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3D Collagen Gels: A Promising Platform for Dendritic Cell Culture in Biomaterials Research

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Abstract: The three-dimensional (3D) cell culture system has garnered significant attention in recent years as a means of studying cell behavior and tissue development, as opposed to traditional two-dimensional cultures. These systems can induce specific cell reactions, promote specific tissue functions, and serve as valuable tools for research in tissue engineering, regenerative medicine, and drug discovery. This paper discusses current developments in the field of three-dimensional cell culture and the potential applications of 3D type 1 collagen gels to enhance the growth and maturation of dendritic cells.

Keywords: Three-dimensional cell culture; Dendritic cells; Type 1 collagen gels; Bovine tendons and rat tails

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1. Introduction

Three-dimensional (3D) cell culture systems have gained significant attention in recent years as an alternative to traditional two-dimensional (2D) cultures for studying cellular behavior and tissue development [1]. These systems aim to mimic the complex cellular microenvironment found in living tissues, providing a more physiologically relevant model for studying cell function, disease progression, and drug responses [2]. In a 3D cell culture system, cells are grown in a matrix or scaffold that allows them to occupy a three-dimensional space. This can be achieved using various techniques, including hydrogels, scaffolds made from natural or synthetic polymers, and organoids. These matrices provide physical support and structural cues to the cells, enabling them to form complex cell-to-cell interactions and tissue-like structures [3].

One of the main advantages of 3D cell culture systems is their ability to recapitulate the organization and functions of specific tissues. By allowing cells to grow in a three-dimensional manner, these systems better represent the *in vivo* cellular microenvironment, resulting in more accurate physiological responses. This is particularly important when studying diseases that involve tissue architecture, such as cancer or organ dysfunction. Another advantage of 3D cell culture systems is that they can better simulate the diffusion of nutrients, oxygen, and waste products compared to traditional 2D cultures. This enhanced nutrient exchange allows cells to maintain their viability and metabolism over a longer period, closely resembling the conditions found in living tissues.

Furthermore, 3D cell culture systems provide a platform for studying cell-cell and cell-matrix interactions. These interactions play a crucial role in various cellular processes, including cell adhesion, migration, and differentiation. By incorporating different types of cells or extracellular matrix components into the culture system, researchers can investigate how these interactions influence cell behavior and tissue formation. In addition to basic research applications, 3D cell culture systems have gained attention in the field of drug discovery and development. Traditional 2D cultures often fail to accurately predict drug efficacy or toxicity due to the lack of physiological relevance. In contrast, 3D models can better mimic the complexity of human tissues, allowing for a more reliable assessment of drug responses.

In conclusion, three-dimensional cell culture systems offer significant advantages over traditional 2D cultures in terms of physiological relevance, tissue organization, and cell-cell interactions. These systems have revolutionized the field of cell biology and provided new opportunities for studying cellular behavior, disease mechanisms, and drug development. As technology continues to advance, 3D cell culture systems will undoubtedly play a crucial role in future research and therapeutic applications [4,5].

Dendritic cells are a type of immune cell that plays a crucial role in the adaptive immune response. They are named for their unique shape, resembling the branching dendrites of a neuron. Dendritic cells are primarily found in tissues that are in contact with the external environment, such as the skin, respiratory tract, and gastrointestinal tract. Their main function is to capture antigens, which are foreign substances or molecules that can trigger an immune response. When dendritic cells encounter an antigen, they take it up through a process called antigen capture [6]. They then process the antigen and present it on their cell surface using specialized molecules called major histocompatibility complex (MHC) molecules. This process is known as antigen presentation. The presentation of antigens by dendritic cells is crucial for initiating an immune response [7]. When dendritic cells present an antigen, they also express co-stimulatory molecules that help activate other immune cells, such as T cells. This interaction with T cells is essential for the adaptive immune response to mount a specific and targeted attack against the antigen.

