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The use of alginate in the cultivation of mesenchymal cells in biomedical engineering

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Abstract: Stem cells are a specialized type of cells in a human or animal organism that have the ability to self-renew and differentiate into various types of cells in the body. They have great potential in medical research and therapies as they can be used to treat a variety of diseases, regenerate tissues and develop new therapeutic approaches. In the article, we describe the cultivation of stem cells with alginate, which is used in 3D cultivation. The biopolymer alginate is preferred in biomedical applications due to its excellent properties. Polysaccharide alginate is isolated from marine, brown algae and bacterial cultures of species, *Azotobacter* and *Pseudomonas* species. Alginate is soluble in water, which allows easy processing and application in the form of various hydrogels or matrices. We present the preparation of alginate gel in the form of beads in combination with multiplied stem cells under in vitro conditions. At the same time, we observed how a natural polymer, specifically alginate, which is used for cultivation, behaves towards cells and how it affects their proliferation and differentiation.

1 Introduction

A characteristic feature of stem cells is the undifferentiated state of cells with the ability to differentiate into any cell type of the human organism and at the same time the ability to self-renew. They are found in embryos, but also in the cells of adult individuals. The division of stem cells is not limited, which allows them to replace other cells in the body again. During the division of each stem cell, there is a possibility that it differentiates into a somatic cell with a specific function, for example, into muscle or nerve cells, or it can remain in the form of a stem cell [1]. Thanks to their properties, stem cells offer enormous potential in the field of regenerative medicine and the therapy of various disorders.

Polysaccharide alginate is isolated from marine, brown algae and bacterial cultures of species, *Azotobacter* species and *Pseudomonas* species. In the case of bacteria, alginate forms a protective capsule, supports the formation of a biofilm and helps with adherence and colonization. Due to its ability to form hydrogels, alginate is widely used as a stabilizing and thickening agent and emulsifier. Alginate is, after cellulose, the most widespread biopolymer available worldwide [2].

1.1 Alginate

The extraction of alginate from natural, plant sources involves several processes. In the first phase, a mineral acid acts, which changes the salts of alginic acid (called alginates) contained in algae into free alginic acid. This step is followed by a process of neutralization with sodium bicarbonate or sodium hydroxide, whereby soluble sodium alginate is formed. The purification of this soluble alginate is carried out with either calcium chloride or mineral acid and the product is insoluble fibers of calcium alginate and

a gel form of alginic acid. The mentioned substances are subsequently mixed with sodium bicarbonate to form sodium alginate.

Sodium alginate is a substance that is the most desired form of alginate salt prepared industrially. In addition to the mentioned form, calcium alginate, potassium alginate and ammonium alginate are industrially prepared [4].

Calcium alginate is isolated by a similar procedure. Potassium alginate and ammonium alginate are made by adding the appropriate hydroxide, usually potassium carbonate or ammonium hydroxide, to the alginic acid gel [5].

1.2 Structure of alginate

Alginates are composed of 1,4-linked β-D-mannuronic acid (M) and 1,4 α-L guluronic acid (G) residues that form blocks of repeating G residues, repeating M residues, and alternating G residues and M (Figure 1). The composition and sequence of G and M residues depends on the type of natural sources used for alginate extraction. It has been proposed that only the carboxylate groups of G residues form conductive bonds with divalent cations such as Ca^{2+}, Mg^{2+} to form hydrogels. Alginates with high G content form tough hydrogels hydrogels, while alginates with high M content form softer elastic hydrogels. The approximate molecular weight of alginate is ranges from 32,000 to 400,000 g/mol. As the molecular weight of alginate increases, its viscosity increases during the preparation of the gel. Although alginate is usually considered a biocompatible, non-toxic and non-immunogenic material, there are opinions that alginate with a high content of the mannuronic (M) component may be more immunogenic compared to alginate with a high content of the guluronic (G) component [2].

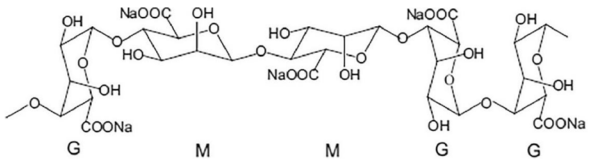

Figure 1 Chemical structure of alginate composed of 1,4-linked residues of β-d-mannuronic acid M and 1,4 α-l-guluronic acid G [2]

1.3 Properties of alginate

The biopolymer alginate is preferred in biomedical applications due to its excellent properties. Its main properties include the ability to form gel structures in the presence of divalent cations, especially calcium (Ca^{2+}).

In addition, alginate is known for its low toxicity to human cells and organisms, making it a safe material for use in biomedicine. Alginate is soluble in water, which allows easy processing and application in the form of various hydrogels or matrices. Its chemical composition contains acid and hydroxyl groups, which allows various chemical modifications, such as cross-linking with divalent cations or modification of properties for specific applications in biomedicine. Combined with these properties, alginate is a useful material for various applications in biomedicine, such as drug carriers, biological matrices for tissue engineering, or the basis for specific types of bioactive materials [3].

