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A Comparative Analysis of Antibiotics in MSCs Cultures

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Abstract

Mesenchymal stem cells (MSCs) exhibit potential in clinical applications, particularly in tissue engineering, because of their regenerative and immunomodulatory characteristics. The use of antibiotics in the culturing of MSCs is common to avert contamination; nonetheless, their impact on MSCs survival, differentiation, and therapeutic efficacy necessitates meticulous evaluation. This study analyzed the impact of different antibiotics specifically penicillin-streptomycin, gentamicin, chloramphenicol, amphotericin B, and tetracycline on the proliferation, viability, and differentiation of MSCs in adipogenic, osteogenic, and chondrogenic lineages. Although antibiotics lessen contamination hazards, their application can compromise the functionality of MSCs, because elevated doses may cause cytotoxicity and diminish differentiation capability. The effects of antibiotic concentrations on MSCs proliferation and survival, together with molecular insights into the problems of antibiotic-induced differentiation, are thoroughly examined. Antibiotic-free cultures present a feasible alternative, promoting enhanced cell proliferation and lineage specific differentiation, however, they also introduce contamination issues. Enhancing MSCs culture methodologies may require reduced antibiotic concentrations or alternative antimicrobial agents to maintain cell integrity and sterility. This study emphasizes the significance of dosage modifications, investigates antibiotic free systems, and assesses innovative antimicrobial techniques to improve the quality of MSCs in both research and clinical applications.

Keywords: *Antibiotics, Antimicrobial Properties, Cell Viability, Mesenchymal Stem Cells, Microbial Infections, Proliferation Rates.*

Introduction

Mesenchymal stem cells (MSCs) are multipotent stromal cells found in several organs, including bone marrow, adipose tissue and the umbilical cord. MSCs possess the capability to develop into a variety of cells, such as osteoblasts, chondrocytes and adipocytes. MSCs have attracted considerable attention in therapeutic use because of their regeneration capacities, immunomodulatory

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characteristics and potential in tissue engineering, particularly in musculoskeletal reconstruction, cardiovascular disease administration and therapy [1].

MSCs research and therapeutic use critically depend on xeno free growing conditions, which reduce the potential for contamination by animal-derived components, including serum. MSCs treatments cannot be safe or effective without these conditions in clinical environments. The use of xeno-free medium, immunogenicity is lowered and cell survival and functioning. Moreover, xeno-free systems are closer to one another with regulatory criteria and are repeatable, making them a better choice for manufacturing cell-based treatments [2].

Many times, when used in culture settings, antibiotics help prevent microbial contamination, thereby affecting the survival of cells and the results of the study [3]. Nevertheless, the use of antibiotics in MSCs cells raises questions. Although they can maintain culture sterility, extended exposure may cause antibiotic resistance and affect the metabolic activity and developmental capacity of MSCs [4]. Antibiotics have to be chosen carefully so that do not compromise MSCs properties.

According to recent studies, antibiotics used in MSCs cultures affect cell growth, survival and differentiation in different ways. While certain antibiotics might have negative effects on the functional properties of MSCs, others are predicted to have notable outcomes [5]. Understanding these processes helps one ensure the greatest results for medical use and create favorable conditions inside civilizations.

Antibiotics in MSCs Cultures: An Overview

The use of antibiotics is crucial for growing MSCs because they minimize contamination [6]. The combination of penicillin and streptomycin is an attractive option among the most popular antibiotics because of its wide range of bacteria it can kill, including those with gram positive and gram-negative infection. The aminoglycoside antibiotic gentamicin is widely used in cell culture because of its potent antimicrobial and broad-spectrum anticontamination capabilities. The wide-ranging properties of chloramphenicol, which make it efficient against gram-positive bacteria, occasionally lead to its selection, although it is not as widely used. Mostly used for their antifungal properties, amphotericin B helps to keep fungal pathogens at distance while cultivating MSCs [7]. Finally, tetracycline is well-known for its antifungal and antibacterial qualities; it acts by preventing the synthesis of proteins by bacteria, thereby reducing the risk of the culture media [8]. These antibiotics combined will maintain mesenchymal stem cell cultures alive and protect them against contamination.

