Integrated UG/PG Biotechnology (Vth Semester)
End Semester Examination, 2013
LBTC 503: Basic Animal Biotechnology

Model Answer (AS-2907)

Section A

Answer 1:

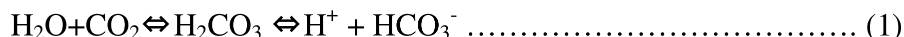
I. (a) Harrison and Carrel
II. (d) Need necessary expertise
III. (b) Bicarbonate
IV. (c) 56^0C
V. (a) Hypoxanthine, Aminopterin and Thymidine
VI. (d) Bovine serum albumin
VII. (a) Gonadotrophic hormone
VIII. (c) Development of transgenic animals
IX. (d) All of the above
X. (b) Casein kinase

Section B

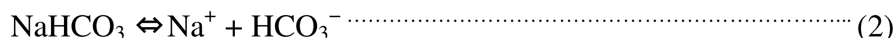
Answer 2:

The pH of culture media must be optimized to the level of physiological pH of cells from where they are derived. Most cell lines grow well at pH 7.4. Although the optimum pH for cell growth varies relatively little among different cell strains, some normal fibroblast lines perform best at pH 7.4-7.7, and transformed cells may do better at pH 7.0-7.4. It is well established that epidermal cells could be maintained at pH 5.5, but this level has not been universally adopted. In special cases it may prove advantageous to do a brief growth experiment, plating efficiency assay, or special function analysis to determine the optimum pH. Phenol red is commonly used as an indicator. It is red at pH 7.4 and becomes orange at pH 7.0, yellow at pH 6.5, lemon yellow below pH 6.5, more pink at pH 7.6, and purple at pH 7.8.

Carbon dioxide in the gas phase dissolves in the medium, establishes equilibrium with $HCO3^-$ ions, and lowers the pH. The atmospheric CO_2 tension will regulate the concentration of dissolved CO_2 directly, as a function of temperature. This regulation in turn produces H_2CO_3, which dissociates according to the reaction


$H_2O+CO_2 \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$ ………………………………. (1)

HCO_3^- has a fairly low dissociation constant with most of the available cations so it tends to reassociate, leaving the medium acid. The net result of increasing atmospheric CO_2 is to depress the pH, so the effect of elevated CO_2 tension is neutralized by increasing the bicarbonate concentration. A buffer may be incorporated into the medium to stabilize the pH. Despite its poor buffering capacity at physiological pH bicarbonate buffer is still used more frequently than any other buffer, because of its low toxicity, low cost, and nutritional benefit to the culture.


$NaHCO_3 \Leftrightarrow Na^+ + HCO_3^-$ ……………………………………………… (2)

The increased HCO_3^- concentration pushes equation (1) to the left until equilibrium is reached at pH 7.4.

Answer 3:

<u>**Ingredients:**</u> There are various ingredients of culture medium for the optimum survival and growth of cells. These ingredients are as follows-

Amino Acids: The essential amino acids are required by cultured cells, plus cystine, cysteine, arginine, glutamine, and tyrosine, although individual requirements for amino acids will vary from one cell type to another. Other nonessential amino acids are often added as well, to compensate either for a particular cell type's incapacity to make them or because they are made, but lost by leakage into the medium. The concentration of amino acids usually limits the maximum cell concentration attainable, and the balance may influence cell survival and growth rate.

Vitamins: Media contain only the water-soluble vitamins (the B group, plus choline, folic acid, inositol, and nicotinamide, but excluding biotin). Biotin is present in most of the more complex media, including the serum free recipes. Fat-soluble vitamins (A, D, E, and K) may also be present specially required media.

Salts: The salts are chiefly those of Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, $SO4^{2-}$, $PO4^{3-}$, and $HCO3^-$ and are the major components contributing to the osmolality as well as buffering of the medium. Divalent cations, particularly Ca^{2+} are required by some cell adhesion molecules, such as the cadherins. Ca^{2+} also acts as an intermediary in signal transduction, and the concentration of Ca^{2+} in the medium can influence whether cells will proliferate or differentiate. Na^+, K^+, and Cl^- regulate membrane potential, whereas $SO4^{2-}$, $PO4^{3-}$, and $HCO3^-$ have roles as anions required by the matrix and nutritional precursors for macromolecules, as well as regulators of intracellular charge.