Dendritic cells are often referred to as “sentinels” of the immune system because they are constantly scanning their surroundings for foreign invaders. They have specialized receptors that recognize pathogen-associated molecular patterns (PAMPs), which are specific molecules found on the surface of pathogens like bacteria or viruses. When these receptors detect PAMPs, they trigger an immune response against the invading pathogen. In addition to their role in antigen presentation, dendritic cells also secrete cytokines, which are small signaling molecules that help regulate the immune response. These cytokines can have different effects on other immune cells, such as promoting inflammation or modulating the activity of specific cell types. Overall, dendritic cells are crucial players in the immune system’s ability to detect and respond to foreign invaders. Their ability to capture, process, and present antigens, as well as their role in activating other immune cells, makes them essential for initiating and coordinating an effective immune response [7,8].

Type 1 collagen is a widely used substrate in cell culture due to its structural and functional properties. It is the most abundant protein in the extracellular matrix of connective tissues, providing essential support and flexibility to cells. Using type 1 collagen as a cell culture substrate offers several benefits. Firstly, it mimics the physiological conditions *in vivo*, providing cells with a more natural environment for growth and interaction. This helps in maintaining cell morphology and function. Collagen also possesses excellent biocompatibility and promotes cell adhesion, migration, and proliferation. It provides a three-dimensional scaffold for cells to attach and spread, aiding in the formation of multicellular structures and tissue-like organization. This promotes cell-cell interactions and enhances cellular responses to growth factors, cytokines, and other signaling molecules [9]. Another advantage of type 1 collagen in cell culture is its ability to support the differentiation of various cell types, including fibroblasts, epithelial cells, endothelial cells, and stem cells. Collagen substrates

can induce specific cellular responses and promote tissue-specific functions, making them valuable tools in regenerative medicine, tissue engineering, and drug discovery research. Furthermore, type 1 collagen can be modified and tailored to meet specific experimental requirements [10]. It can be crosslinked to improve stability and mechanical properties, or functionalized with bioactive molecules, such as peptides or growth factors, to enhance cellular responses and specific tissue formation.

When using type 1 collagen for cell culture, it is important to ensure its purity and quality. Collagen should be sourced from reliable suppliers and should undergo rigorous testing to ensure its absence of contaminants. Additionally, proper handling and storage conditions are crucial to maintain its structural integrity and biological activity [11]. In conclusion, type 1 collagen is a valuable substrate for cell culture due to its physiological relevance, promoting cell adhesion, migration, proliferation, and differentiation. Its versatility and ability to mimic the extracellular matrix make it an indispensable tool for various cellular and tissue engineering applications [12].

2. Dendritic cell culture in biomaterials

Dendritic cells (DCs) are of increasing scientific and clinical interest due to their important role in cancer host responses, their potential use as biological adjuvants in tumor vaccines, and their involvement in the immunobiology of tolerance and autoimmunity. Biological materials are preferred for *ex vivo* or *in vivo* delivery of antigens because they are slightly immunogenic and stimulate DC activation. Biomaterial carriers are used for antigen delivery as they extend the lifetime of intact antigens, increase the uptake and processing capacity of DCs, and improve the controlled delivery of antigens [13]. DCs used in immunotherapy protocols need to mature maximally and stably to promote T cell activation *in vivo*. Therefore, there is increasing interest in maturing human blood-derived DCs through a biologically applicable approach using type I collagen. The finding that culture on type I collagen induces DC maturation has been reported in both mouse and human systems. Mature dendritic cells have a characteristic cell shape, numerous processes (veils, dendrites), and are mobile [14].

In vivo, DCs are in close proximity to extracellular matrix (ECM) proteins. ECM proteins influence DC morphology and function [15]. Peptide-based biomaterial scaffolds, such as cell-seeded collagen, also hold promise for tissue repair and other medical applications. The 3D collagen lattice is a suitable environment for dendritic cell culture and migration [16]. Within the 3D collagen matrix, DCs behave like highly mobile cells, and their migratory properties are strongly influenced by their origin, maturation state, and the structure of the surrounding collagen matrix [17].

Analysis of cell behavior in various natural and synthetic three-dimensional contexts will help improve the study of cell-environment interactions and subsequent materials design. Collagen preparations are occasionally used in tissue culture, usually for the immediate purpose of growing particularly fastidious cells. This is primarily due to the role collagen plays in the maturation of cells in a three-dimensional environment. This microenvironment is specifically engineered to support T cell activation, localize T cells to relevant tissue sites (e.g., tumor biopsy sites), and create a “reservoir” of chemokines/cytokines that support T cell activation. The ability to design such a microenvironment would be an attractive feature of this approach [18].

