UNIVERSITÀ DEGLI STUDI DI TRIESTE

---

Corso di Laurea Magistrale in Genomica Funzionale
Corso di laurea Magistrale in Biotecnologie Mediche e Diagnostiche

**CORSO DI TECNOLOGIE MOLECOLARI E CELLULARI**

**ANNO ACCADEMICO 2024-2025**

# Animal cells: basic concepts

**(for internal use only PLEASE DO NOT DISTRIBUTE)**

**DOCENTE**
**Prof. Daniele Sblattero**
**Dip. Scienze della Vita**

# Animal cells: basic concepts

## 1. Introduction

The term **cell culture** refers to the cultivation of dispersed cells taken from an original tissue, a primary culture, or a cell line. The practice of cultivating cells started at the beginning of the 20th century and was developed from a simple exploratory phase to an expansion phase in the 1950s. For more than 50 years, the culture of cells derived from primary tissue explants has predominated, justifying the original name **''tissue culture.''** Currently, cell culture is in a specialization phase. With the increase of the use of dispersed cells since the 1950s, the term tissue culture was substituted by **cell culture.** At the present time, cell culture techniques allow in vitro propagation of various cell lines including those from insects, humans, mice, rats, and other mammals. Basically, animal cell culture techniques are similar to those employed for bacteria, fungi, and yeast, although there are some characteristic differences. In general, **animal cells are more delicate**, vulnerable to mechanical damage, present lower growth rates, and require more complex culture media and special substrates. Moreover, cell culture has to be performed under **rigorous aseptic conditions**, since animal cells grow more slowly than most usual contaminants, such as bacteria and fungi.

## 2. Establishing a cell line.

**Primary cells are isolated directly from organs or tissues.** Primary cells are normally **heterogeneous** and better represent the tissue from which they originate. These cells have **a finite growth capacity** and can be subcultured for only a limited number of passages. Subcultured cells, which have been selected to form a population of cells of a single type, are designated **cell lines**, and can be finite or continuous. **Finite cell lines** (cells capable of a limited number of generations before proliferation ceases) as well as **continuous cell** lines can be propagated and expanded for the production of well characterized cell banks, where they are preserved by employing cryopreservation techniques.

**A normal tissue usually provides finite cultures**, while cultures obtained from **tumors** can result **in continuous cell lines** (immortal). Nevertheless, there are many examples of continuous cell lines that are obtained from normal tissues and are not tumorigenic, such as BHK 21 (baby hamster kidney fibroblasts), MDCK (Madin-Darby canine kidney epithelial cells), and 3T3 fibroblasts. Immortal cell lines can occur spontaneously (rarely) or after a transformation process (more often), which can be induced by carcinogenic chemical agents, by viral infection, or by the introduction in the cell genome of a viral gene or an oncogene capable of overcoming senescence. Several of the differences between normal and neoplastic or tumor cells are analogous to the differences between finite and continuous cell lines, since immortalization is an important component of the cell transformation process.

**The main advantages** of **continuous cell lines** are:
(i) faster cell growth, achieving high cell densities in culture, particularly in bioreactors;
(ii) the possible use of defined culture media available in the market, mainly serum-free and protein-free media;
(iii) the potential to be cultured in suspension, in large-scale bioreactors.

The **major disadvantages** of these cultures are:
(i) accentuated chromosomal instability;
(ii) the larger phenotype variation in relation to the donor tissue;
(iii) and the disappearance of specific and characteristic tissue markers.

Cells can be cultivated in vitro after a tissue is dissected and totally or partially disaggregated by enzymic treatment. Cell, explants, or organ cultures can be established depending on the degree of disaggregation of the tissue, as shown in Figure 2.2.

**Primary cultures are those obtained directly from organ or tissue fragments,** following enzymic or mechanical disaggregation. For enzymic treatment, trypsin or collagenase may be used and may be mixed with EDTA (ethylenediaminetetraacetic acid). Primary cultures can originate from animal or human tissue. A culture is considered a primary culture up to its first subculture or passage; after that it is named a cell line.

