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Cell Culture: History, Development and Prospects

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A B S T R A C T

Since its inception, the animal cell culture in the twentieth century is related to its usefulness in research. Early methods such as Harrison culture of the inverted drop, Carrel's and Lindbergh's innovations made with the introduction of the use of the infusion pump, the culture of HeLa cells and vaccine design using animal cell cultures are approach which revolutioned the implementation and study of the cell culture. One of the important steps in this technique is the selection of the culture media, which provides the physical and chemical conditions close to those occurring in the natural environment for cell growth, which are crucial for the adhesion, proliferation and cell survival in vitro. In this review, the essential aspects that define this technique are shown, offering a historical overview of the most relevant events and the current prospect in the development of the cell culture, that have been enabled the progress in both, basic and applied research.

Introduction

Historical background

Cell cultures have marked a major change in research during the last decades due to the versatility of studies and field trials to which they apply. The history of this technique dates back to the late nineteenth century when Wilhelm Roux manages to maintain living cells of the neural plate of chick embryos in saline buffer for a few days. In 1887, Leo Loeb achieves culture cells from inside and outside body tissues, using the technique called "tissue culture within the body". He placed skin fragments of guinea

pig embryo in agar and coagulated serum, then inoculated into adult animals and obtains reproduction of mitotic epithelial cells. However, this graft tissues and fluids conducted in a living animal, was not strictly considered a cell or tissue culture. In 1907, Ross Granville Harrison published an article for culturing nerve cells and monitoring the development fibers (Harrison, 1907). Harrison overcomes challenges of basic culture and developed a reproductible technique, consisting in obtaining fragments of neural tube frog embryo by placing a drop of fresh lymph on sterile coverslip. Once coagulated lymph, he inverted the coverslip onto a slide coverslip excavated, generating a hanging drop culture, a widely technique used by microbiologists for studying bacteria but successfully applied to tissue culture (Figure 1). Thus, with this simple experiment began the cell culture as a tool for researching as well as the birth of the main media of production of monoclonal antibodies, vaccines and drugs.

By 1910, Montrose Burrows adapts the hanging drop method for working with warm blood tissues in which the chicken plasma clot was used instead lymph. Then, together with Alexis Carrel, they will culture embryonic and adult tissues of dog, cat, chicken, rat and guinea pig, using fresh plasma from the same source that the tissue cultured. In late 1910, Carrel and Burrows successfully used "plasmatic media" in order to grow chicken, rat, dog and human tumors by the hanging drop method (Carrel and Burrows, 1911). Carrel applied other culture media including the diluted plasma with varying concentrations of salt solution and the use of serum. Subsequently, heelaborated subcultures by samples of tissue placed in fresh plasma clots (Carrel and Burrow, 1911b) that were maintained to grown for several months. Therefore, heevolved the cell culture by developed the first “cell line”. After that, he added chicken embryo extract as supplement to the culture media of animal cardiac cells and he achieved the early indefinite subculture (Carrel, 1912). In the early 1930's, Lindbergh, developed an infusion pump to maintain living tissue, known as "artificial heart" (Hoffman et al., 1998).Nevertheless, by finals of 30’s, cell culture was considered as a failure since it was difficult to manage, not quantitative, not replicable, and has almost not application.

However, in the early 40's, the cell culture blossomed with the design and development of a synthetic culture media for plant cells and animals (White, 1946; Fischer, 1947). Earle obtained the first established cell line, consisting on fibroblasts adapted to undefine growth in culture media (Earle et al., 1943).

In 1951, Dr. Jones diagnosed Henrietta Lacks with cervical cancer using a cervical biopsy. He send a sample to Dr. Gey, director of the Tissue Culture Laboratory, who cultivated the cells and discovered that the cell line was found to be remarkably durable and presented cell division every twenty hours. This new cell line called HeLa (Henrietta Lacks) gave him the best means to develop the poliovirus, allowing the development of the Salk vaccine (Sharrer, 2006). HeLa cells were quickly reproduced in almost any media. Gley distributed to the various laboratories and pharmaceutical companies, thus making them one of the most precious resources for studies in cancer (Sharrer, 2006). In this decade, the “media199” appears with Morgan, Morton and Parker (Morgan et al., 1950), Earle’s group develops protein free media defined for L cells in 1956, Eagle develops Essential Medium (EM) (Waymouth, 1972), and “Dulbecco modified Eagle's medium” adding essential and nonessential amino acids (Figure 2).