Mechanisms of Action and Potential Side Effects in Cells

Although they are vital for controlling bacterial infections, antibiotics may have negative effects on MSCs, which are vital for cell culture stability. Penicillin, for example, prevents the synthesis of bacterial cell membrane components, whereas streptomycin binds to the 30S ribosomal subunit, disrupting peptide synthesis. However, higher doses of these antibiotics could interfere with MSCs development and proliferation. In large doses, it may have cytotoxic effects on MSCs, thereby compromising their ability to survive and usefulness [9]. Gentamicin also binds to the 30S ribosomal subunit to inhibit bacterial protein synthesis. Additionally, linked to unfavorable effects on MSCs growth and function is chloramphenicol, which binds to the 50S ribosomal subunit to lower protein synthesis. Although effective against fungi, amphotericin B weakens the integrity of fungal cell membranes by attaching to ergosterol, possibly resulting in cellular harm in human MSCs. Finally, tetracycline binds to the 30S ribosomal subunit to prevent protein synthesis; thus, long-

term exposure may make it difficult for MSCs to develop and differentiate, thereby lowering their therapeutic effectiveness [10].

Antibiotics are necessary to prevent the contamination of MSCs cells, but they must be used properly. Determining how they work and the impact they might have on MSCs is important for ensuring that the growth conditions are ideal and that cell activity is maintained. In the not-too-distant future, researchers are encouraged to find replacements or methods for using antibiotics as little as possible, which would help MSCs cells stay healthy while still being sterile.

Impact of Antibiotic Concentrations on MSCs Proliferation and Viability

Effects of Different Antibiotic Concentrations (Figure 1 and Table 1)

Penicillin-Streptomycin

Studies have consistently shown that 100 μg/mL streptomycin and 100 U/mL penicillin are appropriate for maintaining the viability of MSCs, even if they have a detrimental effect on growth. According to flow cytometry measurements, higher concentrations (500 U/mL and 500 μg/mL) significantly reduce cell proliferation and survival, leading to an increase in cell mortality [10].

Gentamicin

Gentamicin, when administered at levels ranging from 100 to 150 μg/mL, has been shown to possess the capability of efficiently controlling bacteria while simultaneously promoting the growth of MSCs. There was a considerable reduction in cell viability at concentrations over 130 μg/mL, as shown by the outcomes of the study. It is possible that the nephrotoxic effects of the chemical played a role in this decrease in cell viability. After 48 h, those exposed to a dose of 200 μg/mL exhibited a decrease in reproduction rates of approximately 30% compared with the control groups. This was the result of a study that was carried out [11].

Chloramphenicol

In particular targeting osteogenic and adipogenic lineages, relatively modest doses of chloramphenicol (10-20 μg/mL) reduced the ability of MSCs to differentiate. Decreased colony-forming unit (CFU) tests show that greater amounts (>50 μg/mL) are cytotoxic, highlighting its negative consequences on stem cell activity [12].

Amphotericin B

Research suggests that dosages higher than 0.25 μg/mL can reduce cell viability. In an appropriately designed study, MSCs administered 0.5 μg/mL amphotericin B demonstrated a 40% decrease in cell survival compared with control cells without treatment. This emphasizes the need for an exact dosage, particularly in medicinal applications [13, 14].

Tetracycline

More intensive concentrations (≥20 μg/mL) may produce structural abnormalities and reduced levels of development, even if modest doses (1-10 μg/mL) promoted MSCs survival and growth. A previous study showed a significant decrease in colony forming capacity at dosages of 15 μg/mL [14].

Table 1. Concentration Ranges of Antibiotics on Mesenchymal Stem Cells (MSCs)

S. No	Antibiotics	Concentrations	References
1	Penicillin-Streptomycin	100 μg/mL Streptomycin, 100 U/mL Penicillin	[10]
2	Gentamicin	100-150 μg/mL	[11]
3	Chloramphenicol	10-20 μg/mL	[12]
4	Amphotericin B	≤0.25 μg/mL	[13, 14]
5	Tetracycline	1-10 μg/mL (safe), ≥15 μg/mL (toxic)	[14]

