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Mini Review

A Mini-Review on CO_2 Role in Cell Culture and Medicinal Applications

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ABSTRACT

Cell culture technique has a significant impact on medical sciences. The pH regulation in the cell culture environment is a fundamental biological phenomenon that significantly impacts cell development and metabolism. This review article focuses on the critical elements, *viz* Carbon Dioxide (CO_2), and pH effects in the mammalian culture system.

Keywords: Carbon dioxide; Cell culture; Cell development; CO_2 applications

INTRODUCTION

Cell/cellular biology or cytology is the study of cellular physiology; structure, function, and behaviour of cells; and biochemistry. It can be defined as the sub-culture or transfer of cells onto a new growing medium from a prior culture. It is used in different perspectives such as vaccine development, drug/compound screening, and others. Cell culture requirements are divided into (a) physiological parameters, such as temperature and gas mixture, and (b) growth media requirements. Under the physiological needs, three main factors are (i) appropriate temperature of about 37°C, (ii) CO_2 concentration at 5%, and (iii) humidity.

Similarly, for the growth medium, the required five significant compounds are (a) glucose, (b) amino acids, (c) vitamins, (d) ionic solutions, and (e) bicarbonate. Controlling the physicochemical (i.e., temperature, pH, osmotic pressure, O_2, and CO_2 tension) and physiological environment (i.e., hormone and nutrient concentrations) are the critical advantages for cells propagation. The growth media controls the cultural environment, apart from the temperature. Glucose and glutamine act as a source of energy. Amino acids are building blocks of protein. Vitamins promote cell survival and growth a balance of ions in a solution to maintain osmotic pressure within the cell culture medium.

Carbon dioxide (chemical symbol: CO_2 and molar wt: 44.01 g/mol) is a colourless and odourless gas. It comprises a single carbon atom bound to two oxygen atoms covalently. CO_2 is currently present in our earth's atmosphere at a quantity of roughly 0.04%. CO_2 plays an essential function in aerobic metabolism for oxidation phases in microbial and human cells. It is a crucial component of respiration and is found in aerobic bioprocesses. Microorganisms or mammalian cells are also used to store finished items such as fine compounds and therapeutic proteins. It depends on several factors, including (i) cellular activity, (ii) physical restrictions, (iii) hydrostatic pressure, (iv) aeration and (v) efficiency of the bioreactor. The basic physicochemical properties of CO_2 significantly impact cellular physiology, production processes, metabolic activity, and transcriptional regulation in microbial and human cultures. These qualities can predict the factors that acidify the internal pH and protein. The CO_2 molecule interacts with cellular metabolism, resulting in various transcriptional responses. CO_2 is the end product of respiration and is therefore unavoidable in aerobic microbial and mammalian bio-processes [1]. It presents the optimization of cell culture (Figure 1).

IMPORTANCE OF CO_2 IN CELL CULTURE

In recent days, CO_2 has played a vital role in cell-culture incubators. The significant uses of CO_2 in cell or tissue culture have the following benefits; (a) it maintains an optimum temperature, (b) it ensures the moisture-sterile environment and (c) it sustains an optimum constant physiological pH in the system. CO_2 level mainly depends on the application, whereas maintained between 3%-10%. CO_2 in cell culture aims to maintain a steady-state physiological pH through a CO_2-bicarbonate based buffer system. CO_2 from the environment can dissolve in cell culture media. A tiny amount of CO_2 will react with water to produce carbonic acid. Usually, CO_2 levels of 4%-10% in the air are routinely employed in most cell growth research. Table 1 presents the fundamental components required for the cell culture medium.

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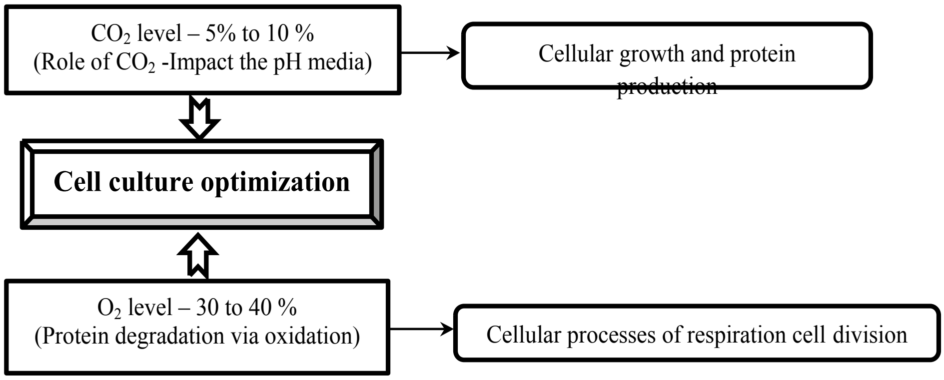

Figure 1: Cell culture optimization.