Glucose: Glucose is included in most media as a source of energy. It is metabolized principally by glycolysis to form pyruvate, which may be converted to lactate or acetoacetate and may enter the citric acid cycle and is oxidized to form CO2 and water. Glutamine may also serve as energy source for some cell lines

Organic Supplements: A variety of other compounds, including proteins, peptides, nucleosides, citric acid cycle intermediates, pyruvate, and lipids, appear in complex media.

Hormones and Growth Factors: Hormones and growth factors like hydrocortisone and dexamethasone, PDGF, EGF, SCF, FGF, IGF-I & II etc. are added in most regular media, although they are frequently added to serum-free media.

Antibiotics: Antibiotics were originally introduced into culture media to

reduce the frequency of contamination. However, the use of laminar-flow hoods, coupled with strict aseptic technique, makes antibiotics unnecessary. Indeed, antibiotics have a number of significant disadvantages and thus may be avoided.

Serum: Serum contains various known and unknown growth factors, which promote cell proliferation, and adhesion factors and antitrypsin activity, which promote cell attachment. Serum is also a source of minerals, lipids, and hormones, many of which may be bound to protein. The sera used most in tissue culture are bovine calf, fetal bovine, adult horse, and human serum. Calf (CS) and fetal bovine (FBS) serum are the most widely used.

Protein: Proteins in culture media is required to act as carriers for minerals, fatty acids, and hormones. Those proteins for which requirements have been found are albumin, which may be

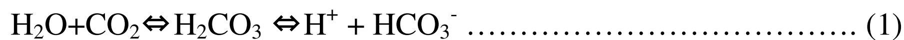
important as a carrier of lipids, minerals, and globulins; fibronectin which promotes cell attachment. Fetuin in fetal serum enhances cell attachment and transferring binds iron, making it less toxic and bioavailable.

Lipids: Linoleic acid, oleic acid, ethanolamine, and phosphoethanolamine are required in small amounts which usually bound to proteins such as albumin.

Physico-chemical properties: Animal tissue culture must be carried out in physiologically controlled environment. The following physiochemical properties of animal tissue culture media must be considered.

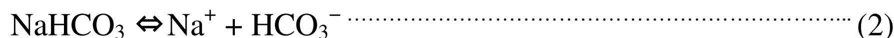
pH:

Most cell lines grow well at pH 7.4. Although the optimum pH for cell growth varies relatively little among different cell strains, some normal fibroblast lines perform best at pH 7.4–7.7, and transformed cells may do better at pH 7.0–7.4. Phenol red is commonly used as an indicator. It is red at pH 7.4 and becomes orange at pH 7.0, yellow at pH 6.5, lemon yellow below pH 6.5, more pink at pH 7.6, and purple at pH 7.8. The atmospheric CO_2 tension will regulate the concentration of dissolved CO_2 directly, as a function of temperature. This regulation in turn produces H_2CO_3, which dissociates according to the reaction



$H_2O + CO_2 \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$ ………………………………. (1)

HCO_3^- has a fairly low dissociation constant with most of the available cations so it tends to reassociate, leaving the medium acid. The net result of increasing atmospheric CO_2 is to depress the pH, so the effect of elevated CO_2 tension is neutralized by increasing the bicarbonate concentration:



$NaHCO_3 \Leftrightarrow Na^+ + HCO_3^-$ ……………………………………………………… (2)

The increased HCO_3^- concentration pushes equation (1) to the left until equilibrium is reached at pH 7.4.

Buffering:

A buffer may be incorporated into the medium to stabilize the pH. Despite its poor buffering capacity at physiological pH bicarbonate buffer is still used more frequently than any other buffer, because of its low toxicity, low cost, and nutritional benefit to the culture. HEPES is a much stronger buffer in the pH 7.2–7.6 range and is used at 10–20 mM.

