ANIMAL BIOTECHNOLOGY - SBM 5108

ANSWERS SHEET

1. **The microbiology approach to the problem of culture heterogeneity is to isolate pure cell strains by cell cloning which is a fairly easy approach. State and explain five conditions that improve clonal growth in animal bio-technology. (10 marks)**
   - The medium – select a rich medium that has been optimized for the cell type in use.
   - The serum – Select a batch for cloning experiments that gives a high plating efficiency during tests. When serum is required, fetal bovine is generally better than calf or horse.
   - Hormones – some hormones such as insulin have been found to increase the plating efficiency of several cell types.
   - Carbon dioxide – C02 is necessary for obtaining maximum cloning efficiency for most cells as it protects the cells from PH fluctuations during feeding and in the event of failure of the CO2 supply
   - Treatment of substrate – it has been found some compounds such as polylysine improve the plating efficiency of human fibroblasts in low serum concentrations
   - Conditioned medium such as trypsin – medium that has been used for the growth of other cells acquires metabolites, growth factors and matrix products from these cells. This conditioned medium can improve the plating efficiency of some cells if it is diluted into the regular growth medium.

2. **In animal somatic cell fusion of two different cells, the production of a hybrid cell has been successfully achieved. State five significant areas in biotechnology where this can be applied. (10 marks)**
   i. Study of control of gene expression and differentiation
   ii. Gene mapping
   iii. Malignancy
   iv. Viral replication

v. Anti body production through hybridoma technology

3. **Define the following terms as they have been used in animal bio-technology. (10 marks)**
   a) **Cell culture –** this is the process by which prokaryotic, eukaryotic or plant cells are grown under controlled conditions. In practice, it refers to the culturing of cells derived from animal cells.
   b) **Primary culture –** this refers to the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate (reach confluence). At this stage, the cells have to be passed to a new stage (subculture) by transferring them to a new vessel with fresh growth medium to provide more room for the continued growth.
   c) **Cell line –** After the first sub-culture, the primary culture becomes known as a cell line or sub-clone. Cell lines derived from primary cultures have limited life spans.
   d) **Cell strain –** if a subpopulation of a cell line is positively selected from the culture by cloning or some other method, this cell line becomes a cell strain. A cell strain often acquires additional genetic changes subsequent to the initiation of a parent line.
   e) **Stem cells –** These are undifferentiated biological cells that can differentiate into specialized cells and can divide, through mitosis, to produce more stem cells. They are found in multi-cellular organisms.

4. **State and briefly explain Five physiochemical properties necessary for Cell Culturing. (10 marks)**
   i. pH – Most cell lines grow at pH 7.4. The optimum pH for cell growth varies relatively little among different cell strains.
   ii. CO2 and Bicarbonate – Carbon dioxide in the gas phase dissolves in the medium, establishing $HC03^-$ ions and this lowers the pH
   iii. Buffering – culture media must buffer under two sets of conditions:
      - Open dishes, wherein the evolution of CO2 causes the pH to rise and;

- Overproduction of CO2 and lactic acid in transformed cell lines at high cell concentrations when the pH will fall. A buffer may be incorporated into the medium to stabilize the pH

iv. Oxygen – the other major significant constituent of the gas phase is oxygen. Whereas most cells require oxygen for respiration in vivo, cultured cells often rely mainly on glycolysis, a high proportion of which, as in transformed cells, may be anaerobic.

v. Osmolality – most cultured cells have a fairly wide tolerance for osmotic pressure. As the osmolality of the human plasma is about 290 mosmol/kg, it is reasonable to assume that this level is the optimum for human cells in vitro, although it may be different for other species.

vi. Temperature – The optimum temperature for cell culture is dependent on;
   a. The body temperature for the animal from which the cells were obtained
   b. Any anatomic variation in temperature
   c. The incorporation of a safety factor to allow for minor errors in regulating the incubator. Thus, the temperature recommended for most human and warm-blooded animal cell lines is 37^0C.