Table 1: Essential components for cell culture.

S. No	Constituents	Functions
1	Amino acids (L-glutamine)	Acts as a starting point for protein synthesis and cell proliferation.
2	Inorganic salt (Ca^{2}+, Na+, K+)	Maintains osmotic equilibrium.
3	Proteins and peptides (Albumin and Fibronectin)	Protein in the blood aids in the binding and transferring of water, ions, free fatty acids, hormones, and vitamins between tissues and cells.
4	Antibiotics (Penicillin, Streptomycin)	Substance inhibits microbes and fungal infections.
5	Vitamins (A, D, E and K)	It works as a cofactor and aids cell development.
6	Growth factors	Promoting cell differentiation and division.
7	pH indicator (Phenol red)	For continuous media monitoring.
8	Buffer solution (HEPES)	pH control is essential for optimal culture conditions.
9	Natural buffer (CO_2)	For balancing CO_2/HCO_3- concentration in the cell culture medium.

Table 2: Role of CO_2 in medical applications [4,5].

Sl. No.	Medical applications	Purpose
1	Invasive surgery- arthroscopy, endoscopy, and laparoscopy	CO_2 is used as an insufflator gas to widen and stabilize body cavities to improve the better visibility of the surgical area
		If necessary, CO_2 is used to expand blood vessels and boost CO_2 levels, followed by rapid breathing.
		CO_2 is used as a carrier gas to deliver drugs/medicine into open wounds.
2	Cryosurgery	CO_2 is used as cryotherapy or cooling agent because it is less hazardous and easy to handle
	Cryotherapy	Process of crystallization destroys the body cells
		Removal of wart, moles and skin tags
		CO_2 is used as respiratory stimulation during and after anesthesia
3	Treatment of respiratory treatment and lung system	CO_2 is used as insufflation or cooling agent for the simulation of breathing
		Carbogen=Medical CO_2 (5%–30 %) + medical O_2 (95%-70 %) used cardiopulmonary bypass surgery and the management of renal dialysis and chronic respiratory obstruction
		During over-breathing (hyperventilation) or respiratory depression, CO_2 is used at the minimum level to maintain constant breathing
4	Open heart surgery	100% CO_2 is injected into an open wound to prevent air embolism and neurological damage.
5	Tissue freezing techniques	CO_2 is in solid form (i.e., dry ice).
	Assessment of tooth sensitivity	CO_2 is used as an arterial contrast agent for various applications (i) evaluate and intervention of lower extremity arterial disease and (ii) reliable diagnosis and intervention in below-knee arteries.
	Angiography	
6	Supercritical CO_2 in medical plastic processing - assisted injection moulding	Non-toxic, non-flammable and chemically inert
		An inexpensive solvent in many polymer processing applications due to its attractive physical properties
		Medical devices currently being produced using this technology include endoscopes, heart pumps, inhalers and nebulizers
		Examined as a sterilant of bioresorbable devices

CO_2 LEVELS IN CELL CULTURE

The pH of the culture is regulated by the growth media, which shields the cells from pH variations. Typically, an organic or CO_2-bicarbonate-based buffer is used to accomplish this process. Because the pH of the medium is dependent on a delicate equilibrium of dissolved CO_2 and Bicarbonate (HCO_3–), changes in atmospheric CO_2 might impact it. Most researchers utilise a CO_2 concentration of 5%–7% in the air, and most cell culture investigations require a CO_2 concentration of 4%–10%. CO_2 is essential for cell culture metabolism and medium dissolution [2]. When a small amount of CO_2 reacts with water, carbonic acid is generated. The amount of Sodium Bicarbonate ($NaHCO_3$) in the medium impacts the CO_2 required to maintain the pH. For normal tissues, the physiological pH is thought to be 7.2 to 7.4 [3]. Provides the role of CO_2 in medical applications (Table 2) [4,5].