Oxygen:

The other major significant constituent of the gas phase is oxygen. Cultured cells often rely mainly on dissolved oxygen in media. Providing the correct O_2 tension is, therefore, always a compromise between fulfilling the respiratory requirement and avoiding toxicity. It has been observed that the requirement of selenium in medium is related to oxygen toxicity, as selenium is a cofactor in glutathione synthesis. Because the depth of the culture medium can influence the rate of oxygen diffusion to the cells, it is advisable to keep the depth of the medium within the range 2–5 mm (0.2–0.5 mL/cm^2*)* in static culture.

Osmolality:

Most cultured cells have a fairly wide tolerance for osmotic. As the osmolality of 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 (e.g., around 310 mosmol/kg for mice. In practice, osmolalities between 260 mosmol/kg and 320 mosmol/kg are quite acceptable for most cells.

Temperature:

The optimal temperature for cell culture is dependent on (1) the body temperature of the animal from which the cells were obtained, and (2) any anatomic variation in temperature. Thus, the temperature recommended for most human and warm-blooded animal cell lines is 37◦C, close to body heat.

Viscosity:

The viscosity of a culture medium is influenced mainly by the serum content and in most cases will have little effect on cell growth. Any cell damage that occurs under these conditions may be reduced by increasing the viscosity of the medium with carboxymethylcellulose (CMC) or polyvinylpyrrolidone (PVP).

Surface Tension and Foaming:

The effects of foaming have not been clearly defined, but the rate of protein denaturation may increase, as may the risk of contamination if the foam reaches the neck of the culture vessel. Foaming will also limit gaseous diffusion if a film from a foam or spillage gets into the capillary space between the cap and the bottle, or between the lid and the base of a Petri dish.

Answer 4:

Animal cell lines have been widely utilized as host for therapeutic protein production like factor

VIII, erythropoietin, β-interferon and tissue plasminogen activator etc. for clinical application

because of their capacity for proper protein folding, assembly and post-translational modification. Thus, quality and efficacy of protein can be superior when expressed in mammalian source. Many of them used for large production of therapeutic proteins undergo apoptic death due to deprivation of nutrients like amino acids, glucose, serum, oxygen etc, in the bioreactor environment. These are required to be monitored carefully to reduce to avoid unintentional transmission of viral contaminants that could infect and to increase yield. For the production of therapeutic proteins, following steps are involved-

1. Identification of gene of interest (GOI) encoding therapeutic protein by direct screening of genome through hybridization or by isolating mRNA and converting into cDNA by reverse transcriptase enzyme.
2. Insertion of GOI in suitable cloning vector along with selectable marker and transformation into suitable host to get sufficient copy of GOI.
3. Insertion of GOI in suitable expression vector along with promoter and other sequences necessary for expression of GOI like selectable markers like hgprt and dhfr etc.
4. Gene constructs transfer in chosen mammalian cell line with desired modifications like codon optimization and addition of sequences of homologous recombination for proper and stable integration in genome and optimum expression.
5. Culture of transfected cells and gene expression will start at this step.

6. Host cell engineering and media optimization.
7. Downstream processing for the purification of therapeutic protein by precipitation, electrophoresis and chromatography methods etc.
8. Lyophilization and preservation of therapeutic proteins

Various therapeutic proteins and their application:

Tissue plasminogen activator- Clot dissolution to prevent coronary artery blockage
Insulin- Diabetes mellitus
Erythropoietin- Anemia
Factor VIII- Hemophilia
Human growth factor- Treating hypopituitary dwarfism in children

Hepatitis-B vaccine- Hepatitis B

Alpha interferon- Leukemia, hepatitis-B

Beta interferon- Sclerosis Chinese Hamster
Gamma interferon- Chronic granulomatus disease
Streptokinase- Acute myocardial infarction

Answer 5:

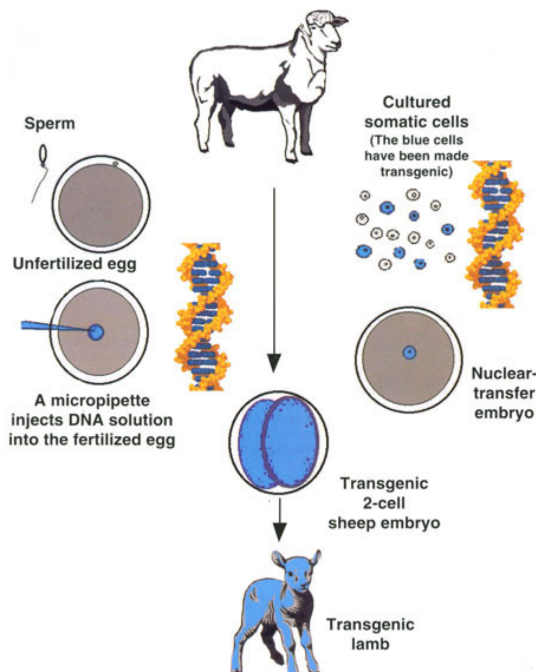
Following points should be discussed.

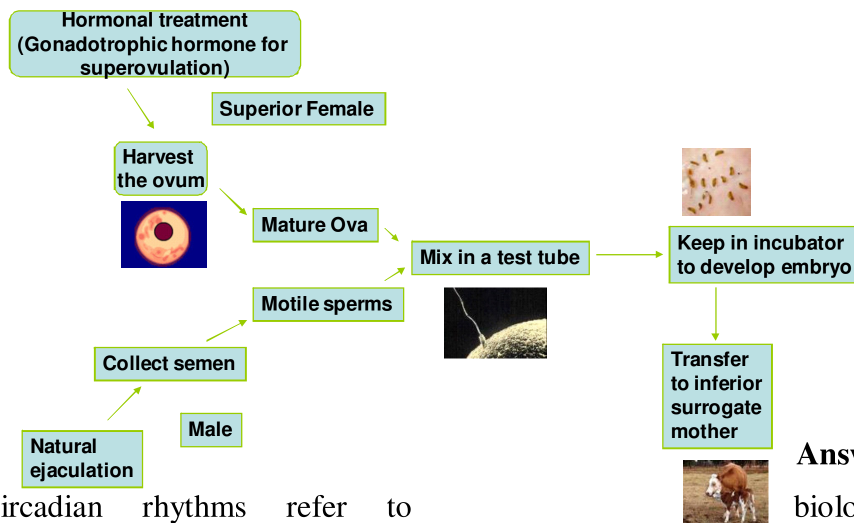
The in vitro fertilization and embryo transfer involve following steps-

1. Collection of semen and washing of semen to isolate sperm
2. Induction of superovulation in superior female by gonadotropin hormone
3. In vitro cultivation of oocytes for maturation
4. In vitro fertilization of the mature oocyte and sperm
5. Development of embryos from fused zygote
6. Transfer of embryo at balstocyst stage in the uterus of inferior surrogate mother
7. Confirmation of pregnancy

Application of embryo transfer:

1. Embryo can be genetically manipulated
2. Genetic improvement
3. Planned mating
4. Genetic testing for Mendelian recessive traits
5. Disease control
6. Salvage of reproductive function





Answer 6:

Circadian rhythms refer to biological variations or rhythms with a cycle of approximately 24 hours. Circadian rhythms are self-sustaining (i.e., free running), meaning that they will persist when the organism is placed in an environment devoid of time cues, such as constant light or constant darkness. The persistence of rhythms in the absence of a dark-light cycle or other exogenous time signal (i.e., a Zeitgeber) clearly seems to indicate the existence of some kind of internal timekeeping mechanism, or biological clock. These cycles persist with a period of close to, but not exactly, 24 hours. If the rhythms were exogenously driven, they should persist with a period of exactly 24 hours. Circadian rhythm has the ability to be synchronized, or entrained, by external time cues, such as the light-dark cycle. If a shift in external cues occurs (e.g., following travel across time zones), the rhythms will be aligned to the new cues. This alignment is called entrainment.