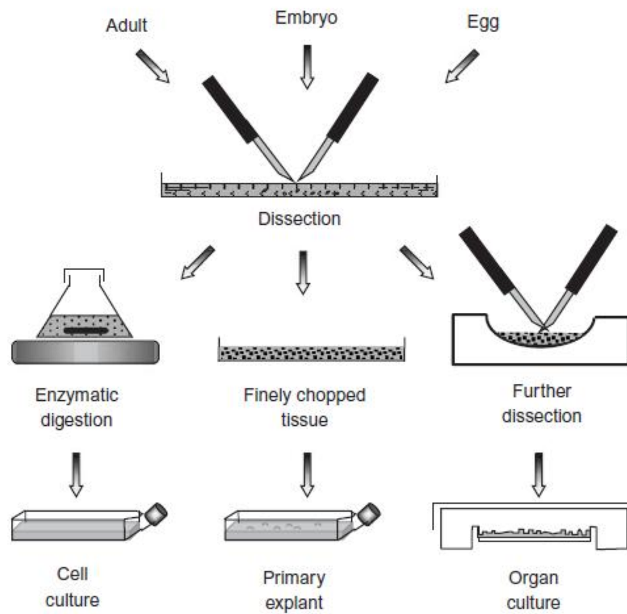


**Figure 2.2**

*In vitro* culture types of animal cells (adapted from Freshney, 2000, 2005).

Figure 2.3 shows a schematic diagram of the **sequence of events associated with subculture of a normal primary cell culture.** Primary cultures are initially heterogeneous, but fibroblasts may become predominant after some growth. Obtaining primary cultures is laborious and cells can be maintained in vitro for only a limited period. During this period, cells generally maintain the differentiated characteristics of the original tissue from which they were harvested. During primary culture, cells may be subjected to considerable stress. For instance, the enzymatic dissociation of organ fragments or adherent cells breaks cell–cell or cell–surface interactions. Dissociated cells generally change their shape, becoming rounded and losing their phenotypic polarity, modifying protein distribution in cell membrane. Certainly not all cells survive cell manipulation but those that survive should be able to correct any injuries and adapt to environmental changes. Culture adaptation is time-consuming and is influenced by culture conditions. Cells condition their environment through the release of substances into culture medium, such as growth factors, which promote cell adherence and proliferation.

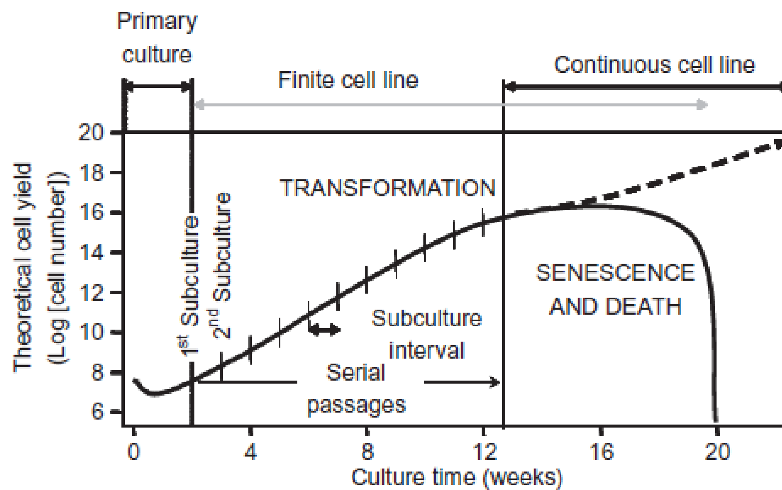


**Figure 2.3**

Typical evolution of a cell line (adapted from Freshney, 2000, 2005).

After successive subcultures of a very heterogeneous primary culture, containing many cell types of the original tissue, a more homogeneous cell line with a higher growth rate may arise. A cell line can be serially propagated in culture, usually for only a limited number of cell divisions. Finite cell lines are generally diploid and maintain some degree of differentiation. Nevertheless, these cell lines die after a limited number of generations, the **Hayflick limit**, which is usually **about 30–50 division cycles depending on the origin of the cells**.