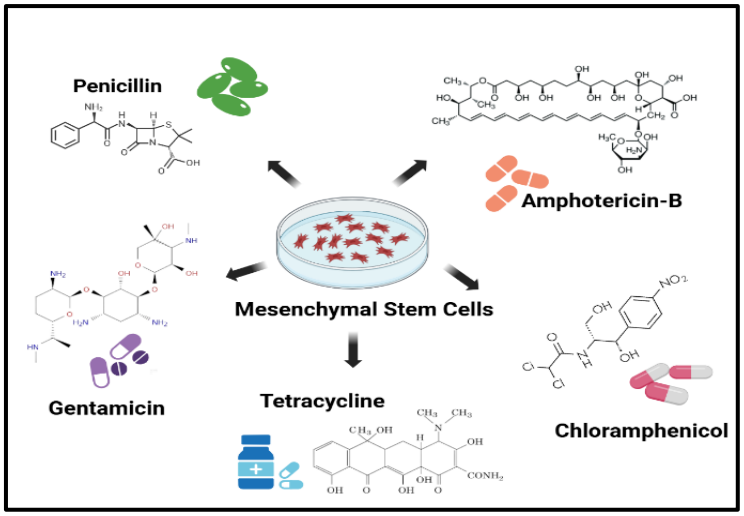

Figure 1. Different Antibiotics Effect on MSCs

Comparative Analysis of Proliferation Rates and Cell Viability

Proliferation Rates

A comparison of the results of many investigations reveals that MSCs continue to exhibit excellent growth frequencies even when subjected to reduced numbers of antibiotics. When analyzed at increased concentrations, the growth rates of MSCs treated with gentamicin at a dose of 100-150 µg/mL revealed a 25% increase [15, 17]. This pattern is observed for many antibiotics, indicating that a delicate equilibrium is required to maintain sterility while simultaneously fostering cell development.

Cell Viability

Viability tests, like MTT, Live/Dead spotting, and Annexin V assays, have shown that lower antibiotic amounts are needed to obtain the largest number of live cells. For example, if you are exposed to 100 U/mL penicillin and 100 µg/mL streptomycin, more than 90% of the people who are exposed will survive [16]. However, as the concentrations increased, the viability rate decreased to less than 70%.

Dose Response Relationships

A dose-dependent impact was observed in all investigations using MSCs cultures. The results of reducing growth and survival through both lower (inappropriate sterility) and higher (cytotoxicity) antibiotic doses frequently follow a bell shape curve. It is essential to determine the ideal antibiotic concentration, and suggestions usually adhere to existing standards (such as that of gentamicin at 100-150 µg/mL).

Antibacterial doses affect the development and survival of MSCs in a complicated interaction that requires careful modification of cell culture techniques. A recent study substantiated the idea that smaller quantities may be conducive to the preservation of both cell integrity and functionality. The subsequent study should aim at building broad guidelines and investigating alternatives to traditional antibiotics. These alternatives may include antimicrobial peptides or natural compounds, both of which can confer sterility despite the cytotoxic effects associated with antibiotics [18, 19]. Consequently, MSCs will be useful in medical applications and will enhance the pharmacological capabilities of these cells (Figure 2).

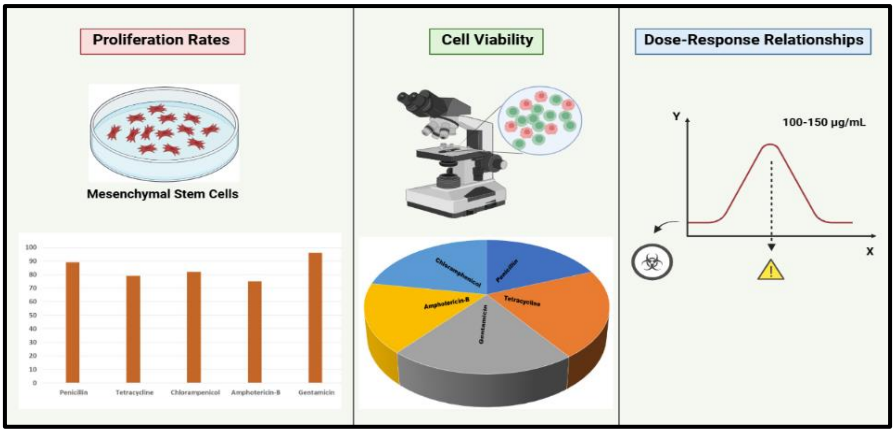

Figure 2. Comparative Analysis

The Influence of Antibiotic on MSCs Differentiation Potential

Effects of Differentiation into Various Lineages

Adipogenic Differentiation

Antibiotics such as gentamicin and chloramphenicol are known to harm the adipogenic development of MSCs. After conducting studies, repeated exposure to the medication at doses over 150 µg/mL has been shown to considerably decrease the buildup of lipids, as determined by Oil Red O staining [20, 21]. According to the previous example, chloramphenicol appears to be linked to a reduction in the production of important adipogenic indicators, such as PPAR-γ and C/EBP-α, which ultimately results in a reduction in the development of fat cells.