There are various types of biological rhythms which are classified as below (Every types/ class should be described in details) -

1. Physical classification:
2. Functional classification:
3. Mathematical classification
4. Descriptive classification
5. Evolutive classification
6. Duration classification
7. Physiological classification
8. Biological classification
9. Resistance classification
10. Ontogenetic classification
11. Structural classification
12. Consistency classification
13. Constitutive classification
14. Hierarchical classification

Answer 7:

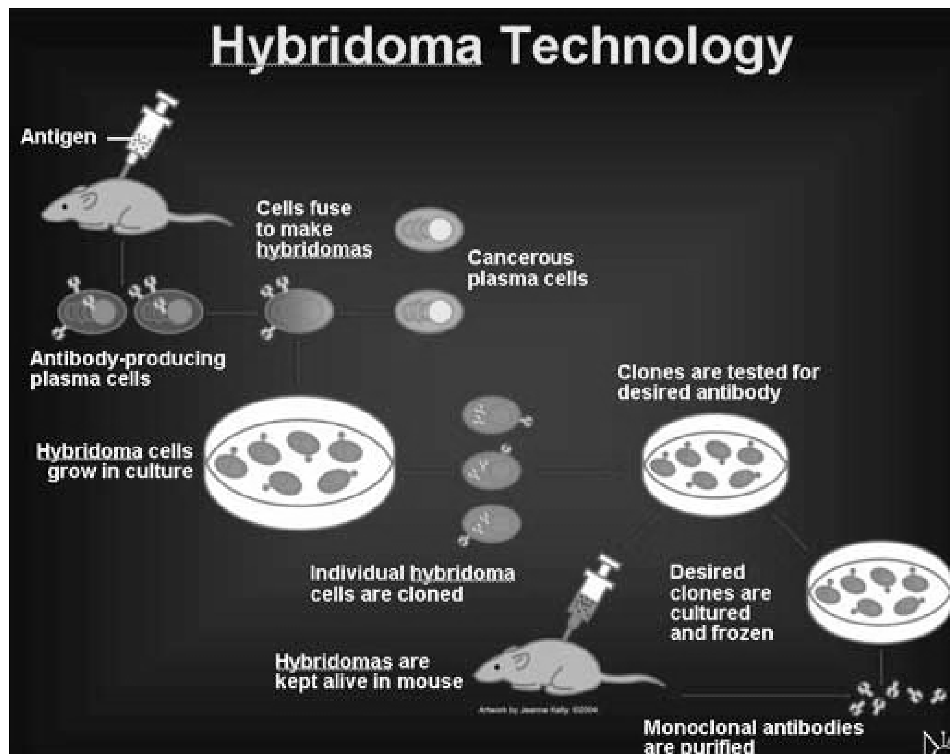
Hybridomas are cells that have been engineered to produce a desired monoclonal antibody in large amounts. Monoclonal antibodies can be produced in specialized cells through a technique

now popularly known as hybridoma technology. Hybridoma technology was discovered in 1975 by two scientists, Georges Kohler and Cesar Milstein who was awarded the 1984 Noble prize for physiology and medicine.

Methodology to produce hybridoma cells and monoclonal antibody:

Explain the following steps-

- Immunization of rat or mice with specific antigen along with adjuvant
- Isolation of B-lymphocytes from spleen of immunized mice or rat
- Selection and culture of cancerous cells i.e. myeloma cells
- Fusion of antibody producing B-lymphocytes and myeloma cells by Polyethylene glycol or Sendai virus
- Selection of hybrid cells by HAT medium
- Screening of hybridoma cells for antibody production by antigen-antibody reactions
- Culture of hybridoma cells in vitro or in vivo in hybrid rat or mice peritoneal cavity
- Extraction of culture medium or ascite fluid of rat/mice for antibody purification
- Downstream processing for purification of monoclonal antibody using precipitation, filtration, ultrafiltration, chromatography techniques