The specific structure of secondary lymphoid organs is thought to make the immune response highly efficient, providing a defined space and auxiliary signals for new lymphoid tissue formation. Scaffolds could support simple injection-based vaccination strategies, superior to purified disease-specific DCs. Collagen provides a suitable environment for cell culture maturation because the fiber distribution and biophysical structure of the collagen lattice closely resemble the reticular stroma of interstitial soft tissue, dermis, and lymph nodes [19].

3. 3D culture systems and cell migration and maturation studies

Cell migration is a fundamental function of normal cellular processes, including cell proliferation and migration. Cell behavior *in vitro* is typically examined in a two-dimensional environment; however, in the body, cells exist in a three-dimensional extracellular matrix environment rich in type I collagen. In fact, cells cultured in 3D matrices better reflect *in vivo* cell physiology compared to traditional 2D systems. Culturing immature dendritic cells (iDCs) with polylactic-co-glycolic acid (PLGA) microparticles (MP) or film resulted in morphology similar to that of LPS-matured DCs and the association, or possible internalization, of PLGA MPs. Furthermore, biomaterial-treated iDCs demonstrated an increase in MHC class II and costimulatory molecule expression compared to iDCs, but to a lower level than that of LPS-matured DCs [20].

Granulocyte-macrophage colony-stimulating factor and IL-4-treated human monocytes were used as precursor cells to investigate the interaction of DCs at different maturation stages with extracellular matrix proteins like fibronectin, collagen type I, and collagen type IV. The binding of monocytes to collagen type I was less strong but induced the maturation of the precursor cells. These results indicate that proteins of the extracellular matrix play an important role in the development and function of human DCs [18].

Lymphocytes did not adhere to or migrate to 2D substrates such as glass coated with serum or fibronectin. They attached to the hydrated 3D collagen lattice and migrated within the lattice. When the collagen was dehydrated to form a two-dimensional surface, lymphocyte adhesion to the collagen was reduced [21].

4. Bio fabrication of 3D collagen gels

Collagen extraction was performed in two batches, yielding 8 grams of collagen from 60 grams of tendons in both batches. In batch II, the tendons were divided into two sets of 30 grams each, and the extraction protocol was followed. Collagen was not obtained from set 1 of batch II. **Figure 1** shows the extracted Type 1 collagen from bovine tendons. Type I collagen is the most abundant collagen type found in bovine tendons, known for its high tensile strength and role in structural support. Type I collagen extracted from bovine tendons is used in various applications, including biomedical devices (e.g., wound dressings, tissue engineering scaffolds), cosmetic products, food supplements, and research purposes (e.g., studying cell-matrix interactions). **Figure 2** shows the Type 1 collagen from rat tail. Extracting Type I collagen from rat tails follows a similar process to that of bovine tendons, but with some adjustments due to the smaller size and different tissue characteristics of rat tails.

