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Expanding Perspectives on Carbon Dioxide in Life Sciences and Clinical Applications

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ABSTRACT

Cell culture technologies are foundational to contemporary biomedical research and therapeutic development, providing controlled platforms to study cellular behavior, disease mechanisms, and drug responses. Among the physicochemical parameters governing in vitro systems, precise regulation of extracellular pH is essential for maintaining cellular viability, metabolic balance, and phenotypic stability. Carbon dioxide (CO_2) plays a central role in this process by modulating bicarbonate-based buffering systems that closely mimic physiological conditions. Beyond pH control, accumulating evidence indicates that CO_2 can directly influence cellular signaling, metabolism, gene expression, and stress responses. This review critically examines the role of CO_2 in mammalian cell culture systems, with emphasis on its interaction with buffering strategies, cellular development, and metabolic regulation. In addition, emerging medicinal and biotechnological applications of CO_2-regulated culture environments are discussed, highlighting their relevance to translational research, tissue engineering, and therapeutic manufacturing. By integrating fundamental principles with recent advances, this article provides a comprehensive perspective on CO_2 as both a regulatory and functional component of modern cell culture and biomedical applications.

Keywords

Carbon dioxide
Cell culture systems
pH regulation
Cellular metabolism
Biomedical applications

INTRODUCTION

Cell biology, also known as cytology, is the scientific discipline dedicated to understanding cellular structure, function, physiology, and biochemical behavior. A core methodological approach within this field is cell culture, which involves the maintenance and propagation of cells in vitro following their transfer from an original biological source or an established culture. Cell culture systems form the experimental backbone of modern biomedical research and are extensively applied in vaccine production, drug and compound screening, toxicology testing, molecular biology, and regenerative medicine research [1]. Their primary advantage lies in the ability to precisely control the extracellular environment, thereby enabling reproducible investigation of cellular responses under defined conditions.

The successful propagation of mammalian cells in vitro depends on the optimization of both physiological parameters and growth medium composition. Key physiological factors include temperature, atmospheric gas composition, and humidity. Mammalian cells are typically maintained at approximately 37 °C to replicate physiological body temperature, while controlled humidity minimizes evaporative loss from culture vessels. A carbon dioxide (CO_2) concentration of around 5% is routinely employed to stabilize extracellular pH and support optimal cellular metabolism [2]. Together, these parameters recreate essential aspects of the in vivo

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cellular microenvironment and are critical for sustaining cell viability, proliferation, and functional stability.

Growth media supply the biochemical components required for cellular survival and division. Essential constituents include carbohydrates such as glucose, amino acids, vitamins, inorganic salts, and buffering agents, most commonly bicarbonate. Glucose and glutamine serve as major energy substrates, while amino acids function as building blocks for protein synthesis. Vitamins support enzymatic activity and redox balance, and ionic solutions maintain osmotic pressure and membrane potential within the culture system. The composition of the growth medium, in conjunction with controlled temperature and gas exchange, governs nutrient availability, waste removal, and intracellular signaling pathways [3].

Carbon dioxide is a colorless and odorless gas with a molecular weight of 44.01 g/mol, composed of a single carbon atom covalently bonded to two oxygen atoms. Although atmospheric CO_2 represents only approximately 0.04% of the Earth's atmosphere, it plays a fundamental role in cellular metabolism and physiological regulation. In mammalian cell culture, CO_2 is primarily used to regulate pH through equilibrium with bicarbonate-based buffering systems, closely mimicking physiological extracellular conditions [4]. This buffering mechanism is essential for maintaining enzyme activity, membrane stability, and metabolic homeostasis.

In addition to pH regulation, CO_2 is intrinsically linked to aerobic metabolism and cellular respiration in both microbial and mammalian systems. It is an inevitable end product of oxidative metabolism and accumulates during cellular growth and bioprocessing. In large-scale cell culture and bio manufacturing platforms, dissolved CO_2 concentrations are influenced by cellular metabolic activity, physical constraints of the culture system, hydrostatic pressure, aeration efficiency, and bioreactor design [5]. Elevated CO_2 levels can alter intracellular pH, protein conformation, metabolic flux, and transcriptional regulation, thereby impacting cell growth and productivity.