A series of multiple achievements was triggered in the following decades. In 1965, Harris and Watkins produced first hybrids mammalian cells, virally fusing human and mouse cells (Harris et al., 1965). In 1975, the Nobel Prize winners for Medicine in 1984, Georges Kohler and Cesar Milstein, accomplished the first monoclonal antibodies. In 1969, Augusti and Sato set tumor lines of mouse nerve cells (neuroblastoma) and isolate clones electrically excitable with nerve prolongs (Augusti-Toccoand Sato, 1965). By 1973, Graham and van der Eb introduced DNA into mammalian cells in culture (Graham and Van Der Er., 1973) and the basis for the development of techniques for incorporating genes into the cellular genome. In 1992 American Type Culture Association, a bank cellswas formed. At the end of the 90's, one of the latest scientific successes in the mammalian cell culture that played a leading and decisive role was happened, the cloning of a mammal by Wilmut, Schnieke and colleagues (Wilmut et al.,1997). Dolly the sheep was the great biological landmark of the late twentieth century. Again animal cell cultures were transformed into an essential tool for the progress of science as a solution to many of the problems of human health.

In terms of technological development, from the 50's, marketing tools cell culture started, turning this technique easy and reproductible, to become one of the most powerful tools widely used in research and development of biotechnology applied to pharmaceuticals.

Fundamentals of Animal Cell Culture: Cell microenvironment

As described, the culture animal cells involves placing one or more living cells of animal origin in an isolated environment whose physicochemical properties are able to emulate the physiological conditions of their origin. There are some important features for a sustainable culture medium for the growth and maintenance of animal cells (Freshney, 2010; Brysek et al., 2013), which are described bellowed (Figure 3).

a) Substrate. The majority of the cultured cells *in vitro* grow as a monolayer inan artificial substrate, although some transformed cell lines and hematopoietic cells grown in suspension. The substrate must be properly distributed to allow cell adhesion and proliferation as well as secretion of cell adhesion factors. In general, most used substrates are glass and plastic for their optical characteristics and regularity. Although synthetic fibers (used in the construction of scaffolds of two and three dimensions), and metals (e.g., stainless steel) are used to transfer the sample to electron microscopy (Freshney, 2010). Furthermore, the substrate properties can be enhanced by treating the surface with products of extracellular matrix collagen and fibronectin with other cell cultures, tissue-derived extracellular matrix in culture and polymers such as poly-L-Lysine or comercial matrices (Freshney, 2010; Ross et al., 2012).

b) Potential Hydrogen (pH). Most animal cells growat optimal pH rangingbetween 7.0 and 7.4. However, this can vary notably in transformed cells (Chaudhrya, 2009).

c) Buffering, carbon dioxide and bicarbonate. Carbon dioxide dissolved in the media, establishes equilibrium with HCO_{3-} ions decreasing pH (Freshney, 2010). Despite its poor buffering capacity at physiological pH, bicarbonate is usually used because of its low toxicity and nutritional benefits

to the culture. The pH of the culture media can be buffered by two types of conditions, the opening of boxes, where CO_2 entrance increases pH; and CO_2 and acid production due to high cell concentrations that causes a decrease in pH (Frahm et al., 2002; Freshney, 2010).

d) Oxygen. It is another important constituent part of the gas phase of the culture media, since generally most cells requires it for respiration *in vivo*, although some of transformed cells can be anaerobic. However, oxygen is still required and its concentration varies depending on the kind of culture; being the low concentrations, better for most cells (Freshney, 2010).

e) Osmolarity. The majority of the cultured cells have a wide tolerance to osmotic pressure. As human plasma osmolality is near 290mOsm/kg is reasonable to assume that this is the optimal level for cells *in vitro*, although it can vary to other species. In practice, the osmolarities between 260mOsm/Kg and 320mOsm/Kg are acceptable for most cells, but once selected, should be maintain constant to ± 10mOsm/Kg (Freshney, 2010).

f) Temperature. The body temperature of the animal of origin determines the optimal temperature for cell culture and anatomic variations as the case of the skin and testicles, which are usually, lower than the rest of the body (Lee et al., 2013). It is important to consider some safety factor to maintain in a minor error at the regulation of the incubator as it can be to maintain an alarm incubator as 1-degree ranges above and below at the desired temperature. The recommended for most cell lines warm-blood animals' temperature is 37°C.

