CELL CULTURE TECHNOLOGY

SQG 3242

ASEPTIC TECHNIQUE AND LAB DESIGN

DR. SITI PAULIENA MOHD BOHARI

What are we trying to do?

- Maintain isolated cells or pieces of tissue in a **sterile controlled environment** in order to study its functions
  - Separate from other tissues/organs
  - Response to specific stimuli
  - Addition or removal of specific molecules/ genes
- Why?

Aseptic Technique

- For best results in tissue culture, we want to work to keep microbial (bacteria, yeast and molds) contamination to a minimum. To do this, there are certain things you must be aware of and guidelines to follow.
- Work in a culture hood set-aside for tissue culture purposes. Most have filtered air that blows across the surface to keep microbes from settling in the hood. Turn off the UV/antimicrobial light and turn on the hood 30 minutes prior to entering the hood.

- Wear short sleeves or roll your sleeves up. Turn your baseball caps back if you MUST wear them, tie long hair back and remove rings and watches.
- Wash hands with soap and water before beginning the procedure and rewash if you touch anything that is not sterile or within the hood.
- Spray down your hands, work surface, and anything that will go into the hood with 70% ethanol. Rewipe at intervals if you are working for a long time in the hood. This will reduce the numbers of bacteria and mold considerably.
- Do not breathe directly into your cultures, bottles of media, etc. This also means to keep talking to a minimum. No singing or chewing gum.

- Work as quickly as you can within limits of your coordination. Also, keep bottles and flasks closed when you are not working with them. Avoid passing your arm or hand over an open bottle.
- Use only sterilized pipets, plates, flasks and bottles in the hood for procedures.
- Take special precautions with the sterile pipets. Remove them from the package just before use. Make certain to set up the numbers on the pipet so that they face you. Never mouth-pipet, use the pipetting aid. Change pipets for each manipulation. If the tip of the pipet touches something outside of the flask or bottle, replace with a new one. Never use a pipet twice.

Basic Cell Culture Procedure for Anchorage Dependent Cells

- View cells using inverted phase microscope
- Aseptically aspirate media
- Rinse media with PBS
- Add Trypsin-EDTA to cells
- Aspirate Trypsin-EDTA
- Incubate cells with layer of Trypsin-EDTA at 37° C
- Resuspend cells with fresh media
- Take sample and count cells
- Calculate how many cells are needed to add to new plate or flask

Antibiotics and anti-fungal agents

- Added to media to prevent or control infection by micro-organisms (e.g. bacteria, yeast)

- Normally use penicillin and streptomycin (most bacteria, but not fungi)
- Anti-fungal??
  - Gentamycin
- Particularly important in serum-containing media (rapid bacterial growth)

Remember

- **Some volumes don't need to be exact in cell culture**
- Rinsing volume of PBS (as long as it fits in the dish and is sufficient to rinse the serum).
- Volume of trypsin EDTA as long a bottom of plate or flask can be covered.
- Volume of media used to resuspend your cells. The same number of cells will be there despite the volume of media used.
  - Too little resuspension media will result in very high cell count and would require more dilution (and higher dilution factor). The volume needed to seed your next plate would then be very small, maybe too small to work with.
  - Too much media would result in low cell count/ml and you may need a large volume to add to your new plate.

- Volume of cells removed for cell counting.
  - You want enough to work with, but not take all of your cells from your plate. If you want a dilution factor of 2, just add an equal amount of trypan blue.
  - 100µl of cells +100µl typan blue=1:1
- Exact # of cells to be plated
  - If you want to plate 2×10^5 cells onto your plate, but you have 2.1×10^5 cells/ml, plating 1ml will be easier than plating .953 ml.

Discussion?

- Use formula **M1V1=M2V2**
  - **M1** (cell number you wants)
  - **V1** (Volume you wants)
  - **M2** (cell numbers that you know from cell counting)
  - **V2** (Volume of the cell aliquots that you need)
- Problem:
  - You wants to plate in your 6 well plate (1×10^6 cells/ml) in each well (6 well). You have 2×10^6 cells/ml from the counting using the haemocytometer. How many ml of cell aliquots you need?