5. State <u>five</u> applications of cell culture. (10 marks)

i. The mitotic process and its modification by stimulus or suppressors have been studied in many cell types.

ii. Visible light has some inhibitory effects upon living cells. The lethal effects of X-rays can be quantified on mouse cells and the effects of radiation upon cell constituents and upon DNA and RNA synthesis can be studied.

iii. Differentiation at the cellular level has mostly been studied in organ rather than cell cultures

iv. The uses of tissue culture in the study of cancer can be studied

v. Comparison of enzyme activities in cells in culture with those from the mouse have been made

6. **State and explain <u>three</u> ways in which stem cells are different from other cells. (10 marks)**
    i. They cannot continue to divide for long periods of time: Most cells such as the skin cells cannot replicate themselves after a certain period of time. Stem cells are self-sustaining by replicating themselves for a much longer period of time.
    ii. They are unspecialized: Stem cells have unspecialized capabilities and do not have tissue-specific structures to perform specialized functions. Specialized cells have specific capabilities that allow them to perform certain tasks for example a red blood cell contains hemoglobin that allows it to carry oxygen.
    iii. They can give rise to specialized cells: stem cells go through a process called differentiation and create special types of cells for instance, muscle, nerve, skin etc.

7. **Discuss briefly the difference between embryonic stem cells and adult stem cells. (10 marks)**

    Embryonic stem cells are the cells within the prospective layer of the blastocyst. They are pluripotent. This means they can develop into any of the cells of the adult body. Because they are pluripotent and easy to grow, they have the best potential for replacing damaged or lost tissue or body parts. **Adult stem cells,** also known as progenitor cells or somatic stem cells, are located in small quantities throughout the body and generate specialized cells for the area they are located. These cells do not renew themselves as well as embryonic stem cells. Still, if these cells are put in a different environment, they may produce a different type of cells from the originating cell.

8. **What is Transgenesis and what is a transgenic animal? (5 Marks)**

Transgenesis is the process of introducing an exogenous gene called a transgene into a living organism so that the organism will exhibit a new property and transmit that property to its offspring. Transgenesis can be facilitated by liposomes, plasmid vectors, viral vectors, pronuclear injection, protoplast fusion and ballistic DNA injection.
A transgenic animal is an animal that carries a foreign gene that has been deliberately inserted into its genome. The foreign gene is constructed using recombinant DNA methodology. Mostly, transgenic mice are used for research and testing.

9. **What is artificial insemination? (5 Marks)**

   This is the deliberate introduction of sperm into a female's uterus for the purpose of achieving a pregnancy through in vivo fertilization. It is a fertility treatment for humans as well as in animal breeding like dairy cattle etc.

10. **Viral vectors are tools commonly used by molecular biologists to deliver genetic materials into the cells. This process can be performed inside a living organism (in vivo) or in a cell culture (in vitro). State and explain FIVE key properties of a viral vector. (20 Marks)**

    i. Safety: Although viral vectors are occasionally created from pathogenic viruses, they are modified in such a way as to minimize the risk of handling them. This usually involves the deletion of a part of the viral genome critical for viral replication. Such a virus can efficiently infect cells but once the infection has taken place, they require a helper virus to provide the missing proteins for the production of new virions.

    ii. Low toxicity: the viral vector should have a minimal effect on the physiology of the cell it infects.

    iii. Stability: some viruses are genetically unstable and can rapidly rearrange their genomes. This is detrimental to predictability and reproducibility of the work conducted using a viral vector and is avoided in their design.

    iv. Cell type specificity: Most viral vectors are engineered to infect as wide a range of cell types as possible. However, sometimes, the opposite is preferred. The viral

receptor can be modified to target the virus to a specific kind of cell. Viruses modified in this manner are said to be pseudo-typed.

v. Identification: Viral vectors are often given certain genes that can help identify which cells took up the viral genes. These genes are called markers. A common marker is antibiotic resistance to a certain antibiotic. The cells can then be isolated easily as those that have not taken up the viral vector genes and do not have the antibiotic resistance and so cannot grow in a culture with antibiotics present.