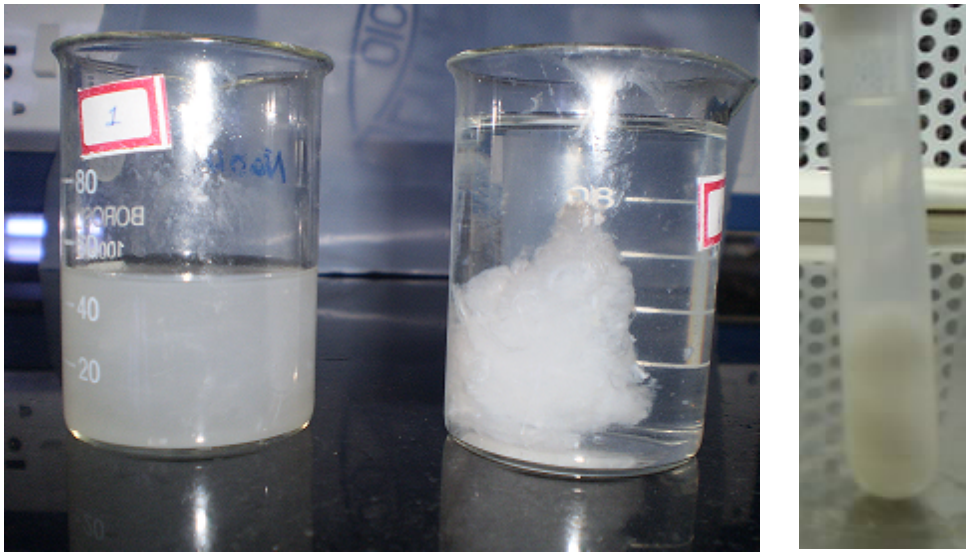

Figure 1. Bovine tendon collagen extraction

Figure 2. The pellet of collagen obtained from rat tail

The 3D collagen gel consists of type 1 collagen, which forms a delicate gel with a difficult-to-control pH and does not adhere to the walls of the well plate. Adding agarose to collagen increases the gelation time. Good gelation occurred with a gel consisting of 1% agarose. A gel containing 0.5% agarose was also gelled. Collagen solution in acetic acid with 0.5% agarose in different proportions (2:1, 1:1) was tested with rat tail and bovine tendon collagen, resulting in gelation with a pH around 3-4. The gel and medium were kept overnight in a CO_2 incubator, causing the pH to change.

A solution of collagen in acetic acid with 0.5% agarose and 0.1 N NaOH showed that the pH rose to 5, but the collagen started to precipitate (since 0.1 N NaOH was used, the same volume as acetic acid was required to neutralize the acid). A solution of collagen in acetic acid with 0.5% agarose and 1 N NaOH, when the pH rose to 5, also caused collagen to begin precipitating. Using exactly the amount of NaOH needed to neutralize 1 ml of acetic acid, light precipitation was observed, but the gel remained intact with a pH of around 7. A solution of collagen in acetic acid with 0.5% agarose, which was allowed to dry for approximately 5 hours and thoroughly washed with PBS, provided a pH value of 7 for the medium added to this gel. The gel was viewed under an inverted microscope, revealing the porous nature of the scaffold (**Figure 3**).

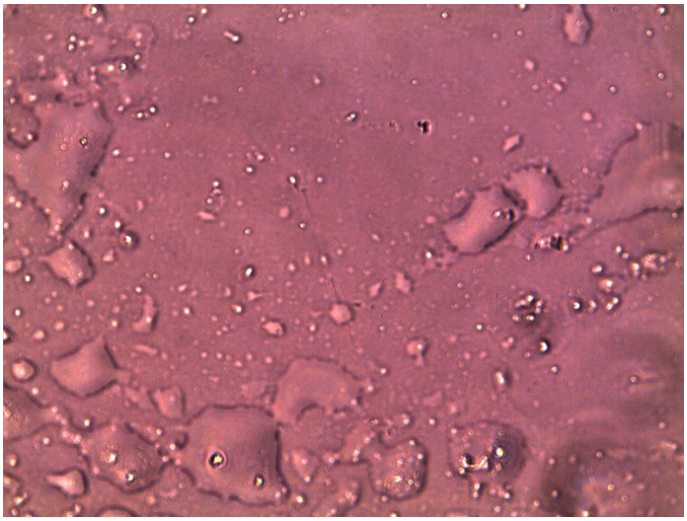

Figure 3. Porous collagen scaffold observed under the inverted microscope (40×)

5. Dendritic cell isolation and culture

From the peripheral blood, lymphocytes and monocytes (buffy coat) were separated (**Figure 4**). The monocytes from peripheral blood were cultured in RPMI medium. After a period of 8 days, the cell culture was observed under an inverted microscope, revealing the characteristic processes present on the surface of the dendritic cells (**Figure 5**).