g) Viscosity. The viscosity of the culture media is influenced by its content in serum. It becomes important when a cell suspension is stirred or when the cells are dissociated after trypsinization. Any damage in these conditions is reduced by increasing the viscosity of the culture medium (Freshney, 2010).

h) Aminoacids and vitamins. The essential aminoacids are a requirement for the cells cultured together with cysteine, arginine, glutamine and tyrosine. Although the amino acid requirements vary from one cell to another type. In the case of Eagle's minimum essential media, it contains water with soluble vitamins (group B, choline, folic acid, inositol, nicotinamide, excluding biotin), and others requirements are derived from serum (Genzela et al., 2004).

i) Ions and glucose. The ions found in the culture media are Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, SO_4^{2-}, PO_4^{3-}, and HCO_3-, which contribute to the osmolarity of the media. Glucose is included in most of the media as an energy source (Kwong et al., 2012).

j) Organic supplements. The media can be added to a variety of components, such as proteins, peptides, nucleosides, citric acid intermediates, pyruvate and lipids, which usually occur in complex media and it's necessary adding these as reduces the use of serum by their function as helpers in the cloning and maintenance of specialized cells (Freshney, 2010).

k) Hormones and growth factors. They are generally added to serum-free media or

provided by the added serum. The main factors found are growth factor derived from platelet, fibroblastic growth factor, epidermal growth factor, vascular endothelial growth factor and insulin-like growth factor. The most common hormones used are insulin and hydrocortisone (Stryer, 1995; Onishi et al., 1999; Freshney, 2010; Aden et al., 2011).

l) Antibiotics and antifungal. They are used in conjunction with laminar flow hoods or security flow hoods to reduce the frequency of contamination by bacteria and fungi (Freshney, 2010).

m) Serum. Serum presents growth factors that promote cell proliferation besides adhesion factors and antitrypsin activity that promote cell adhesion. It is also a source of minerals, lipids and hormones which may be linked to proteins. Serum from cow, human, horse and fetal calves are usually employed (Mojica-Henshaw et al., 2013).

Cell culture applications

With the advancement of biochemistry, molecular biology, cell biology and other areas of biological knowledge has been possible to introduce new techniques to produce different cell lines, characterize and differentiate them in order to perform studies of cell interactions, host-parasite interaction, restore or improve organs function damaged by disease or trauma, and so on. Animal cell culture technology has advanced significantly over the last few decades and is now generally considered a reliable, robust and relatively mature technology (Li et al., 2010).

Since the standardization of cell cultures it has made a technological revolution that goes from rich media development, genetic manipulation of cell lines, development of increasingly sophisticated incubators that provide the desired atmosphere, vaccine design, development of new adhesion surfaces, 3D scaffolds, robotization process, etc. Moreover, a range of biotherapeutics are currently synthesized using cell culture methods in large scale manufacturing facilities that produce for both commercial use or applied technology and clinical or research studies (Li et al., 2010).

Basic Cell Culture Research

In basic research studies intracellular activity includes : DNA transcription, protein synthesis labeled with radioactive isotopes or fluorescence, metabolism studies using immobilization techniques, specific cell lines, assays of cell cycle and senescence, characterization, proliferation, differentiation and apoptosis(Hoffman et al., 1998; Andreeff et al., 2000; Kourtis and Tavernarakis., 2009). In addition, studies of biomolecules intracellular flow as RNA processing, protein transport, assembly and disassembly of microtubules, allow modeling cell lines for research. Within the field of genomics and proteomics, this technique is used in genetic analysis assays, infection, cellular transformation, immortalization, gene expression, metabolic pathways, and cellular interactions as morphogenesis, proliferation, adhesion and extracellular matrix interaction with host parasite interactions (Andreeff et al., 2000).