Emerging evidence further suggests that CO_2 may function beyond a passive metabolic by-product, acting as a regulatory signal that modulates gene expression and cellular stress responses. CO_2-mediated effects on transcriptional networks and metabolic pathways have been reported in both microbial and mammalian cells, highlighting its broader biological relevance [6]. Collectively, the physicochemical properties of CO_2 exert substantial influence on cellular physiology, metabolic regulation, and bioprocess performance (Figure 1). A comprehensive understanding of these interactions is essential for optimizing cell culture systems and improving outcomes in biomedical research and therapeutic production. An overview of the role of CO_2 in optimizing cell culture conditions [7-11].

Importance of CO_2 in Cell Culture

Carbon dioxide (CO_2) is a critical component of modern cell culture systems and plays an indispensable role in maintaining optimal in vitro growth conditions. Contemporary CO_2 incubators are designed to regulate multiple environmental parameters simultaneously, including temperature, humidity,

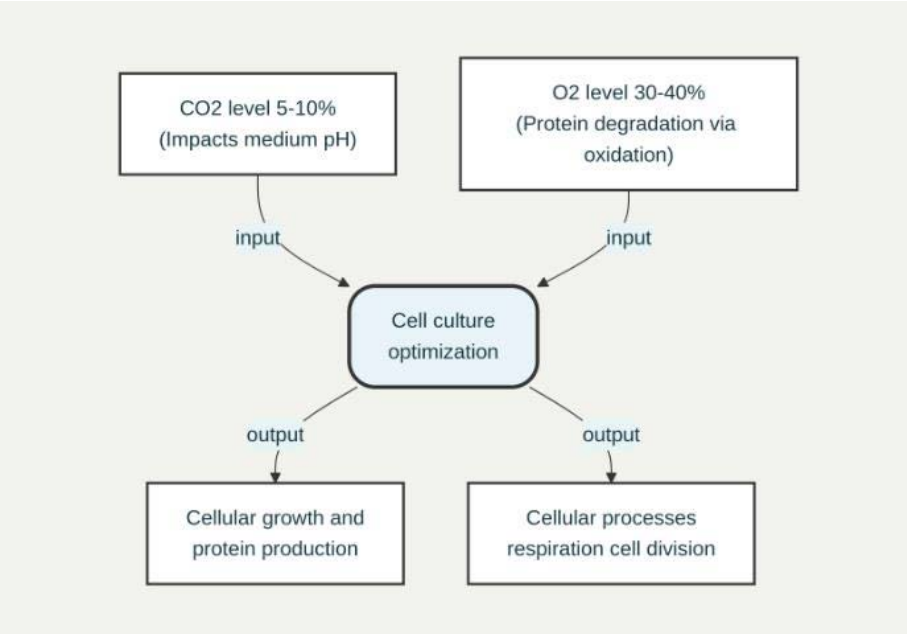

Figure 1: Cell culture optimization.

and gaseous composition, thereby closely mimicking physiological conditions. The primary functions of CO_2 in cell and tissue culture include maintenance of a stable physiological pH, preservation of a humid and sterile environment, and indirect support of optimal cellular metabolism. Depending on the specific application and cell type, CO_2 concentrations are typically maintained within a range of 3%–10%, with 5% being the most widely adopted standard for mammalian cell culture systems [12].

The principal role of CO_2 in cell culture is to sustain a steady-state physiological pH through a bicarbonate-based buffering system. CO_2 from the incubator atmosphere readily dissolves in the aqueous culture medium, where a fraction reacts with water to form carbonic acid. This reaction establishes an equilibrium between dissolved CO_2, carbonic acid, bicarbonate ions, and hydrogen ions, thereby regulating extracellular pH. Most mammalian cell lines exhibit optimal growth within a narrow pH range of 7.2–7.4, which closely resembles physiological conditions in vivo. Deviations from this range can adversely affect enzyme activity, membrane integrity, and overall cellular viability [3]. The fundamental components of a standard cell culture medium and their functions are summarized in Table 1.