**The immortalization of a cell line** can be accomplished as a spontaneous process, by an oncogene or virus or by chemical treatment (see later). This can lead to a **continuous cell line** that can be propagated for an undetermined period. If such changes occur with an effect on cell cycle control, the cell line can be designated a **transformed cell line.**

## 3. Generation of immortal cell lines

Normal cells are unable to replicate past several rounds of proliferation (termed the Hayflick limit) as with each round of proliferation the telomeres shorten. When the telomeres reach a critically reduced length, DNA damage is triggered leading to cellular senescence.

Therefore, if you tried to culture a primary cell population it would eventually die unless the cells were manipulated in some way to circumvent the process of senescence and become immortal. Here we discuss various ways to overcome the hayflick limit and induce immortality in cultured cells.

**Sources of Immortality**

**3.1 Spontaneously Immortalized Cells**

The best example of this would be cancer cells, which may have undergone genetic changes to resist senescence and are immortal. However, many cancer cell lines may not have these changes, in fact, George Gey, the scientist who created the first immortalized and arguably the most famous cell line: HeLa cells, had to test hundreds of cancer lines before stumbling upon the highly metaplastic ovarian cells of Henrietta Lacks. Thus, other methodologies may be required to help even cancer cells become immortal.

### 3.2 Introduce a Viral Gene that Overrides the Cell Cycle

Many viral genes affect the cell cycle and thus can be used to overcome senescence by removing the biological brakes on proliferative control. Many of these are tumor suppressor genes, since they require suppression for tumorigenesis to occur. The most common of these is over-expression of the **Large T-antigen of the simian virus (SV-40),** which represses the retinoblastoma (Rb) and p53 genes, both critical controllers of the cell cycle.

One example of a cell line immortalized with SV40 is **HEK293T**, which are also known as 293T cells, a cell line widely used to express viral particles and for many cellular assays, since they are very easily transfected. Other viral genes include those from the **human papilloma virus (HPV) such as E6 and E7,** which also target Rb and p53.

### 3.3 Expression of Genes that Confer Immortality

The most well-known immortality gene is **Telomerase (hTERT).** A ribonucleoprotein, telomerase is able to extend the DNA sequence of telomeres, thus abating the senescence process and enabling the cells to undergo infinite cell divisions. Indeed, telomerase has recently been heralded as a potential mechanism to reverse aging. The issue with using telomerase to reverse ageing is that increased telomerase expression can also induce tumor growth. Indeed, many of the same genes used to create immortalized cell lines such as hTERT and SV40 induce tumor formation. Thus, it is unsurprising that hTERT is often found over-expressed in human tumors, thus imparting one of the key hallmarks of cancer: unrestrained proliferation. Exactly what we use it for when immortalizing a primary cell line!

### 3.4 Combining Tumor Suppressor Inactivation and Telomerase Expression

In some cell types, using only one immortalization method may yield low numbers of cells that have become immortal. Therefore, depending on your cell line, it may be beneficial to combine both suppression of a tumor suppressor (such as the cell cycle inhibitors mentioned above) and expression of hTERT to immortalize a larger number of cells. This dual method is also suggested if you wish to both immortalize and transform (i.e. make tumorigenic-like) your cell line, as evidence suggests that some cell lines do not undergo efficient transformation without some immortalization.

### 3.5 How to Introduce Immortality to a Primary Cell Line

Many of the above sources of immortality are based on genetic manipulation of your primary cell line. This requires the introduction of foreign DNA into your cells. As many primary cells lines are frustratingly difficult to transfect, the easiest and most effective way to introduce genetic changes into a primary cell line is through viral infection.

The most popular method is through replication-deficient lentiviruses, since they are relatively safer then adenoviruses which have the ability to re-infect cells and thus contain live virus for a much longer period of time. Retroviruses can also be used to transfect cells, however, they can only infect actively dividing cells, thus reducing the number of cells that may be transduced with virus.

Words of Caution: Immortality May Not be the Best Route!

By introducing genetic changes into your cells, you may be profoundly altering the phenotype of your cell line. Although this does make your cell line more useful in some ways: it may make your cells more homogeneous allowing for replication of results, you can create large stocks of cells for future use and they may be easier to experimentally, there is still many benefits to using primary cell lines.

