

Alginate encapsulation induce colony formation with umbilical cord-derived mesenchymal stem cells

Erkan Gumus¹, Burcin Irem Abas², Evrim Cevik³, Bilge Kocabiyik⁴, Melike Cenik⁴, Ozge Cevik²

¹Department of Histology and Embryology, Aydin Adnan Menderes University, School of Medicine, Aydin, Turkey

²Department of Biochemistry, Aydin Adnan Menderes University, School of Medicine, Aydin, Turkey

³Department of Machinery and Metal Technologies, Aydin Adnan Menderes University, Kocarli Vocational High School, Aydin, Turkey

⁴Department of Molecular Biotechnology, Aydin Adnan Menderes University, Graduate School of Health Sciences, Aydin, Turkey

ABSTRACT

Aim: The umbilical cord (UC) is a rich source of mesenchymal stem cell (MSC) isolation. Since the MSCs isolated from here have high self-renewal capacity and differentiation potential, production through biofabrication is essential for clinical treatments. For the cells to be stored for a long time and presented ready for use, encapsulation is required. In this study, UC-MSC cells were encapsulated with alginate using three different methods: alginate drop, alginate coating, and alginate sphere.

Methods: The cell viability, live/dead cell ratio, and colony formation capacities of the encapsulated cells were examined for 14 days.

Results: In the study, it was found that the most effective method was the alginate sphere form and that the structure of the cells should be preserved by injecting them into biomaterials in encapsulation. Colony formation potential was found to be high in biomaterials with alginate spheres.

Conclusion: As a result, the preservation of UC-MSC cells with alginate sphere encapsulation via biofabrication and their clinical use availability may be beneficial for treating of many diseases.

Keywords: Alginate, hydrogel, mesenchymal stem cell, umbilical cord.

✉ Dr. Ozge Cevik

Department of Biochemistry, Aydin Adnan Menderes University, School of Medicine, Aydin, Turkey

E-mail: ozge.cevik@adu.edu.tr

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Introduction

Mesenchymal stem cells (MSCs) are multipotent cell types obtained from a wide variety of tissues such as adipose tissue, bone marrow, dental pulp, placenta, and umbilical cord [1]. MSCs are different from other cells with its features of proliferation, differentiation,

and self-renewal. Although it plays a vital role in development during the embryonic period, they now have the therapeutic potential [2–4]. MSCs have been challenging a role therapeutics in clinical ameliorating from cancer to central nervous system diseases [5,6]. MSCs can inhibit the immune system, increase cell proliferation, induce angiogenesis, or migrate toward damaged tissues. Furthermore, the multifunctional capabilities of MSCs increase their use in both the medical and pharmaceutical industries. In the world, stem cell and cellular therapy companies make

significant investments in obtaining MSCs and keeping them for a long time without disturbing their activity [7]. The umbilical cord (UC) is a non-invasive and ethically trouble-free source of stem cells, as it is isolated from tissue taken at birth. UC contains MSC cells with high availability and high growth capacity of embryonic origin. Compared to cells taken from adult tissues, UC cord cells are a very attractive resource as they have spread faster [8,9].

Isolation processes of MSCs from UC are laborious and higher costly, require a great deal of experience. Isolations can be made differently; the most used methods are enzymatic digestion for separation or explant culture method [10]. After isolation of MSCs, studies are carried out to be stored for a long time and used therapeutically. Biopolymers with high biocompatibility such as alginate, chitosan, agarose, collagen, poly(lactic-co-glycolic acid) (PLGA), poly(ethylene glycol) (PEG), (poly(lactic acid) (PLA) and poly(glycolic acid) (PGA) are used for microencapsulation of MSCs [11]. Alginate is a natural polysaccharide and a biopolymer with high biocompatibility, stability, and non-antigenicity. Alginate shows gelation by crosslinking with calcium ions. These formed hydrogels are used as encapsulation material and can be produced by bio fabrication in various applications. Their use in medicine and pharmacy has been increasing rapidly in recent years due to its non-toxicity and gelling. Microencapsulation of UC-derived MSCs with a highly biocompatible polymer such as alginate after isolation may increase their use in stem cell treatments. New microencapsulation methods should be developed in order for UC-derived MSCs to maintain their rich content and rapid growth capacity without losing their capabilities. In this study, we investigated the

colony formation potential of UC-MSCs in the encapsulation of alginate

Materials and Methods

Cell culture and conditions

Human umbilical cord-derived mesenchymal stem cells (UC-MSC) were provided by ATCC (PCS-500-010). Cells were cultured in DMEM (Dulbecco's Modified Eagle's medium) (Gibco, USA) supplemented with 15% fetal bovine serum (FBS; Gibco), 2 mM L-glutamine, and 100 U/mL penicillin, and 100 µg/mL streptomycin, at 37 °C in a humidified atmosphere containing 5% CO₂. The medium was changed every three days.