CO_2 is ubiquitous in our world, and measuring it has been a goal for many academics from various professions, each with its own set of constraints and objectives. CO_2 sensing is employed in multiple applications, including food storage and agri-food production, medical science, maritime and environmental studies, and analytical chemistry laboratories. CO_2 monitoring in human subjects is most critical in medical practice. Transcutaneous CO_2 sensing combined with dye-based fluorescence CO_2 sensing appears to give the most possibility for producing a future non-invasive clinical CO_2 monitor because of the latter clinical restrictions. The accurate monitoring of vital signs is critical in medical practice for providing appropriate and effective care to patients. Monitoring blood gases, specifically Dioxygen (O_2) and Carbon Dioxide (CO_2), provides respiratory and circulatory signals about a patient's condition. CO_2 monitoring techniques give a precise picture of a person's health and offer an accurate health condition state. CO_2 monitoring is vital in humans for the medical field. It is performed in three different ways: (i) (arterial) blood puncture, (ii) airway capnometry, and (iii) transcutaneous capnography [6]. In medical applications, CO_2 measurement is a well-established approach for noninvasively monitoring a patient's respiratory function that is commonly utilized in an emergency, intensive care, and anaesthesia. The multi-sensors are used to evaluate multi-parametric monitoring factors are connected to examine the patient status during intensive care units [7].

DISCUSSION

CO_2 plays a vital role in medical research because cell cultures must be controlled and monitored in the most sterile and precise manner possible. It is necessary to keep undesirable bacteria and unwanted germs out of the cultures, allowing them to flourish in optimal conditions. Henceforth, it is possible to achieve the most significant outcomes in stem cell treatment, regenerative medicine, and plastic surgery. CO_2 provides the optimum atmosphere for a variety of reasons: (i) incubating human skin cells for life-saving skin transplants; (ii) stem cell research to fight diseases; (iii) stem cell therapy as a realistic surgical alternative (iv) plastic surgery, anti-ageing therapies, tissue repair, and dermatological treatments; and (v) regenerative medicine, multiplying human skin cells for healing processes. One of the most promising areas in modern study is regenerative medicine. By replenishing defective cells, dead tissue and injured organs can be repaired. To accomplish this, the patient's skin is taken, duplicated in a CO_2 incubator, and then implanted back into the patient. This novel treatment method is revolutionary for victims of severe burns, as it avoids the considerable scarring that can occur as a result of typical transplantation operations. Progress in stem cell research is critical in the fight against diseases, particularly cancer therapy. *In vitro* generated tissues can mend or even replace damaged cells and organs *via* stem cell treatment. As a result, stem cell research is at the heart of regenerative medicine research. When handling cell cultures, maintaining sterile conditions and ensuring optimal safety are critical. As a result, an automated sterilization feature should be included in CO_2 incubator. This ensures that the risk of external contamination is minimum. In stem cell therapy, regenerative medicine has revolutionized cardiac surgery research. The Open-heart surgery is increasingly being pushed aside in favour of stem cell therapy. The procedure is as follows: tissue and cell clusters are cultivated in a CO_2 incubator before being transplanted into the patient *via* cell transplantation. These stem cells can be made in one of two ways: (a) the first one is the patient's cells are taken and multiplied in a CO_2 incubator, and (b) the second one is that the living tissue such as heart valves and blood arteries are created from scratch by using a potential CO_2 incubator. In addition to tailored autologous stem cell therapy, CO_2 incubators are crucial instruments. The own body's cells are used in various applications such as plastic surgery, anti-ageing therapies, tissue repair, and dermatological and orthopaedic treatments, among other things. The stem cells are collected and separated from the tissue after being retrieved. This increases contamination potential, so thorough sterility tests are carried out in the CO_2 incubator, where the cell cultures will eventually multiply. At the final stages, the genetic tests are carried out with maximum safety in a CO_2 incubator to ensure that the cells are as safe as possible before being implanted back into the patient. CO_2 incubator-grown skin cell transplants are used for patients with severe heat-related ailments and illnesses. In modern medicine, regenerative medicine is the best recovery method by using cell multiplication in a CO_2 incubator to cure significant heat-related injuries.

CONCLUSION

Cell culture is critical in modern research because it may simplify and comprehend the mechanism of massive complexity within an organism. CO_2 is gaining much interest in mammalian cell culture because it influences primary cell attributes such as growth rate, product quality/production rate, and intracellular pH. It acts as a natural product, critical substrate, and regulatory trigger in microbial and mammalian production. As a result, assuring the suitable CO_2 and pH usage mechanism in the cell culture system will undoubtedly lead to exploring vast concepts in the current critical study. Overall, CO_2 levels are essential for cellular metabolism, product quality, productivity rate, process performance and intracellular pH stability.

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