As primary lines adapt to being culture and being immortalized, cell populations and cellular mechanisms are altered. This may confound your experiments and lead to inconclusive or erroneous results. In addition, there is much debate about how accurately immortalized cells model real tissue. For example, although controversial, it is unlikely that SV40 can infect humans, although the mechanism of action of SV40: p53 and Rb mutations can be

very common mutations in human tumors. Thus, it is important to identify the best genetic manipulation to use to immortalize your cell type.

Therefore, although immortalized cells are immensely powerful in their utility for experimental research, some caveats must be accepted in their use. As with any experimental model, immortalized cells are simply a model for your intended cellular system.

**The main characteristics of transformed cell lines are:**
(i) altered cell morphology (smaller, less adherent, or more rounded cells, with a higher nucleus to cytoplasm ratio);
(ii) higher growth rate (duplication times decrease from 36–48 hours to 12–36 hours);
(iii) less dependency on blood serum or selected growth factors;
(iv) increase in cloning efficiency;
(v) increase in heteroploidy (chromosomal variation between cells) and in aneuploidy (divergence from the original diploid number);
(vi) increase in tumorigenicity. Some of these characteristics are associated with malignant transformations.
**The main advantage of transformed** cells for cell culture is the almost unlimited cell supply. However, a **disadvantage** is that the cells generally maintain few characteristics of their original tissue.

## 4. Cell line maintenance

When a primary culture or a cell line is initiated, **a periodic change of culture medium is necessary**, followed by subculture if the cells are proliferating. Culture medium changes should be performed even in cultures showing no cell proliferation, since cells can metabolize and deplete nutrients from the medium. Also, some nutrients may degrade spontaneously. Intervals between medium changes and subcultures may vary depending on the cell line, growth rate, and metabolism. An increase in cell density, pH decrease, nutrient depletion in the medium, or alteration in cell morphology indicate the need for culture medium replacement. Animal cells may be anchorage-dependent. Cells that depend on a solid substratum for growth are named **adherent cells.** These cells normally proliferate in monolayers and show contact inhibition, with the maximum cell yield generally limited by the available surface of the culture vessel. The yield of cells in suspension is not dependent on a solid substratum. Table 2.1 shows some adherent and suspension cell lines that are commonly cultured. Cell shape usually reflects their origin. Blood cells (such as those derived from lymphomas) generally grow in suspension, while cells derived from solid tissues (such as kidney and liver) are adherent cells.
Since adherent cells proliferate only after cell surface adhesion, an understanding of the steps of this process is very important.
**The first step** consists of the adsorption of adhesion factors to the substratum, such as vitronectin and/or fibronectin glycoproteins and often associated to $Ca^{2+}$ ions. These factors can be derived from serum or can be produced by the cells themselves.
**The second step** consists of contact of the cell with the surface, while in the **third step**, cells attach to the covered surface, producing multivalent heparin sulfate proteoglycans, which bind to cell membrane glycoproteins. **In the fourth step**, cell spreading over the solid surface occurs.

**Table 2.1** Adherent and suspension cell lines commonly employed in animal cell culture

| Name | Cell type and tissue origin | Morphology |
|---|---|---|
| Adherent cells | | |
| MRC-5 | Human lung | Fibroblast |
| HeLa | Human cervix | Epithelial |
| Vero | African green monkey kidney | Epithelial |
| NIH 3T3 | Mouse embryo | Fibroblast |
| L929 | Mouse connective tissue | Fibroblast |
| CHO | Chinese hamster ovary | Fibroblast |
| BHK-21 | Syrian hamster kidney | Fibroblast |
| HEK-293 | Human kidney | Epithelial |
| HEPG2 | Human liver | Epithelial |
| BAE-1 | Bovine aorta | Endothelial |
| Suspension cells | | |
| NS0 | Mouse myeloma | Lymphoblastoid-like |
| U937 | Human hystiocytic lymphoma | Lymphoblastoid |
| Namalwa | Human lymphoma | Lymphoblastoid |
| HL60 | Human leukemia | Lymphoblastoid-like |
| WEHI 231 | Mouse B-cell lymphoma | Lymphoblastoid |
| YAC 1 | Mouse lymphoma | Lymphoblastoid |
| U 266B1 | Human myeloma | Lymphoblastoid |
| SH-SY5Y | Human neuroblastoma | Neuroblast |

Adapted from ECACC Handbook, 2005.