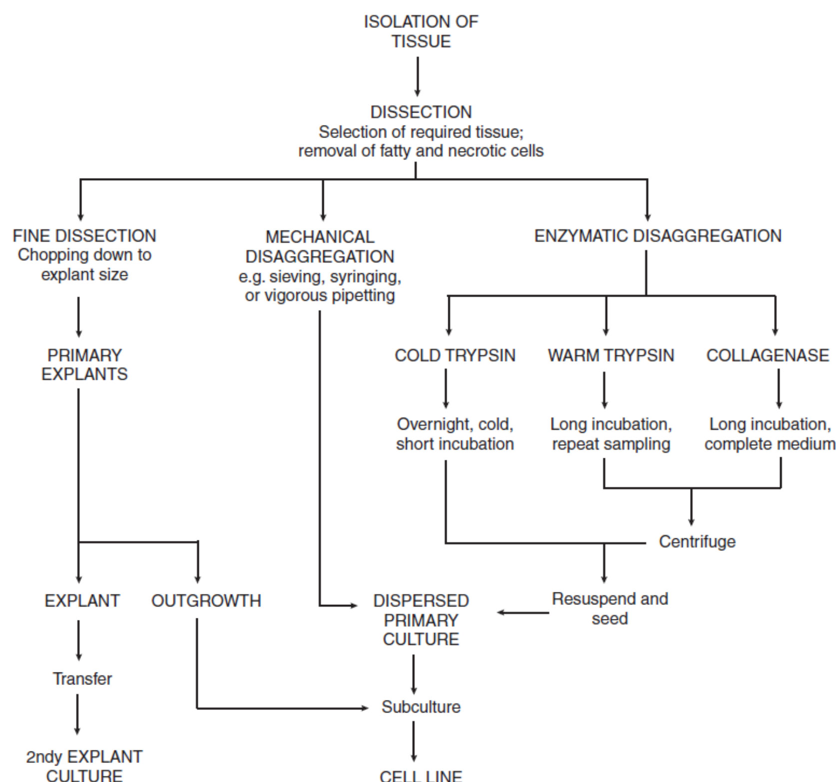
Applications (Explain followings): Monoclonal antibodies or specific antibodies, arc now an essential tool of biomedical research and are of great commercial and medical value. For instance, ABO blood groups could be earlier identified with the help of human sera carrying antibodies of known specificity. These human sera have been replaced by monoclonal antibodies produced by hybridomas, for the identification of ABO blood groups. Thus the diagnostic and screening value of the monoclonal antibodies through serological tests has been demonstrated. Besides the use of monoclonal in identification of blood groups, following three uses for monoclonal are described:

- **Diagnosis (including ELISA test and western blotting for detection of viruses and imaging and confirmation of pregnancy etc.)**
- **Humanized and chimeric antibody production**
- **Immunopurification**
- **<u>Therapy:</u>**

  Non Hodgkin's lymphoma- RituxanR
  Breast cancer- herceptineR
  Inflammatory disease- InfliximabR
  Allergic asthma- VomalizumabR
  In type -1 diabetes- muromunabR
  Clumping of platelet- AbiciximabR
  Hybridoma secreting MAbs against 17-hydroxyprogesterone (17OHP)

Answer 8:

(a)



(b)

Transgenic animal technology is one of the fastest growing biotechnology areas. It is used to integrate exogenous genes into the animal genome by genetic engineering technology so that these genes can be expressed and inherited by offspring. The transgenic efficiency and precise control of gene expression are the key limiting factors in the production of transgenic animals. For the development of transgenic animals, stable gene integration in the genome is very crucial step. For stable gene integration, various methods of gene transfer are available, each of which has its own advantages and disadvantages.
In animal cells, gene/DNA can be transferred by various methods-

(I) Physical methods

1. Microinjection 2. Biolistics/ Gene gun 3. Electroporation

(II) Chemical methods

1. Gene transfer by calcium phosphate method
2. Transfer of DNA by use of polyethene glycol
3. Use of DEAE-Dextran for gene transfer
4. Liposome mediated gene transfer

(III) Viral methods

1. Retrovirus
2. Adenovirus
3. Adeno-associated virus
4. Bacculovirus
5. Herpes virus
6. Simian Virus 40