Osteogenic Differentiation

There are conflicting effects of antibiotics on osteogenic differentiation. Investigations have shown that the combination of penicillin and streptomycin, when administered at a lower concentration (100 U/mL and 100 µg/mL), is not associated with a harmful impact on osteogenesis. This occurs because it maintains the activity of alkaline phosphatase (ALP) and the capacity for calcification. Compared with lower doses, larger doses can prevent mineral accumulation and diminish the activity of osteogenic markers such as Runx2 and OPN (osteopontin). When administered at low concentrations, tetracycline can promote particular components of osteogenic differentiation; however, when administered at larger concentrations, it may also display adverse effects [20, 22].

Chondrogenic Differentiation

Antibiotics can influence the chondrogenic capacity of MSCs. For instance, researchers have shown that amphotericin B can hinder chondrogenic development, thereby diminishing the level of production of significant biomarkers such as Aggrecan and COL2A1 when the quantity of amphotericin B exceeds 0.25 µg/mL. Moderate concentrations of antibiotics such as penicillin-streptomycin, on the other hand, appear to preserve the chondrogenic capacity of MSCs, facilitating the synthesis of cartilage matrix [23, 24].

Mechanistic Insights and Experimental Findings

Adipogenic Differentiation Mechanisms

Antibiotics such as gentamicin are hypothesized to work by disrupting pathways of cell signaling pathways essential for the proliferation of fat cells, resulting in the inhibition of adipogenic differentiation via their use [20]. The influence associated with elevated antibiotic levels and the impairment of transcription factors like PPAR-γ may decrease the differentiation and functioning of adipocytes.

Osteogenic Differentiation Mechanisms

Antibiotics may reduce bone formation by regulating many intricate processes. To achieve this, we must first regulate the signaling pathways that are fundamental to bone formation. MSCs may undergo stress responses when exposed to high concentrations of chloramphenicol and gentamicin. These responses may lead to considerable gene expression alterations, which in turn disrupt the course of osteogenic selection [20]. Furthermore, the use of gene expression sequencing in research has resulted in the discovery that high antibiotic dosages can potentially to inhibit the Wnt/β-catenin signaling pathway, which is an essential regulator of osteogenic differentiation.

Chondrogenic Differentiation Mechanisms

It is possible that antibiotics like amphotericin B can hinder chondrogenic growth. In addition, they may trigger oxidative stress and mitochondrial dysfunction, which are detrimental to cell growth and function. In addition to increasing apoptotic signals, our experiment shows that MSCs exposed to higher concentrations of amphotericin B reduce their production of extracellular matrix components necessary for cartilage formation [22]. Adipogenic, osteogenic and chondrogenic lineages may develop from MSCs. Antibiotics can impact this possibility in various ways. Some antibiotics may help with differentiation at minimal dosages, but when used in greater quantities, they often have negative side effects, such as blocking processes and indicators that are critical for determining lineage. These mechanisms must be understood to maintain the usefulness of MSCs in regenerative medicine and cultural management [24]. Shortly after disassembly, researchers should find new approaches to preserve integrity and detect promise.

Comparative Analysis: Antibiotic-Free vs. Antibiotic-Supplemented Cultures

Yield and Quality of MSCs Prepared Under Different Culture Conditions

Compared with cultures that have not been treated with antibiotics, those that were recently exposed to antibiotics often showed superior exploratory numbers of MSCs. Bacterial contamination can be effectively defended against antibiotics, which is the reason for this. Integration of antibiotics during the first phase of culture might result in a 20-30% increase in MSCs yield. In contrast, conditions in which antibiotics are absent are very unlikely to occur [25]. When antibiotics are not present, this is in contrast to the scenario that has arisen.

This allows for increased cell proliferation. It is especially important to take into consideration this benefit when the potential for contamination is considerable. Nevertheless, although the initially observed yields could be higher in cultures that have been treated with antibiotics, antibiotic free systems are capable of achieving equivalent yields over time. It is common for MSCs grown in antibiotic free environments to have increased growth rates as the culture equilibrium [26]. This phenomenon is particularly prevalent in later stages when the lack of antibiotic-induced stress promotes healthier cell proliferation.

