vitro has become the most important technical challenge to the studies of angiogenesis. In vitro assays are very useful to study the various steps occurring in the angiogenesis such as cell proliferation, cell migration, and cell differentiation [13]. Usually cell will undergo proliferation within the process of angiogenesis. Cell proliferation assay are commonly used because this assays are easily to perform and highly reproducible [14].

Cell proliferation is defined as the number of cell divide. In this assays, increased in cell number is measured by using haemocytometer. Another in vitro assay is cell migration assay by which during angiogenesis, cells are stimulated to degrade the basement membrane and migrate toward the angiogenic factor. The most frequently used assay to assess the migration of cells is Boyden chamber. In this assays, cells are placed on top of the layer of filter and permit the cell to migrate towards the angiogenic factor that placed in the lower chamber [14]. Finally, cell differentiation is the last assays that will stimulate the formation of tubules. This assays involved plating cells onto the layer of gel matrix consists of collagen, fibrin or Matrigel where it will stimulate the proliferation, attachment, migration and differentiation of the cells into tubules so that it can mirror the in vivo environment

[13]. In other word, this assay can be used to study the ability of cells to form a three- dimensional structure. Besides that, the rate of cell differentiation depends on the type of matrix used [15].

Angiogenesis also have been studied in vivo which has been initially developed to become more reliable and easy method that can permit better quantification. In vivo assays include chick chorioallantoic membrane (CAM) assay, corneal angiogenesis assay and many more. CAM assays are the most popular that have been used for in vivo assay. This is because, CAM assays is relatively simple and inexpensive for a large- scale screening. However, the limitation of CAM assays is that CAM has a well developed network, thus making it hard to distinguish new capillaries from the existing ones. Another assay known as corneal angiogenesis assay whereby this assay is reliable and easy to be monitored but expensive as compared to CAM assay therefore it is impractical for large-scale screening. In this assay, corneal is known as avascular tissue so any newly vessel formed, it can be readily visible and can be quantified. [16].

3. Extra-cellular matrix (ECM) for angiogenesis study

Angiogenesis is a dynamic process during physiological condition especially wound healing where a new blood capillary is developed and remodelled again to replace the damaged vessel. During angiogenesis process where migration of cell and development of new capillaries occurred, they will not only depend on the cell and cytokines present but also the production and organization of extracellular matrix component. Formation of a new blood capillary will also depend on the extracellular matrix component because ECM will not only serve as structural support during the developing vasculature but also providing some information guidance for new capillaries [17]. Besides that, the interaction between extracellular matrix and cell are very crucial and further required for complete the vessel maturation. Besides gives support to the cell and tissues, ECM also support the adhesion of cell and transduces cell signal [18]. In addition, ECM is mainly composed of two classes of macromolecules which are glycosaminoglycans and fibrous protein such as collagen, elastin, fibronectin and laminin [12]. Collagen is one of the major structural proteins in the ECM where many researchers have used it as a potential scaffold in vascular tissue engineering. However, there are some limitations of using collagen where it does not possess high mechanical strength which may result in a weak construction for implantation [19].

Besides collagen, fibrin is also known to be one of the native ECM component that involved angiogenesis. This is because; fibrin shows excellent properties as the matrix where it has been widely used to investigate the angiogenic behavior of different types of endothelial cells in vitro with the presence of growth factor [20]. Fibrin is one of the natural proteins that shows to support the vascular growth meanwhile another synthetic materials are effectively inhibit angiogenesis unless they are impregnated with growth factor [21].

4. Angiogenesis assessment and imaging

Angiogenesis is known as a basic process that involved in a variety of physiological as well as pathological condition. Physiologically, angiogenesis is essential for the wound healing, ischemia, and during development of embryo. Pathologically, it is associated with diseases such as cancer, blinding ocular disorders, rheumatoid arthritis and psoriasis. Besides that, the realization of angiogenesis as one of the essential process that potentially induce the growth of tumor, has led to enormous interest in the

development of drugs that can target these molecules as well as discovery of angiogenesis stimulus. However, the difficulties in designing clinical trials for anti-angiogenic drugs causes some of the potential drugs can't live up to the expectation of preclinical trials [25]. Recently, imaging modalities may be a better alternative to assess and detect angiogenesis due to the fact that it is non-invasive and can be assess in a larger volume. Examples of modalities used are X-ray computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET) and ultrasound.

MRI is one of the practical modality that has been used widely in assessing angiogenesis. MRI is a method used to estimate angiogenic blood flow and volume. Besides MRI, CT imaging is another modality that has been used to assess angiogenesis and shows more quantitative data than data obtained by MRI. Other than CT scan and MRI, ultrasound imaging also can be used to assess angiogenesis. Although ultrasound imaging is portable, widely available and inexpensive but it produce images that low resolution compared with those produces by CT imaging ad MRI. Another approach for the imaging of angiogenesis vasculature is the use of molecular markers. The example of molecular markers that have been identified including vascular endothelial growth factor receptor (VEGF), $\alpha_v\beta_3$ integrins, endoglin and endosialin [26]. These molecular markers will bind to the molecular agents to assess high specificity of angiogenesis epitope.

Currently, there are several experimental methods available to study on the quantification of angiogenesis activity instead of angiogenesis imaging. Method available include capillary density estimation, enzyme linked immunosorbent assay (ELISA), hemoglobin determination in matrigel plugs and semi-automated vessel quantification. Capillary density estimation is one of the methods that have been mostly used in the quantification of angiogenesis activity because this method is easy to perform in laboratory and less cost [27]. Besides that, enzyme linked immunosorbent assay (ELISA) is known as a test that used antibodies and color change to identify a substance. However, this method also can be used to estimate angiogenic growth factors [28]. Another method known as matrigel vessel quantification in which this method is known as the simplest method to determine the amount of micro-vessels within the matrigel [29-30].

5. Concluding Remarks

Current directions in angiogenesis research have become numerous and exciting around the world. This is approximately because of the role of angiogenesis process where it is very fundamental to many physiological conditions. This event plays an important function in wound healing, tissue repair pregnancy as well as exercising. Yet, the abuse of angiogenesis process could lead to pathological condition for example tumor growth. However, angiogenesis is one of the activities that are central to the development and also tissue maintenance. This is why; researchers need to increase their understanding on angiogenesis although this process is still in its early stages. A better understanding on angiogenesis assays could lead to a profound therapeutic outcome of artificial organs development.

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