As vertebrate cells have negative charges non-uniformly distributed over their external membrane surface**, solid substrata with a hydrophilic surface are required**, with an adequate distribution of the surface charge.

**For the subculture of adherent cells**, removal of culture medium and the detachment of cells from the monolayer are necessary. This detachment is usually performed with trypsin, but other proteases, such as pronase, dispase, and collagenase, can be employed.

In general, a chelating agent, such as EDTA, is also added to capture the Ca2þ ions involved in the cell adhesion process. Some cell lines bind weakly to surfaces and, in small scale cultures, can be removed mechanically by gently tapping or hitting the culture flask by hand.

**For cells growing continuously in suspension**, the subculture process can be performed similarly to the method used for microbial cultures. Trypsin treatment is not required and subculture is faster and less traumatic for the cells. Total medium exchange is not generally performed for these cultures since it would require a centrifugation step. Culture maintenance can be performed by dilution with fresh medium after adequate cell growth.

## 5. Influence of environmental conditions on animal cell culture

Effective in vitro maintenance and growth of animal cells requires culture conditions similar to those found in vivo with respect to temperature, oxygen and carbon dioxide concentrations, pH, osmolality, and nutrients. Within normal tissue in vivo, animal cells receive nutrients through blood circulation. For growth in vitro, animal cells require an equivalent supply of a complex combination of nutrients. For this reason, the first attempts
in animal cell culture were based on the use of biological fluids such as plasma, lymph and serum, as well as on extracts from embryonic-derive tissue.

Medium composition is one of the most important factors in the culture of animal cells. Its function is to provide appropriate pH and osmolality for cell survival and multiplication, as well as to supply all chemical substances required by the cells that they are unable to synthesize themselves. Some of these substances can be provided by a culture medium consisting of low molecular weight compounds, known as basal media. However, most basal media fail to promote successful cell growth by themselves and require supplementation with more complex and chemically undefined additives such as blood serum.

Many attempts have been made to develop culture media that do not need this type of supplementation, that is, serum-free media formulations. These serum-free media present several practical advantages. However, the

formulations that have been developed are specific for certain cell types and a universal culture medium suitable for the culture of all animal cell
types seems, so far, unreachable.

**5.1 pH control** is fundamental for in vitro cell culture. Most mammalian cell lines proliferate at pH 7.4. Although the optimal pH value for cell growth does not vary too much for different cell lines, some normal fibroblasts proliferate well at a pH range between 7.4 and 7.7, and transformed cells have an optimal growth at a pH varying from 7.0 to 7.4. It is possible to monitor pH variation by adding a pH indicator. The compound most used is **phenol red,** which is rose-colored at pH 7.8, red at pH 7.4, orange at pH 7.0, and yellow at pH 6.5. Nevertheless, it is convenient to point out that most commercially available phenol red contains impurities that could influence cell behavior. Also, this compound can interfere with the interpretation of experimental data obtained by the use of fluorescence and absorbance techniques.
Culture medium needs to be buffered to compensate for CO2 and lactic acid derived from glucose metabolism. Most culture media employed for animal cells are buffered with CO2 originating from the gaseous phase, in equilibrium with sodium bicarbonate (NaHCO3) added to the culture medium, as described by the following equilibrium reaction:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \qquad (3)$$

$$NaHCO_3 \leftrightarrow Na^+ + HCO_3^- \qquad (4)$$

An increase in CO2 tends to increase Hþ and HCO3– in reaction (3), consequently increasing medium acidity. In compensation, the increase in HCO3– causes NaHCO3 formation through reaction (4), until an equilibrium is reached at pH 7.4. In summary, a medium pH decrease due to a CO2 increase in the gas phase is neutralized by the action of sodium bicarbonate, with the pH stabilizing at 7.4. Traditionally, culture media are buffered with sodium bicarbonate at a final concentration of 24 mM.

When cells are growing at low densities or are in a lag phase, they do not produce CO2 in sufficient quantities to maintain the pH at an optimal value. Decreasing the CO2 content in the gaseous phase results in an increase in the culture equilibrium pH. Therefore, the control of CO2 concentration allows appropriate maintenance of culture pH.