To improve the physical characteristics of alginate, other substances are often added, resulting in alginate composites. Other substances are often added to improve the physical properties of alginate, resulting in alginate composites. Natural polymers, collagen, chitosan and gelatin, as well as synthetic polymers, polylactides and polypyrrole, and inorganic compounds, tetraethylorthosilicate (TEOS) and hydroxyapatite (HA) are most often used as additives for alginate composites. The mixture was also tested with other types of materials such as ceramics, bioglass, inorganic nanoparticles and inorganic carbon materials.

Multiple alginate composites are currently used for clinical application in the form of gel wound treatment. For the use of alginate composites in various biomedical applications, they are molded into fibers, beads, hydrogels or 3D-printed materials for the specific requirements of each biomedical application [6].

1.4 The role of alginate in biomedical engineering

Alginate is characterized by its unique properties advantageous in biomedical applications, but it also has certain disadvantages. Mutual interactions between monovalent cations and alginate blocks cause instability of the gels. Unbound polymer blocks are not naturally degraded by enzymatic processes in humans. Although the resulting gel material dissolves in the organism, the alginate molecules themselves are difficult to completely eliminate, because the average molecular weight of commercially available alginates exceeds the renal clearance limit of the kidneys. However, alginate as a biopolymer of natural origin, which exhibits many unique properties and is more suitable for tissue engineering, has several significant disadvantages [7].

However, alginate is characteristically non-degradable for mammals, because there is no enzyme (alginase) that could break down the polymer chain of alginate. Partially oxidized alginate can degrade in water, which makes alginate a promising material for drug and cell delivery in various applications. Sodium periodate is used to oxidize alginate, which can break the carbon-carbon bonds in the *cis*-diol group of uronate. This process changes the conformational structure of alginate to an open chain and promotes the decomposition of the main structure of alginate [3].

Another disadvantage of alginate is its weaker ability to allow cell adhesion, resulting in less cell interaction and adhesion with both the 3D and 2D surroundings.

However, alginate hydrogels can support minimal protein adsorption due to its hydrophilic property. Therefore, alginate hydrogels alone are unable to support

cell migration and adhesion but can be refined on a 2D surface or inside a 3D hydrogel, where they form multicellular bundles. To make alginate a suitable material for tissue engineering applications, it is necessary to increase its degradation rate through chemical modifications such as gamma irradiation or partial oxidation and by adding cell-bound materials such as RGD-containing proteins or peptides. These materials can be conjugated with alginate to promote cell adhesion. The chemical structure of RGD is shown in Figure 2 [3].

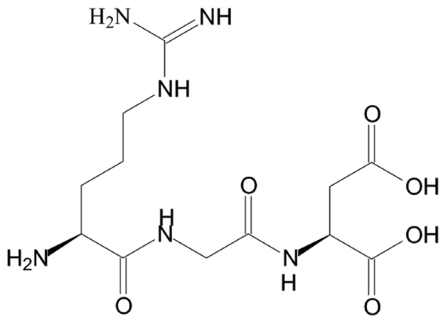

Figure 2 Chemical structure of RGD [3]

2 Methodology

2.1 Isolation of stem cells

The process of isolating stem cells from the amnion-chorion membrane begins with obtaining these fetal membranes after a planned section at the Gynecology and Obstetrics Clinic of the Agel Košice-Šaca Hospital from healthy pregnant women with their prior consent. This material is then transferred to the sterile laboratory conditions of KBIaM, where it is further processed in a laminar box to maintain its sterility.

2.2 3D cell culture in alginate

The process of 3D culture of stem cells in alginate starts with trypsinization of chorionic mesenchymal stem cells (CMSCs) when they are at the stage of approximately 80% formation of the monolayer of adherent cell layer. At the beginning, the cultivation bottle T75 is prepared. Subsequently, we added 4.0 ml of 0.25% trypsin EDTA to each bottle, which helps to disrupt intercellular junctions and release adhered cells from the surface. Trypsinization was observed under a microscope to assess its effectiveness and was allowed to act for another 3 minutes. After the completion of trypsinization and inactivation of trypsin by adding a 20% solution of bovine serum in DMEM, we prepared the cells for 3D cultivation. First, we prepared a 1% solution of alginate in physiological solution, which we sterilized by filtration. To create gelling spheroids, the so-called "alginate beads", we used a solution of 0.1 M $CaCl_2$. CMSCs cells were resuspended in a 1% alginate solution at a concentration of 1 million cells/ml and, using a 2 ml pipette, we applied the solution drop by drop into the $CaCl_2$ solution. In contact with $CaCl_2$, rapid polymerization of alginate occured, while the cells were trapped inside the spheroids. $CaCl_2$ solution was aspirated from the polymerized spheroids and they were washed with the medium without additives. To ensure optimal conditions for the cells, we placed the spheroids in a complex culture medium (e.g. alpha MEM / DMEM with 10% FBS and 1% antibiotics/antimycotics) (Figure 3) and incubated at 37 °C and in an atmosphere with 5% CO_2. We regularly changed the culture medium every 2-3 days, which ensured a constant supply of nutrients and removal of metabolic waste. We monitored the overall condition and development of the cell culture in a 3D environment regularly under a microscope to monitor the morphology and health of the cells.