Cell culture in Applied Research

The cell culture has been indispensable to study virology and has made obtaining virus by using special animals since they provided large numbers of cells suitable for virus isolation, facilited control of contamination with antibiotics, clean-air equipment, and

helped decrease the use of experimental animals and virus isolation in cell cultures (Lindenbach et al., 2005; Leland et al., 2007). Further morre, plant cell cultures (Mokili et al., 2012), vaccine production, and biotechnology studies from drug production in bioreactors (interferon, insulin, growth hormone, etc.) have also been made(Rates, 2001; Li et al., 2010).Otherwise, cell culture applications in pharmacology and toxicology studies are testing the effect of different drugs, interactions of drug-receptor type, resistance phenomena, cytotoxicity, mutagenesis, carcinogenesis, among others. One field of application rather studied in recent years has been the tissue engineering, for example, the production of tissue *in vitro* as skin or cartilage for treatment of burns, autografting, differentiation and induced differentiation (Naderi et al., 2011).

Cell cultures in cancer drug discovery have been very usefull and new techniques are developed every day. One example is the use of three-dimensional (3D) cell culture models (Smalley et al., 2008) which could be non-adherent (anchorage-independent) or adherent to a substrate (anchorage-dependent). In the 3D anchorage-independent culture the aggregation of cells can be achieved by using low-attachment plates and through coating surfaces (e.g., poly-hydroxyethyl methacrylate and agarose) (Friedrich et al., 2007; Lovitt et al., 2014).

In the anchorage-dependent model, 3D environment can be established with the use of pre-fabricated scaffolds, which consist of porous materials to support the growth of 3D structures called multilayered cell cultures (MCCs) which are resulted from cells adhering to specific substrates that are composed of tumor cells cultured on a membrane specifically designed to allow measurement of drug diffusion. Microfluidics channels are also able to support the formation of 3D cell cultures in addition; ECM can also be added into these chambers to allow ECM-to-cell interactions (Toh et al., 2007; Lovitt et al., 2014). The advantages of exploiting cells grown in 3D culture conditions are: oxygen and nutrient gradients increased cell-to-cell interactions, different rates of cellular proliferation and more (Lovitt et al., 2014).The primary goals for developing 3D cell culture systems vary widely from engineering tissues for clinical delivery through to the development of models for drug screening (Haycock, 2011).

Tissue Engineering

Certainly one of the most promising developments in the applications of cell culture is the development of tissue engineering, through which it seeks to develop and establish cell growth *in vivo* in order that in the future to be used as replacement tissue damaged or malfunctioning of the patient requires it. Despite limited success in some complex organs, the promise of substitute tissues has been fulfilled for some targets. The clinical successes in skin (Lazic and Falanga, 2011), cartilage (Roberts et al., 2008), in bladder (Atala et al., 2009) and trachea (Macchiarini et al., 2008) have already shown that tissue engineering can fill a gap in the biomedical field (Zorlutuna et al., 2013).

Development of artificial extracellular matrix (ECM) structures either based on ECM components or synthetic materials is another area where tissue engineering provides methods for development of cellular microenvironments (Zorlutuna et al., 2013). Thus, tissue engineering are now considered as end products for regenerative medicine as well as enabling technologies for other fields of research ranging from

drug discovery to biorobotics (Zorlutuna et al., 2013). This revolutionary technique involves the development of new concepts and cell culture technology because, in contrast with conventional techniques of cell culture, tissue development depends on a three-dimensional array of cells and the formation or synthesis of an appropriate extracellular matrix (ECM) (Sittinger et al., 1996), emphasizing the development of this matrix and cell differentiation in an artificial tissue. Moreover, individual components of ECM have been widely used as scaffold materials in tissue engineering (Zorlutuna et al., 2013).

Thus, we should not lose sight of the design of a good combination of the three main elements of the tissue engineering: cells (embryonic stem cells (ESCs) as cell source, induced pluripotent stem cells (iPSCs), differentiation of stem cells using biomaterials, cell sheets of mesenchymal stem cells (MSCs)), scaffold (biodegradable synthetic polymers such as poly-L-lactic acid (PLLA), poly L-lactic-co-glycolic acid (PLGA) and poly-caprolactone (PCL), ECM components such as collagen, fibronectin, laminin (Gomes et al., 2012; Zorlutuna et al., 2013), and growth factors (e.g., growth factor β1 (TGF-β1), insulin-like growth factor binding protein 4 (IGFBP4), etc.). Once the primary culture established, the cells responsible for the synthesis of the parent of the new tissue, the scaffold will serve as an ideal environment that will promote proper cell function and growth factors will facilitate the regeneration, differentiation and cell multiplication (Ikada, 2006).