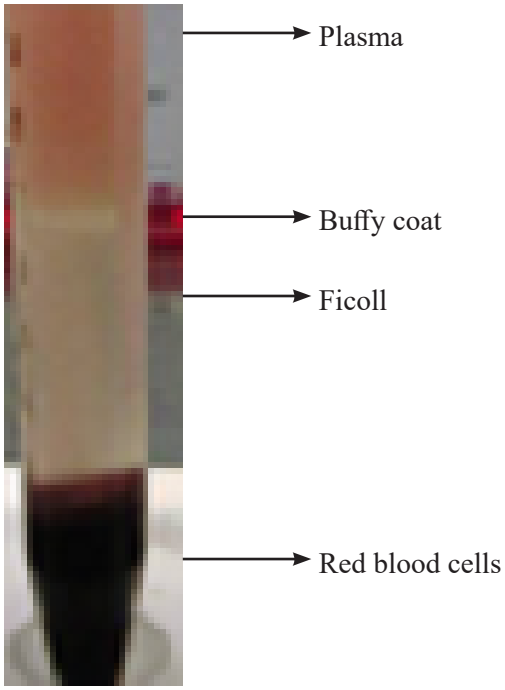

Figure 4. Buffy coat (second layer) isolated from PBMC

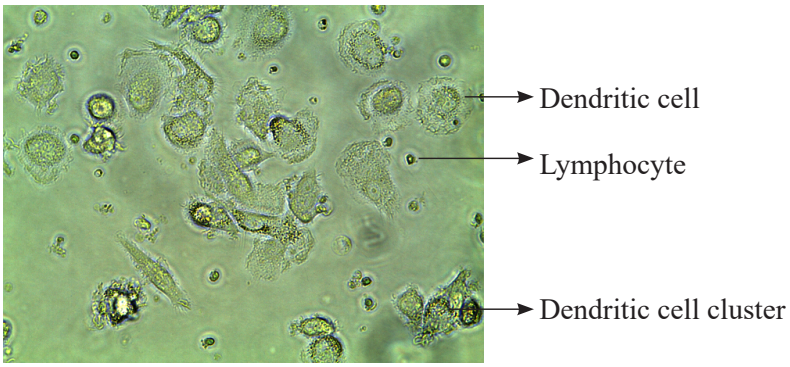

Figure 5. Observation of dendritic cells cultured without collagen after 8 days under the inverted microscope (40×)

6. Seeding of dendritic cells in the collagen scaffold and its maturation study

Monocytes from peripheral blood were observed after 24 hours of culture in the RPMI medium. The figure shows the immature dendritic cells (**Figure 6**). These cells were then injected into the collagen scaffold (various percentages of gel) and were observed after 7 days by removing the gel and placing it on a glass slide (**Figure 7**). The images of the collagen gel were observed under 40× magnification using an inverted microscope (**Figure 8**).

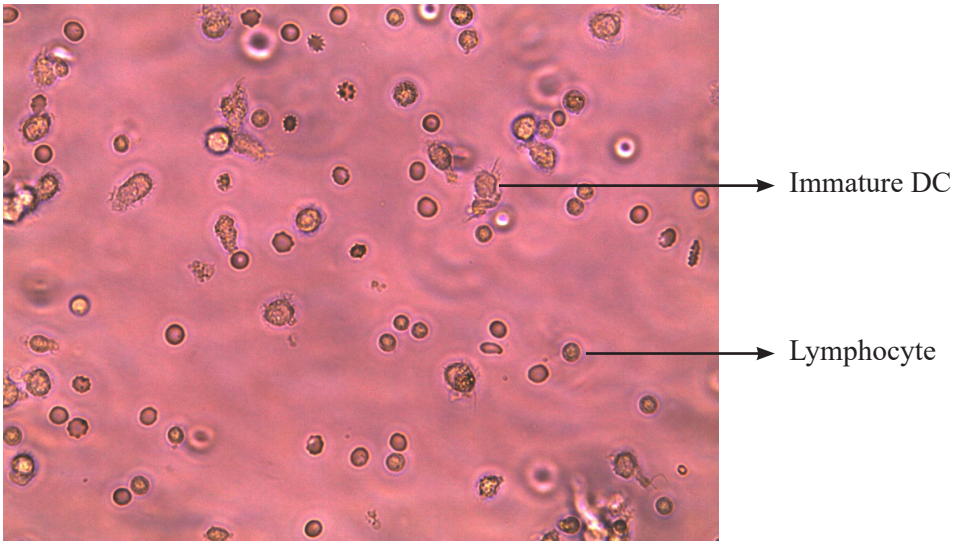

Figure 6. Observation of dendritic cells after 24 hours under the inverted microscope (40×)