Figure 3 Change of the culture medium

3 Results

Isolation and cultivation of stem cells: After the successful isolation of stem cells, their cultivation followed. This process was aimed at monitoring cell proliferiation growth in a controlled environment. During the first 2-3 days after isolation, the culture medium was regularly checked and changed when necessary, which helped to eliminate non-adherent erythrocytes and other unwanted cells. This care allowed the cells to adapt to the new environment. The culture medium was systematically changed twice a week, which ensured optimal conditions for the growth and development of the culture. This approach not only promoted cell growth, but also minimized the risk of contamination. After two weeks of intensive care and monitoring, we were able to see the formation of a monolayer culture of adhered cells. This culture was characterized by its purity, as it did not contain any hematopoietic cells, indicating a high efficiency of the isolation and culture process. The stem cells showed a healthy morphology and a consistent proliferation rate, indicating their good adaptation to the culture environment (Figure 4). Thanks to regular checks and maintenance of

the medium, we have recorded minimal to no contamination, which additionally confirms the effectiveness of our sterilization and cultivation techniques.

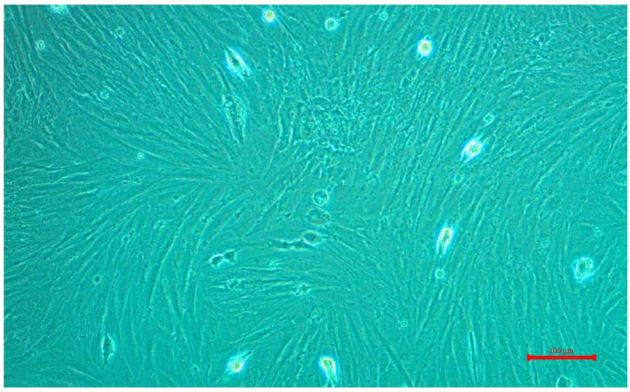

Figure 4 Stem cells: day 6 passage (magnification under the microscope 10x)

3D cell culture in alginate: During our 14-day 3D cultivation of stem cells in alginate, we regularly changed the culture medium every 2-3 days, thereby ensuring optimal conditions for cell growth and development. Systematic monitoring of the cell culture under the microscope allowed us to observe their proliferation, morphology and overall health in a three-dimensional environment. The results of our observation showed that the cells in the alginate matrix maintained their natural morphology and showed significant proliferation. These results confirmed that the 3D culture environment provides conditions that effectively mimic the natural cellular environment, which is critical for studies related to cell interaction and differentiation (Figure 5).

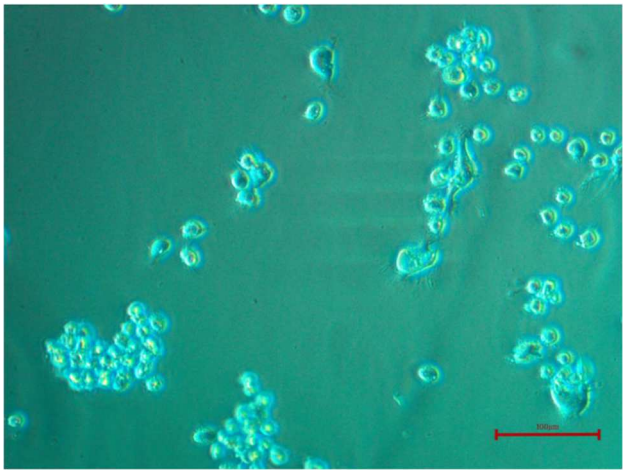

Figure 5 Stem cells: passage day 6 in alginate (magnification under the microscope 20x)

4 Conclusion

This work dealt in detail with the study of 3D cultivation of stem cells using a natural polymer, specifically alginate. In the theoretical part, the basic characteristics and possibilities of using alginate in biomedical engineering were discussed. The practical part of the work demonstrated the application of theoretical knowledge in the cultivation of stem cells from the amnion-chorion membrane in an alginate matrix.

Experiments have confirmed that alginate provides an effective environment for the growth and differentiation of stem cells, which supports their use in biomedical engineering. The overall findings of this work point to the great potential of natural polymers in biomedical applications and open the way for further research that could lead to new therapeutic strategies for the treatment of various diseases. The findings from this study show us that the 3D culture technique in alginate is an effective tool for biomedical research and tissue engineering applications. Cells cultured by this method show promising results for further experimental and therapeutic use, while their 3D arrangement greatly increases the relevance of the results for clinical applications.

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