It is noteworthy that cell sources for tissue engineering have expanded significantly since differentiated cells, progenitor and stem cells mean for this field holds great promise for the creation of highly complex tissues in the future serve as a replacement (Shamblott et al., 1998; Morrison et al., 1999). However, it remains to understand these great potential synced stem cells and progenitor cells of various origins have been shown to differentiate into a variety of cell types and in some cases forming functional tissue which remains challenge power control these characteristics and to make this distinction in a controlled, efficient and reproducible functional structures to generate.

Applications such as 3D tissue models for drug testing (to evaluate toxicity anticancer drug efficacy, cellular immunity by cytokine release, monitoring events such as proliferation and apoptosis, among others, therapeutic drug testing), development of multicell type artificial cornea mimics (Vrana et al., 2008; Götz et al., 2012), cancer models, and in biorobotics by using cell based systems as "actuators" (e.g., *vorticella*, cardiomyocytes, myoblast) with the ability to respond to multitude of inputs based on piezoelectric materials (Ueda et al., 2010; Zorlutuna et al., 2013) are another remaining of the amazing use and potential of tissue engineering.

Cell culture in Mexico

In Mexico, this technique has been developed for different purposes, from the generation of cell lines allowing pharmacological and toxicological studies in which one of the main objectives is to understand the morphological changes experienced by cells when exposed to different concentrations of metals, a present problem in the local environment (Fuente et al., 2002; Carranza et al., 2005) to observe the role of drugs on response and protection systems in order to establish therapeutic treatments (Gutiérrez et al., 2006).

Figure.1. Model cell culture exposed by RG Harrison in 1907

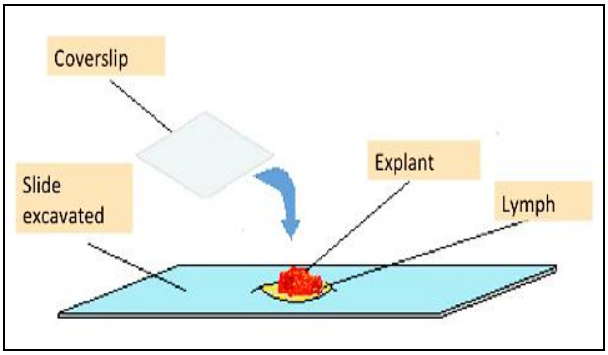


Figure.2 Mammalian cell culture technique. a) Organ from which the explant is obtained; b) the organ is segmented into pieces of about 1 mm^3; c) the fragments are placed in specific areas, growth factors, antibiotics and other supplements are added; d) the culture dish is placed in an atmosphere with 95% air flow and 5% CO_2 and incubated at 37°C; e) the culture is maintained and observed at inverted microscope