CO_2 Levels and pH Regulation in Cell Culture

The pH of cell culture systems is primarily governed by the buffering capacity of the growth medium, which protects cells against abrupt fluctuations in acidity or alkalinity. Both organic buffers and CO_2–bicarbonate buffering systems are employed; however, the latter remains the most physiologically relevant and widely used approach. Because pH regulation depends on a delicate balance between dissolved CO_2 and bicarbonate ions (HCO_3^-), alterations in atmospheric CO_2 concentration can directly influence medium pH. Consequently, precise control of incubator CO_2 levels is essential for experimental reproducibility and cell health [13].

Most routine mammalian cell culture experiments utilize atmospheric CO_2 concentrations ranging from 5% to 7%, although some specialized applications require levels as low as 4% or as high as 10%. The sodium bicarbonate concentration in the culture medium determines the exact CO_2 level needed to maintain physiological pH. Media containing higher bicarbonate concentrations require correspondingly higher CO_2 levels to achieve equilibrium. This interdependence underscores the importance of matching medium formulation with incubator settings to ensure stable culture conditions [3]. The roles of CO_2 in biomedical and medical applications related to cell culture are outlined in (Table 2) [14,15].

CO_2 Monitoring and Medical Applications

Carbon dioxide is ubiquitous in both environmental and biological systems, and its accurate measurement has long been a focus across diverse scientific disciplines. CO_2 sensing

Table 1: Role of CO_2 in Mammalian Cell Culture Systems.

Parameter	Typical Range	Functional Role of CO_2	Impact on Cell Culture
CO_2 concentration	4–10% (commonly 5%)	Maintains CO_2–bicarbonate buffer equilibrium	Stabilizes physiological pH (7.2–7.4)
pH regulation	7.2–7.4	CO_2 reacts with water to form carbonic acid	Prevents pH-induced cellular stress
Bicarbonate ($NaHCO_3$)	15–44 mM	Works with CO_2 to buffer culture medium	Supports optimal enzymatic activity
Temperature interaction	37 °C	Influences CO_2 solubility and dissociation	Ensures consistent metabolic function
Gas equilibrium	CO_2/O_2 balance	Regulates dissolved gas composition	Supports aerobic respiration and growth

Table 2: Medical and Biotechnological Applications of CO_2-Controlled Cell Culture.

Application Area	Role of CO_2 Control	Clinical or Research Relevance
Stem cell research	Maintains pH and metabolic stability	Enables proliferation and differentiation
Regenerative medicine	Supports tissue growth under sterile conditions	Used in skin grafts and organ repair
Cancer research	Ensures reproducible tumor cell culture conditions	Drug screening and mechanistic studies
Vaccine production	Maintains viability of host cells	Large-scale viral propagation
Tissue engineering	Promotes scaffold-based cell growth	Development of functional tissues
Cell-based therapies	Ensures genetic and physiological stability	Safe autologous and allogeneic treatments

technologies are employed in numerous applications, including food preservation, agricultural systems, environmental monitoring, analytical chemistry, and biomedical research. In medical science, CO_2 monitoring is particularly critical, as it provides essential information regarding respiratory and circulatory function. Monitoring CO_2 levels in patients offers valuable insight into ventilation efficiency, metabolic status, and overall physiological stability [16].

Clinically, CO_2 monitoring is performed using three primary approaches: arterial blood gas analysis, airway capnometry, and transcutaneous capnography. Among these, non-invasive techniques—particularly transcutaneous and fluorescence-based CO_2 sensing—have gained increasing attention due to their potential for continuous, real-time monitoring without patient discomfort. Accurate assessment of CO_2 levels, in conjunction with oxygen measurements, enables clinicians to evaluate respiratory adequacy and detect early signs of physiological compromise [17].

In critical care, emergency medicine, and anesthesiology, CO_2 measurement is a well-established standard for monitoring respiratory function. Advanced multi-sensor platforms now integrate CO_2 sensing with additional physiological parameters, allowing comprehensive, multiparametric patient monitoring in intensive care settings. These technologies enhance clinical decision-making and contribute to improved patient outcomes by providing continuous, high-resolution data on vital physiological processes [18].