Cultures that use antibiotics and those that do not may produce MSCs of completely distinct purity. Although MSCs viability can be affected over time, early production may be substantially better under antibiotic treatment conditions. Prolonged antibiotic treatment changes the structure of MSCs and reduce the expression of important stemness related surface markers, including CD73 and CD90. This may significantly impede their capacity to differentiate themselves from the crowd and perform well in general [27]. On the other hand, there is a greater possibility that MSCs that are cultivated in an environment that does not contain antibiotics will retain their functional

capabilities. Certain traits include increased vitality and a stronger tendency for multilineage differentiation, notably in the adipogenic and osteogenic lineages, for example. These qualities are among the most prominent [28]. According to the findings of preclinical research, these cells have an increased sensitivity to differentiation signals, which has resulted in promising potential therapeutic applications in the future. Consequently, the insertion of antibiotics into cell cultures has the potential to hasten the early phases of their development; however, cultures that do not contain antibiotics have a propensity to retain the quality and functioning of mesenchymal stem cells for a longer, extended length of time [29].

Advantages and Disadvantages of Antibiotic-Free Cultures

One of the many advantages of antibiotic-free cultures is the promotion of stronger and more efficient MSCs development. Because antibiotics do not cause any physiological stress, MSCs can maintain their stem cell characteristics for a longer period and even multiply more efficiently [30]. When involving regenerative medicine, antibiotic-free cultures are preferable because they are equipped with the ability to produce higher levels of cell proliferation and differentiation than antibiotic-containing cultures. The lack of antibiotics in cell culture procedures has become an increasingly pressing issue. This is because bacterial strains that are resistant to antibiotics are less likely to emerge when these drugs are not available. Promoting a more ethical perspective on research and therapeutic applications helps ensure a cultural system's sustainability eventualy. Tissue engineering and transplantation-related endeavors have achieved better success rates [31].

Cultures that do not have access to antibiotics suffer substantial obstacles. There is a significant risk of contamination when there is increased susceptibility to bacterial and fungal infections. This vulnerability may harm the quality and output of cells. Because of the increased risk, it is necessary to adhere to stringent aseptic practices and exercise cautious supervision. This often involves more resources and training for workers. In addition, it is not yet known whether the lack of antibiotics leads to an extension of the lifespan of cultures; scientists may need to devote more time and effort to address contamination concerns and keep tissues sterile. Consequently, achieving the requisite cell densities in antibiotic-free systems may require more time, thereby delaying research timelines [32-34]. Moreover, the use of antibiotic-free cells may require other methods to maintain cell viability. Enhanced cultivation needs may be attained, for example by using a medium that is devoid of xenobiotic substances. To optimize the use of MSCs in research and therapeutic applications, it is crucial to strike a balance between their advantages and disadvantages.

Conclusion

Finally, the antibiotic research conducted in MSCs clarified the importance of cellular activity and sterility. Sterile cell cultures cannot be cultured without antibiotics. However, long term antibiotic therapy may change the morphology, immunophenotype and proliferation. While it is true that certain antibiotics promote cell proliferation, it is also true that they may lessen the efficacy of other antibiotics by causing cellular stress, aging or genetic instability. Immune response, cytokine production and growth factor secretion are all processes that MSCs regulate. These processes may be affected by these changes. Worries over the treatment's therapeutic safety have been heightened by the finding that long-term antibiotic use is associated with an increased risk of chromosomal abnormalities.

One of the primary objectives of future research should be to develop methods that may lessen the need for antibiotics. Many techniques, such as the implementation of

stringent aseptic procedures, investigation of additional antibacterial medications, and adjustment of culture conditions, might be included in this category. These techniques might help lower the dangers associated with antibiotic exposure. When it comes to antibiotics in culture systems, we need to have a better understanding of how precisely they modify MSCs so that we can make judgments based on accurate information. When considering the fact that antibiotics affect mesenchymal stem cells, it is feasible that the technique for selecting cultures can be improved. This paper makes the crucial necessity of MSCs treatment and research abundantly clear and describes it without sacrificing clarity. This method simultaneously reduces the toxicity of antibiotics while fostering the formation of mesenchymal stem cells. The results of this study can make MSCs based regenerative treatment more efficient and less risky.

Conflict of Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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