Preparation of alginate constructs

Sodium alginate was purchased from Sigma (W201502) and dissolved with sterile PBS (phosphate-buffered saline) solution. Alginate solution was modified prepared different concentration as 0.625%, 1.25% and 2.5% w/v via encapsulation methods [12]. Each solution was passed through 0.45 sterile filters. Sterile calcium chloride solutions (2% w/v) were prepared and used for reducing agent to crosslink the alginate at 25 °C. Calcium and alginate complex was left for 5 minutes to form a hydrogel, and then it was taken into DMEM medium. The alginate encapsulation's rheological behaviors were determined using a rheometer (HAAKE MARS 40 Rheometer, Invitrogen). The viscosity measurements were done at 25°C and calculated by shear rate. Alginate deformation rate was calculated as the change in viscosity over 14 days without cells at 37 °C.

Encapsulation of UC-MSC

Experimental design on encapsulation was included in Figure 1. The encapsulation method

was modified by the amount of alginate and calcium and according to the selected cell [13].
Alginate Drop: Alginate solution (1.25%) and UC-MSC cells (500 cells) were mixed. 10 μL volume of the mixture was dropped into calcium solution. It was incubated for 5 minutes to crosslink, and after washing with PBS, 100 μL of cell medium was added into each well of the 96-well plate.
Alginate Coating: 50 μL alginate solution (1.25%) was coated into each well of the 96-well plate, and 50 μL calcium solution was added to crosslink. After the washing with PBS, UC-MSC cells (500 cells) were seeded with 100 μL of cell medium was added into each well of the 96-well plate.
Alginate Sphere: 10 μL alginate solution (1.25%) was added into each well of the 96-well plate, and 25 μL calcium solution was added to the alginate. After the crosslinking, UC-MSC cells (500 cells) were injected into the alginate sphere, and 100 μL of cell medium was added into each well of the 96-well plate.

Cell viability and live/dead cells

The encapsulated UC-MSC cells were incubated for 7 days and 14 days. At the end of the incubation, alginate encapsulations were dissolved in sodium citrate-EDTA buffer (55 mM Na-citrate, 20 mM EDTA). Each well was incubated with 10 μL MTT solution (MTT; Vybrant, Invitrogen) for 4 hours at 37°C, 5% CO₂. After incubation, solubilization was done in 100 μL SDS buffer to formazan precipitates [14]. Color changes were measured at 570 nm using a microplate reader (Epoch, Biotek). Dissolved cells were suspended in PBS and measured in a cell counter (Invitrogen Countess II).

Colony formation

The encapsulated cells were examined morphologically [14] at the end of the 14th day

under an inverted microscope (Zeiss Axiovert, Germany). The areas inside the capsules in the cells were focused. The ratio of colony-forming cells among total cells was calculated in each well.

Statistical analyses

Data were analyzed with GraphPad Prism software (GraphPad Inc., San Diego, CA, USA). All values were presented as mean \pm SD. Between study groups, the obtained data were compared by using a non-paired t-test and two-way ANOVA. Differences were considered statistically significant if the p-value was less than 0.05.

Results

Alginate viscosity was measured in a shear rate range of 1–1000 s^{-1} by increasing the shear rate every 10 s for 1 min. For 2.5% alginate, the low shear viscosity at 25 °C was found to be 282 mPa s; for 1.25% alginate, the moderate shear viscosity at 25 °C was 365 mPa s; for 0.625% alginate, the high shear viscosity at 25 °C was 687 mPa s (Fig 2a). The effect of shear stress on the viscosity was different for each alginate. In alginate deformation, the deformation rate was significantly higher in the coating of 0.625% alginate on the well surface ($p < 0.0001$) while there is no deformation in the other concentration for encapsulation with drop and sphere of alginate (Fig 2b). Cell viability activities of UC-MSC cells were checked on the 7th day, as the adhesion and attachment of stem cells were difficult. A certain number of stem cells were planted in each well, it was observed that the cells maintained their viability in the alginate drop group ($p > 0.1350$), but cell viability decreased significantly in the alginate coating group compared to the control ($p < 0.0001$). The alginate sphere group