DISCUSSION

Carbon dioxide (CO_2) occupies a central position in contemporary biomedical research due to its indispensable role in maintaining tightly controlled cell culture environments. Precise regulation of CO_2 concentration is fundamental for preserving sterility, physiological pH, and optimal metabolic conditions, all of which are prerequisites for reliable in vitro experimentation. In mammalian cell culture systems, deviations in CO_2 levels can result in pH instability, impaired cellular metabolism, and compromised experimental reproducibility, underscoring the importance of continuous monitoring and environmental control [19].

The relevance of CO_2-regulated culture systems is particularly evident in stem cell research, regenerative medicine, and translational clinical applications. CO_2 incubators provide a controlled microenvironment that supports the expansion and differentiation of human cells for therapeutic use. These systems enable the cultivation of autologous and allogeneic cells under conditions that closely resemble the in vivo physiological milieu, thereby preserving cellular functionality and genetic stability. Applications include the expansion of human keratinocytes for skin grafting, generation of stem cells for disease modeling, and development of tissue constructs for reconstructive and plastic surgery [20,21].

Regenerative medicine represents one of the most promising frontiers in modern healthcare, with CO_2-regulated cell culture serving as a foundational technology. By enabling the controlled proliferation of patient-derived cells, regenerative approaches facilitate the repair or replacement of damaged tissues and organs. In the context of severe burn injuries, autologous skin cell expansion in CO_2 incubators allows for transplantation with reduced immunogenicity and minimal scarring compared with conventional grafting techniques. This strategy exemplifies how optimized CO_2 environments directly contribute to improved clinical outcomes [22].

Advances in stem cell biology further highlight the importance of CO_2 control in disease treatment, particularly in oncology and cardiovascular medicine. In vitro–generated tissues and stem cell–derived constructs are increasingly explored for repairing cardiac tissue, reducing the need for invasive surgical interventions. In such applications, cells are cultured, expanded, and functionally validated under CO_2-regulated conditions before transplantation. Both autologous approaches—using patient-derived cells—and bioengineered tissues fabricated de novo rely on stringent CO_2 control to ensure safety, viability, and therapeutic efficacy [23].

Maintaining sterility throughout cell culture workflows remains a critical concern, especially during cell isolation, expansion, and genetic testing stages. CO_2 incubators equipped with automated sterilization systems significantly reduce the risk of microbial contamination, thereby enhancing biosafety and compliance with clinical manufacturing standards. This is particularly important in personalized cell therapies, where cells undergo multiple processing steps prior to implantation. Comprehensive sterility and genetic stability assessments conducted within controlled CO_2 environments are essential to minimize clinical risks and ensure therapeutic reliability [24].

Beyond regenerative medicine, CO_2-regulated systems support a wide array of biomedical applications, including dermatological treatments, orthopedic tissue repair, and anti-aging therapies. The capacity to expand patient-specific cells under physiologically relevant conditions enables personalized treatment strategies while reducing immune rejection. Collectively, these applications demonstrate that CO_2 incubators are not merely supportive tools but essential platforms driving innovation across multiple domains of modern medicine [25].

CONCLUSION

Cell culture technologies are integral to modern biomedical research, providing critical insight into cellular behavior, disease mechanisms, and therapeutic development. Among the environmental parameters governing cell culture systems, carbon dioxide plays a particularly significant role due to its direct influence on intracellular pH regulation, metabolic activity, growth kinetics, and product quality. CO_2 functions not only as a buffering agent but also as a regulatory signal that affects gene expression and cellular homeostasis in both microbial and mammalian systems. Appropriate control of CO_2 concentration and pH is therefore essential for maintaining experimental consistency, optimizing cellular productivity, and ensuring translational relevance. As cell-based therapies and regenerative medicine continue to evolve, the importance of optimized CO_2 management will further increase. Careful integration of CO_2 regulation strategies into culture system design will facilitate advancements in tissue engineering, stem cell therapy, and precision medicine. Overall, maintaining optimal CO_2 levels is fundamental to enhancing cellular metabolism, process performance, product quality, and the long-term success of cell culture–based biomedical applications.

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