Figure 7. The scaffold gel on a glass slide

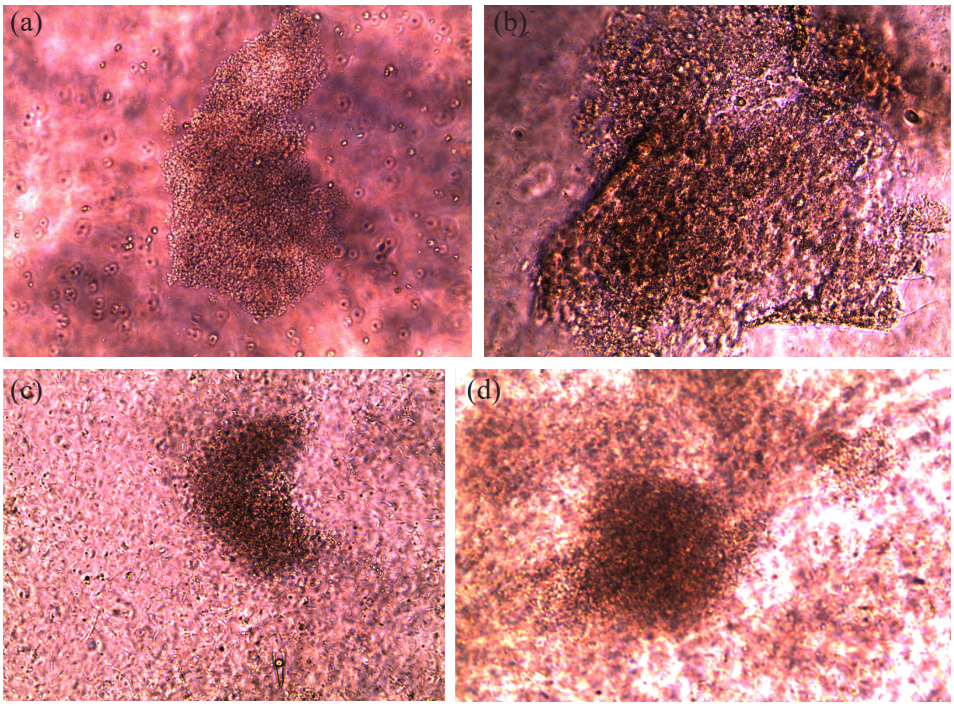

Figure 8. (a) Growth of dendritic cells into tissue in the 3D collagen scaffold 10% gel (rat tail collagen); **(b)** Growth of dendritic cells into tissue in the 3D collagen scaffold 15% gel (rat tail collagen); **(c)** Growth of dendritic cells into tissue in the 3D collagen scaffold 10% gel(bovine tendon collagen); **(d)** Growth of dendritic cells into tissue in the 3D collagen scaffold 15% gel (bovine tendon collagen)

7. Discussion

Type 1 collagen is extracted from bovine tendon, an inexpensive source, and from rat tail tendons, where collagen exists in pure form. Recent advances in medical technology have enabled the development of 3D DC culture systems that simulate the *in vivo* environment. Hydrated collagen gels are used in 3D cell culture systems because the structure of the collagen network resembles that of several tissues, including the dermis and the reticular stroma of lymph nodes [22,23].

In this study, a collagen-agarose scaffold was prepared and used for DC culture. Dendritic cell populations were isolated from monocytes in peripheral blood and seeded into the collagen-agarose scaffold. The results were consistent with previous findings [19]. DC maturation is a topic of current interest due to the wide array of potential applications for these cells in augmenting immune responses *in vivo*. Collagen, a ubiquitous and important component of the extracellular matrix, could mediate such maturation [24].

Dendritic cell maturation was studied in the presence and absence of collagen. In the absence of collagen, individual cells developed visible dendrites after 6–7 days of culture, and clusters of dead cells were seen after 10 days. Culturing follicular dendritic cells in a 3D collagen matrix allows physiological maturation to occur [17]. Exposure to type I collagen can promote the maturation of immature DCs [18]. Culturing peripheral blood mononuclear cells (PBMCs) with GM-CSF and agarose or polylactic acid results in the detachment of cells from the culture dish and the formation of cell clumps, characteristic of DC development. DCs cultured on agarose membranes showed visible cell detachment and cluster formation, possibly due to the hydrophilic nature of the agarose membrane [25].