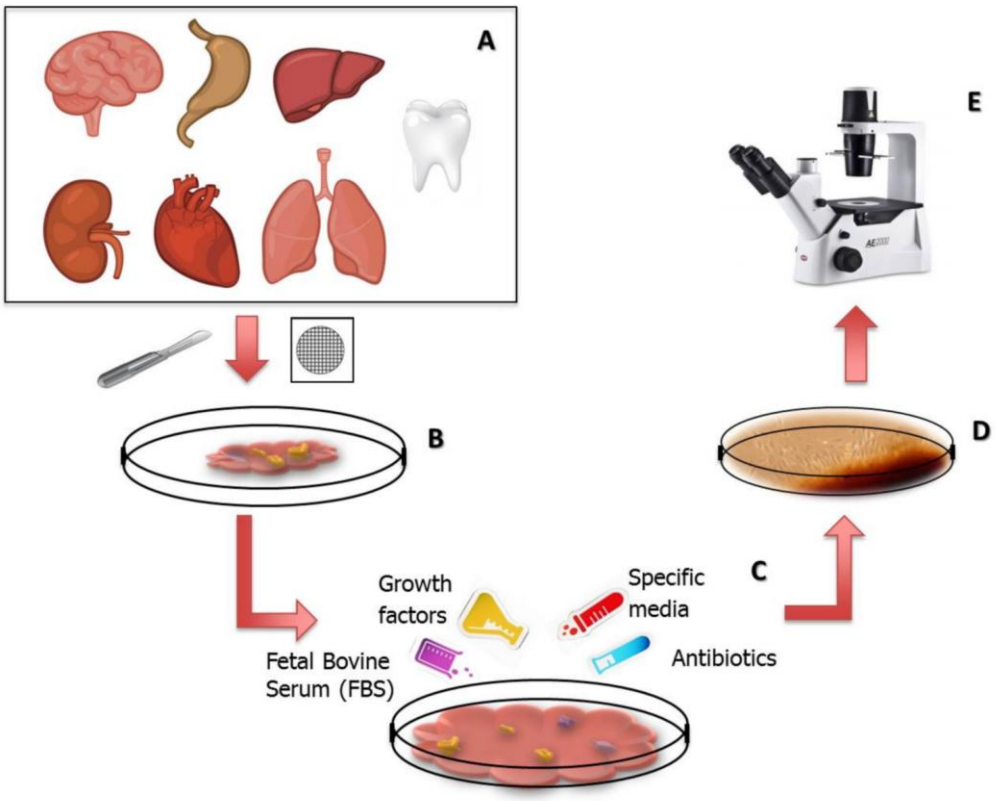
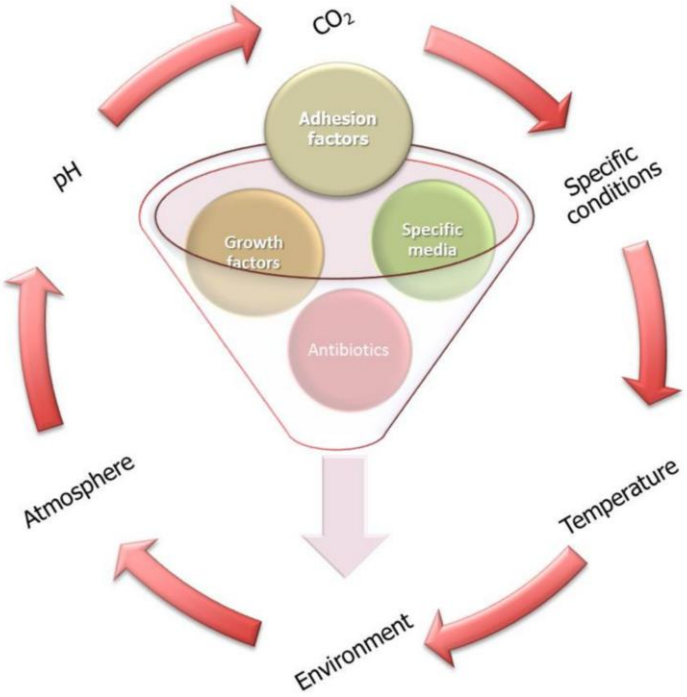


Figure.3 Parameteres for the growth and maintenance cell culture. The factors that make successful cell culture should be manteinedbalanced and just right to allow the development of experiments that yield results in which cell behavior is similar or as close to the origin tissue



Perhaps one of the newly studied fields and high impact in the country has been the skin culture by the Department of Cell Biology and Tissue Center of Research and Advanced Studies of Instituto Politécnico Nacional (IPN), together with the Mexican Institute of Social Security, and which are developed autologous transplants for skin, as well as epidermal cell cultures conducted by the Department of Cell Biology and Tissue of the Universidad Nacional Autónoma de México UNAM (Metcalfe and Ferguson, 2007). Recently a group published what appears to be the start of assays of differentiation from stem cells for the production of insulin-producing pancreatic cells in Mexico (Vázquez-Zapién et al., 2013).

Evaluations of the clinical and sequential imaging follow-up results at a mean of 36 months after an arthroscopic technique for implantation of matrix-encapsulated autologous chondrocytes for the treatment of articular cartilage lesions on the femoral condyles have also been made (Ibarra et al., 2014). These are some of the examples that support development of this technique which always seeks to improve depending on the model established for the purposes of interest.

In conclusion, the development of new technologies is fundamental in the basic and applied cell culture research. This research tool has a growing future in the generation of new alternatives ranging from innovation

in components and constructs scaffolds up the possibility of developing the necessary methodologies for tissue engineering that will restore more natural structure and/or same functionality of organs and tissues that have been damaged. Significant efforts are made in Mexico to contribute to the development of tissue culture.Prospects in Mexico should include the development of tissue engineering and the factors that define it. The cell culture and tissue engineering should be opened with caution due to the clinical practice to offer and develop the results so far obtained in the laboratory.

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