This type of buffering is of **low cost, non-toxic,** and also provides other chemical benefits for the cells. Each basal medium has its own bicarbonate

**5.2 Temperature** plays a very important role in cell culture. It has a critical influence on cell growth and it affects the solubility of various medium components, especially of gases such as CO2 and O2, which have low solubilities. Most mammalian cells have optimal growth rates within the range of 35–37°C. Insect cells grow at optimal temperatures of 26–28°C, while cold-blooded vertebrate cells normally grow well at lower temperatures.

**5.3 Composition and nature of the substratum for cell adhesion**
Adherent animal cells (anchorage-dependent) need a surface for adhesion, spreading, and proliferation. Glass and plastic are the most common materials employed as a solid substratum. For several decades, glass was the only substratum used for animal cell culture. As a consequence, the common term ''in vitro'' means ''in glass.'' Animal cells present good adhesion on glass surfaces, especially borosilicate surfaces, that have a high silica content. Glass is still used as a solid substratum on a small scale, but very rarely in cultures aiming at high cell quantities. The use of plastic materials for routine cell culture on the laboratory
scale was introduced at the end of the 1960s, and some characteristics of glass surface, such as hydrophobicity and negative charge, were maintained in these materials**. Polystyrene is the most widely used plastic material for animal cell adhesion at present**, because of its surface characteristics, its low cost, and also its

transparency. For more demanding cell lines, the surface has to be submitted to a treatment that involves coating with proteins such as poly-lysine, poly-ornithine, or extracellular matrixderived proteins such as fibronectin, laminin, and collagens.

### 5.4 Main components of animal cell culture media

#### 5.4.1. Water

One of the basic components of a culture medium, and also one of the most critical, is water. Animal cells are extremely sensitive to water quality, since this can be the source of contamination that can affect cell growth. Potential contaminants include inorganic compounds (heavy metals, iron, calcium, and chloride), organic compounds (detergents), microbial-related contaminants (endotoxins and pyrogens), besides particles and colloids from several different origins. Since the presence of any of these contaminants can prevent cell culture on any scale, strict purity standards of water quality must be maintained. Efficient water purification systems are, therefore, required, and can be based on multiple-distillation
systems or on equipment combining deionization, microfiltration, and reverse osmosis.

#### 5.4.2 Glucose

Glucose is usually the main carbohydrate for animal cell growth, acting as source of both carbon and energy. Normally, this compound is added to the culture medium in concentrations varying from 5 to 25 mM (0.9–4.5 g/L), but may be up to 56 mM (10 g/l).

#### 5.4.3 Amino acids

Essential amino acids are those not synthesized by the organism and are required by animal cells in culture. These include specific amino acids such as cysteine and tyrosine but the requirement varies between cell lines. Amino acids are necessary for protein, nucleotide, and lipid synthesis and, in addition, may be used as an energy source. Traditionally, from 0.1 to 1 mM of each amino acid is added to the culture medium, including both essential and non-essential amino acids.

#### 5.4.4 Vitamins

Many cells require media supplemented with complex B vitamins, while other vitamins are presumably supplied by the addition of serum to culture media. Nevertheless, when serum-free media are employed, not only the water-soluble vitamins should be provided, but also the lipidsoluble ones, such as biotin, folic acid, niacin, panthotenic acid, thiamine, and ascorbic acid, as well as the vitamins B12, A, D, E, and K. Vitamins are used in very low amounts as enzyme cofactors, essential for general cell metabolism. While ascorbic acid acts in collagen synthesis,vitamin A affects cell growth and differentiation.

#### 5.4.5. Salts

The salts most commonly added to the culture medium are Naþ, Kþ,Mg2þ, Ca2þ, Cl –, SO42–, PO43–, and HCO3–. These ions are important in the maintenance of the ionic balance and osmotic pressure, besides acting as enzymatic cofactors. Salts are the components that contribute most tothe increase in culture medium osmolality.