The presence of collagen in the study led to tissue maturation and aggregation in collagen-agarose gels made from bovine and rat tail tendons. The triple helical nature of collagen and the three-dimensional structure of the scaffold promoted the growth of DCs in tissues. Without completely depleting all LPS or collagen in solution, a collagen-mediated effect during DC maturation cannot be ruled out [24]. Consistent with previous findings, this study observed that collagen supported DC maturation.

8. Biomedical application of dendritic cells

Dendritic cells (DCs) are a type of white blood cell that play a crucial role in the human body's immune system. DCs have many functions, including the ability to recognize pathogens, activate other immune cells, and present antigens to other immune cells. In recent years, they have become an important tool in biomedical research and have been used to treat a variety of diseases, including cancer, HIV/AIDS, and autoimmune disorders. In cancer therapy, DCs have been employed to stimulate the body's natural immune response to attack tumor cells. In HIV/AIDS therapy, DCs have been utilized to reduce the viral load and improve patients' quality of life. In autoimmune diseases, DCs have been used to reduce inflammation and modify the immune response.

DCs have also been instrumental in the development of vaccines. By introducing a specific antigen to DCs, they can induce an immune response to that particular antigen, leading to the development of vaccines for diseases such as influenza, hepatitis B, and HPV. Additionally, DCs have been used to deliver gene therapy. By introducing a gene into DCs, they can deliver it to other cells in the body. This approach has been applied to treat genetic diseases such as cystic fibrosis and to deliver gene therapies for cancer. Lastly, DCs have been used in regenerative medicine. By introducing stem cells into DCs, they can stimulate the growth of new cells and tissues, which have been used to regenerate damaged tissues, such as heart muscle after a heart attack [26].

Dendritic cells are highly specialized immune cells known for their unique ability to capture, process, and present antigens to other immune cells, thereby initiating and shaping immune responses. Due to these

remarkable properties, dendritic cells have been extensively studied for their biomedical applications. Notable applications include:

(1) Cancer immunotherapy: Dendritic cell-based immunotherapies have shown promise in cancer treatment. DCs can be isolated from a patient's blood or generated in the laboratory, loaded with tumor-specific antigens, and re-introduced into the patient's body. These antigen-presenting dendritic cells help stimulate the patient's immune system to recognize and attack cancer cells more effectively.

(2) Vaccines: Dendritic cells play a key role in vaccine development. They can be loaded with specific antigens, such as viral or bacterial antigens, to initiate a targeted immune response. By presenting these antigens to T cells, dendritic cells help activate and educate the immune system, leading to the production of pathogen-specific antibodies and memory T cells for long-term protection against infectious diseases.

(3) Autoimmune diseases: Dendritic cells can be manipulated to regulate immune responses in autoimmune diseases. Through various strategies, such as targeting specific antigens or modifying their maturation and activation status, dendritic cells can be used to induce immune tolerance, preventing the immune system from attacking self-tissues in conditions like rheumatoid arthritis, multiple sclerosis, and type 1 diabetes.

(4) Allergies and asthma: Dendritic cells also play a role in allergic reactions and asthma. By modulating dendritic cell function, researchers aim to develop therapies that can reduce allergic responses and promote immune tolerance to allergens, potentially offering relief to individuals suffering from these conditions.

(5) Transplantation: Dendritic cell-based approaches are being explored to improve the success of organ and tissue transplantation. By manipulating dendritic cells, scientists aim to promote immune tolerance towards transplanted organs, reducing the need for lifelong immunosuppressive drugs and improving long-term graft survival.

(6) Infectious diseases: Dendritic cells are key players in the immune response against pathogens. Research is ongoing to better understand the interactions between dendritic cells and various infectious agents, such as HIV, tuberculosis, and malaria. This knowledge can help in developing novel approaches to enhance immune responses and combat infectious diseases.

9. Conclusion

The extraction of type 1 collagen from bovine tendons and rat tails is more cost-effective compared to commercially available collagen from Recombinant DNA Technology and other 3D cell culture systems. This collagen can be used as a three-dimensional scaffold for cell culture systems, as culturing dendritic cells on this scaffold resulted in mature tissue aggregates of dendritic cells. The phenotypic changes and antigen-presenting abilities of these mature dendritic cells were investigated. These 3D collagen gels can serve as a platform for studying cell microenvironments and conducting various cancer immunology research.

Disclosure statement

The author declare no conflict of interest.

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