**Assessment of your knowledge**

(a) Answer the following questions to assess your command of terminology, facts, concepts, and

theories learned in this chapter:

1. External inosculation is a process where:

a. an autonomous vascular network develops within the implanted construct

b. a preexisting vascular network develops interconnections with the host microvasculature outside the space of the implanted construct

c. a vascular network fully surrounds the space of the implanted construct

d. vascular cells develop vessel structures out of their physiological environment

2. The detection of ALU sequences is used to discriminate cells

a. of endothelial origin

b. of ectodermal origin

c. of human origin

d. in quiescent state

3. Platelet-rich plasma is

a. isolated from blood samples

b. an expensive biomaterial for growth factors provision

c. a cost-effective biomaterial with a well-standardized composition

d. shows a reduced donor-to-donor variability

4. Hypoxic preconditioning of cells can be used to:

a. decrease the metabolism of cells before implantation

b. potentiate the angiogenic nature of cocultures and increment the secretion of proangiogenic factors

c. promote the occurrence of necrosis at the scaffold borders

d. downregulate the expression of the transcription factor hypoxia-inducible factor 1, alpha (Hif1α)

5. The CAM:

a. is technically simple but expensive

b. can only be used for studying nonmammalian xenografts because of its nonspecific inflammatory reactions

c. understates limited ethical regulation as compared to other animal models

d. does not allow for large screenings

6. Intussusceptive angiogenesis occurs

a. when new vessels arise from previous vascular loop-shape networks

b. only if vascular cells are biochemically preconditioned in vitro

c. in early stages of the neo-vascularization process

d. when a new blood vessel is created by splitting of an existing blood vessel in two.

7. Endothelial progenitor cells have been explored as angiogenic cell source. Which statements are correct?

a. Endothelial progenitor cells show greater proliferation capacity compared to mature endothelial cells.

b. Endothelial progenitor cells show lower proliferation capacity compared to mature endothelial cells.

c. Endothelial progenitor cells can be isolated from peripheral blood.

d. The prime function of endothelial progenitor cells is to support sprouting angiogenesis.

8. Mural cells are often applied in combination with an endothelial cell source. What is the rationale behind this?

a. Mural cells prevent a too fast formation of vascular sprouts.

b. Mural cells support the sprouting process, stabilize newly formed vessels, and guide their maturation.

c. Mural cells secrete proangiogenic factors and guide new vessel formation by building

gradients of these factors.

9. Photoacoustic imaging is:

a. An optical technique for angiogenesis visualization

b. A technique purely applied to excised tissues

c. A purely optical technique for visualization of blood flow

d. A hybrid technique revealing the photo-absorption of hemoglobin

10. The orthotopic model:

a. Allows assessing graft functionality independently of the influence of tissue-specific biochemical factors.

b. Allows assessing graft functionality within the patho-physiological context of its target implantation site.

c. Is exclusively generated into small animals.

d. Is obtained by creating subcutaneous pockets into immune-deficient animals.

11. Microvascular fragments (MVFs) are suggested as vascularization units for tissue engineering. Why are they superior when compared to single cells?

a. MVFs already represent biologically intact vessel segments with a physiological tubelike structure.

b. After their seeding onto biomaterials/scaffolds, MVF can bridge relatively wide distances due to their lengths, leading to rapid creation of a mature microvasculature.

c. After their isolation, MVFs contain stem cells within their physiological niches.

12. The dorsal skinfold chamber model is frequently used for assessing the vascularization of scaffolds implanted in vivo. What makes this model special?

a. It allows continuous access to the implant through the observation window.

b. It allows studying the individual cell types by means of specific immuno-staining approaches.

c. The size of the skinfold chamber can easily be adapted and used for all sizes of rodents.

d. It can be used to even analyze large scaffolds up to multiple centimeters.

13. In vitro prevascularization generally involves the combination of:

a. Growth factors and endothelial cells

b. Tissue-specific cells and growth factors

c. A vascular tree and proangiogenic factors

d. Endothelial cells and tissue-specific cells

14. Cell survival inside an engineered tissue upon in vivo implantation can also be improved by:

a. A preconditioning of the tissue with gamma irradiation

b. The use of a material inducing inflammation of the host tissue

c. Incorporation of glucose inside the construct

d. The use of immortalized cells

15. Endochondral ossification occurs through:

a. A direct differentiation of mesenchymal cells in bone-forming cells

b. The generation of a cartilage template that gets remodeled to bone upon vascularization

c. Endothelial cell differentiation into bone-forming cells

d. Differentiation of osteoclasts into bone-forming cells

16. Decellularized matrices

a. Are made of synthetic polymers

b. Are the result of chemical and enzymatic treatments of natural tissues

c. Can be produced using 3D printing technology.

d. Can only be produced from bone and cartilage.

17. What is a typical method to assess the angiogenic potential of cells?

a. An MTT assay.

b. The assessment of network formation on Matrigel.

c. Measurement of collagen deposition.

18. What might be the challenges for the clinical application of angiogenic cells?

a. The heterogeneity of EPC populations.

b. EPC cannot be obtained in an autologous setting.

c. The lack of established characterization methods.

19. What are the important characteristics of a scaffold for a successful neo-vascularization:

a. The presence of interconnected pores

b. Its price and available size

c. Its tubular shape

d. Its micro/macro topography

20. Is it possible to develop a “universal” proangiogenic scaffold for all tissues?

a. Yes, as soon as it promotes vascularization.

b. Yes, vessels are the same everywhere.

c. No, microvessels from different tissues have different structures/components.

d. No, the scaffolds also need to fulfill the specific requirements of the target tissue.

(b) Answer the following questions to assess your ability to apply the concepts and theories learned in this chapter in real-life, clinical, and scientific situations.

1. Describe the advantages and disadvantages in using ectopic and orthotopic animal models to study vascularization of tissue constructs.

2. Describe the main types of imaging used for the assessment and monitoring of the implant vascularization in vivo.

3. Describe at least three methods to promote tissue-engineered construct vascularization, including advantages and disadvantages.

4. What are key factors to promote the neo-vascularization of constructs and why?

5. Describe the vascular tree.

6. Describe the main strategies to modify the physico-chemical properties of scaffolds to enhance their vascularization.

7. Describe the functional characteristics of endothelial cells.

8. Can different vascularization strategies (prevascularization, proangiogenic materials, in situ vascularization) be combined? Imagine and describe a possible tissue product based on a such strategy.

9. What are the advantages of decellularized tissues? Please describe a concrete example.

10. Discuss why the lack of vascularization is a problem and give two possible strategies to solve it.