#### 5.4.6 Serum

Blood serum, usually bovine-derived (calf or fetal bovine), contains aminoacids, growth factors, vitamins, proteins, hormones, lipids, and minerals. Besides fetal bovine serum, serum from horse (equine), and even from humans (less common) can also be used. **The main functions of serum are to stimulate growth and other cellular activities through hormones and growth factors,** to increase cellular adhesion through specific proteins, and to supply proteins for the transport of hormones, minerals, and lipids. Supplementation with bovine fetal serum is performed at concentrations **from 2 to 20% in volume**.

## 6. Cryopreservation and storage of cell lines

Cells can be unstable when in culture for long periods, exhibiting alterations in their morphology, function, growth pattern, and karyotype. Animal cells show in vitro changes related to age and some cultures can suffer spontaneous transformation, showing altered growth or changes in functional characteristics. When adequately frozen, cells can be preserved for long periods without alterations in viability or in other characteristics. Thus, a cell bank, consisting of frozen and stored cell lines, makes it possible to maintain the cultures as a renewable source. This procedure enables **repeated cultures to be performed with cells having equivalent characteristics, with consistent passage numbers.** Cells stock also minimizes **the risk of losing a culture due to accidents** such as contamination by microorganisms or other cell lines, or due to failure of equipment such as CO2 incubators. Culture conditions influence the survival of cells submitted to cryopreservation.

To be frozen, cells should be in an active growth phase, with a viability greater than 90% and free of contaminants. The optimal cryopreservation conditions are different for each cell line. When a cell is exposed to low temperatures, ice crystals are generally formed and can disrupt the cell membrane, causing death. Therefore, **cells should be treated with a cryoprotector** to support the freezing and thawing processes. The optimal cell concentration in a suspension of cryoprotector medium depends on the cell type and is determined empirically, but is generally within the range of 1-3 $10^6$ to 1-3 $10^7$ cells/ml. When added to medium at concentrations between 5 and 10% (v/v), **cryoprotectors such as glycerol or dimethylsulfoxide (DMSO) affect membrane permeability, allowing water release from the cell interior during cooling.**

Cryoprotectors decrease the freezing point, and ice crystals start to be formed at –58C: Water efflux plays a key role in the freezing process. With cell dehydration, occurring at between -58C and –158C, ice crystals are formed around the cells and not inside them. The cooling velocity is also critical and should be low (about 1°C/min). Freezing conditions should minimize crystal formation in the cell interior, preventing lysis. For cells cultivated in the presence of serum, the cryopreservation medium should also contain fetal bovine serum to protect cells from the stress associated with the freezing and thawing processes. Generally, serum is added at concentrations above 20%, and can attain 90% of volume of the cryopreservation medium. Frequently cells are stored immersed in liquid nitrogen at –196. °C. However, storage in vapor phase liquid nitrogen (at –140°C to –180°C) is recommended, avoiding possible contaminations or cell death in case of cryotube rupture.

During **cell thawing**, the heating rate should be high. As a general practice, flasks containing frozen cells are immersed in water at 37°C, and thawing occurs in about 90 seconds. Some cells are particularly susceptibleto osmotic shock during thawing and culture medium transfer processes.

For cell line storage, **a cell bank is generally established**, with initially three to five flasks. One of these flasks is then thawed and the cell population is expanded to produce a master bank with about 10 to 20 flasks, depending on future requirements. The size of this bank will depend on the future demand. At this stage it is important to confirm that the master and the work banks are genetically identical.

**6. Culture quality control and laboratory safety**

Cell culture practice requires rigorous control with respect to the quality of material and reagents, the origin and integrity of the cell lines, and the absence of microbial contamination.

The culture system should be totally free of compounds that can cause toxic or inhibitory effects. **Microbial contamination** is the most frequent problem in cell culture. The main contaminant types are bacteria, fungi, mycoplasmas, and viruses. **Bacterial and fungal** contamination is detected by a rapid increase in medium turbidity or by a rapid pH change. After contamination, animal cells generally survive for a short period. **Mycoplasma** contamination is the most difficult to detect and can cause a reduction in cell growth rate, morphological alterations, chromosomal aberrations, and changes in metabolism of amino acids and nucleic acids. **Virus contamination** causes changes in cell growth rate, and fetal bovine serum is usually the main source of this kind of contaminant.