- M1V1=M2V2
  - **$(1\times10^6)(6)=(2\times10^6)(V2)$**
  - **$6\times10^6= 2\times10^6 xV2$**
  - **$6\times10^6/2\times10^6=V2$**
  - **V2= 3ml**
    - ❖ 3ml of cell suspensions + 3ml of media
    - ❖Why?
      - ❖Cells suspension is more concentrated (high amount of cells) that what you need to be plated in your each well.

Laboratory design

- The major requirement to distinguishes tissue culture from most other laboratory techniques is the need to maintain asepsis (free from disease causing contaminants).
- Several consideration need to be taken in planning new laboratory
  - Ventilation- pressure balance, laminar flow hood
  - Accommodation- staff numbers/ space, aseptic area, hoods, incubation, preparation area, servicing aseptic areas and storage
  - Renovation-choose the location carefully to avoid space constrain
  - Access-the space are wide and height enough to allow installation of equipment
  - Quarantine facilities

Layout of Aseptic room

- Small tissue culture laboratory - suggested layout for simple, self-contained tissue culture laboratory for use by two or three person. Dark shade areas represent movable equipment, lighter-shaded areas fixed of movable furniture.

- Medium-sized tissue culture laboratory-suitable for five or six person, with washing up and preparation facilities located elsewhere.

Who Is responsible for Safety?

- General
  - All employees are responsible for a safe working environment
  - Management is responsible for providing:
    - Safe working conditions
    - Safe equipment
    - Training

Employee

- Employees are responsible or reviewing all pertinent safety programs
- Complying with all safe work practices, rules and regulations set forth by Safety & Health
- Recognizing hazards in the work place
- Reporting unsafe conditions to their supervisor or Safety & Health immediately

Safe Work Practices

- Maintain a clean, uncluttered work area
- Working alone after hours is prohibited
- Know the location of the nearest emergency exit and safety equipment (shower, eye wash)
- Report all accidents or near misses to your supervisor & Safety & Health

Good Laboratory Practices

- No smoking, food or beverages in the labs
- Required PPE(personal protective equipment) must be worn while in the lab
- Aisles & exits are free from obstruction
- Lab benches must be cleaned, disinfected or decontaminated after work is completed

Good Laboratory Practices

(continued)

- Do not use hoods for storage
- Work surface is protected from contamination
- Heavy objects are confined to lower shelves
- Glassware is free from crack, breaks or chips
- Broken glass containers are available & in use

Personal Protective Equipment

- PPE must be worn when in the laboratory

  - **Eye protection**
  - **Protective Clothing:**
    - Labs coats or tyvek garments, sleeve covers
  - **Gloves**
  - **Shoes**
    - No open-toed shoes allowed in laboratories
    - Safety shoes issued as required

Hazard Communication

- Primary & secondary containers are labeled with identity of material, expiration date & hazard warning
- Signs on storage areas are consistent with hazards within (I.e. biohazard, flammable)
- MSDS (material safety data sheet) binders are available and employees know location of them

Chemical Storage

- Incompatible materials must be segregated
- Corrosives & flammables must be stored below eye level
- Unused & outdated materials must be disposed of properly
- Safety carriers must be used to transport all chemicals
- Lab carts must have side rails

Compressed Gas Cylinders

- Gas cylinders must be properly secured
- Cylinder caps must be in place when cylinders are not in use or transported
- Gas cylinders are labeled with their contents
- Empty gas cylinders are marked “EMPTY”
- Check hoses, tubing and regulators daily

Waste Disposal

- Do not drain dispose of liquid waste
- **Separate waste streams by type** (biological, chemical)
- Use approved containers for each waste stream
- Label all containers with approved labels
- Virkon (detergent)
- Do not allow waste to accumulate on floors, in corners or under shelves

Safety Equipment

- Know the location of safety equipment
  - Safety showers, eye wash stations & fire extinguishers
- Make sure safety equipment is not blocked
- Spill team list and emergency numbers must be posted in labs next to phones

Emergency Evacuation

- Know your exit route
- Know the location of fire alarm pull-stations
- Notify your supervisor and Safety & Health of any unsafe conditions

References

- Freshney, R.I. (2000) Culture of Animal Cells: A manual of Basic Technique. 5fifth edition. New Jersey: John-Wiley & Sons, Inc.
- Freshney RI. Culture of Animal Cells: A Manual of Basic Technique. New York: Wiley-Liss, 2005.
- Bernhard, O., & Bhatia, N. (2004) Tissue Engineering. Pearson Prentice Hall Bioengineering