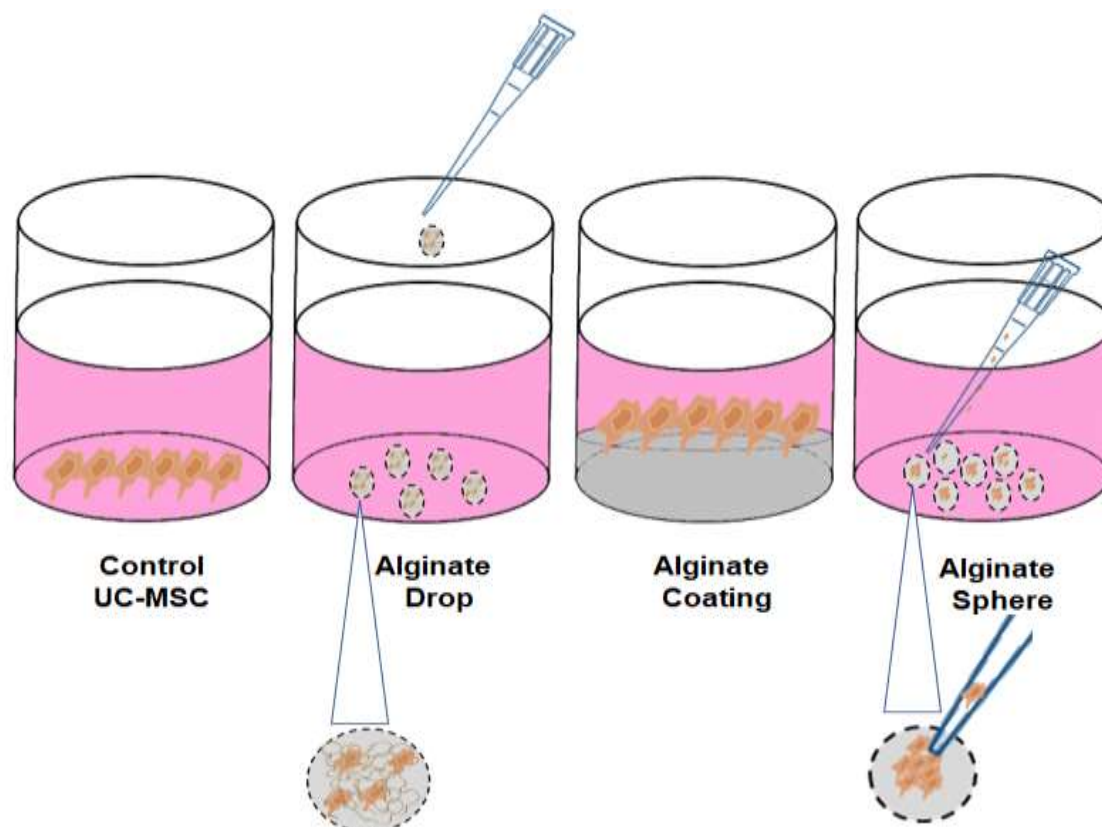


Figure 1. Experimental design for the umbilical cord- mesenchymal stem cells (UC-MSCs).

observed that the cell viability levels increased significantly compared to the control group ($p < 0.0098$, Fig 3a). The live and dead cell proportions of the UC-MSC cells after 14 days were evaluated. It was observed that the number of dead cells increased slightly in the alginate drop group compared to the control group ($p < 0.0166$), and the change in the number of live cells was not significant ($p > 0.0594$). It was observed that the number of dead cells increased significantly in the alginate coating group ($p < 0.0001$) and decreased in the alginate coating group of living cells compared to the control group ($p < 0.0001$). It was observed that the number of live cells increased significantly in the alginate sphere group compared to the control group ($p < 0.0403$), and there was no change in the number of dead cells ($p > 0.1708$, Fig 3b).

The colony formation potential of UC-MSC cells after 14 days was evaluated

microscopically (Fig 4). Since cells in the control group tend to adhere to cell culture plates, their colony-forming potential is limited. The colony-forming potential of cells in the alginate drop increased significantly compared to control group cells ($p < 0.0002$). When the alginate coating group was compared with the control group, it was observed that there was no change in colony formation potential ($p > 0.3780$). The colony-forming potential of UC-MSC cells in the alginate sphere increased significantly compared to control group cells ($p < 0.0001$, Fig 4b).

Discussion

MSCs are rich in content, and they interact with other cells through bioactive mediators in their structure, such as hormones, growth factors, cytokines, and extracellular vesicles that exert angiogenic and anti-inflammatory effects. MSCs can show immunosuppressive, anti-

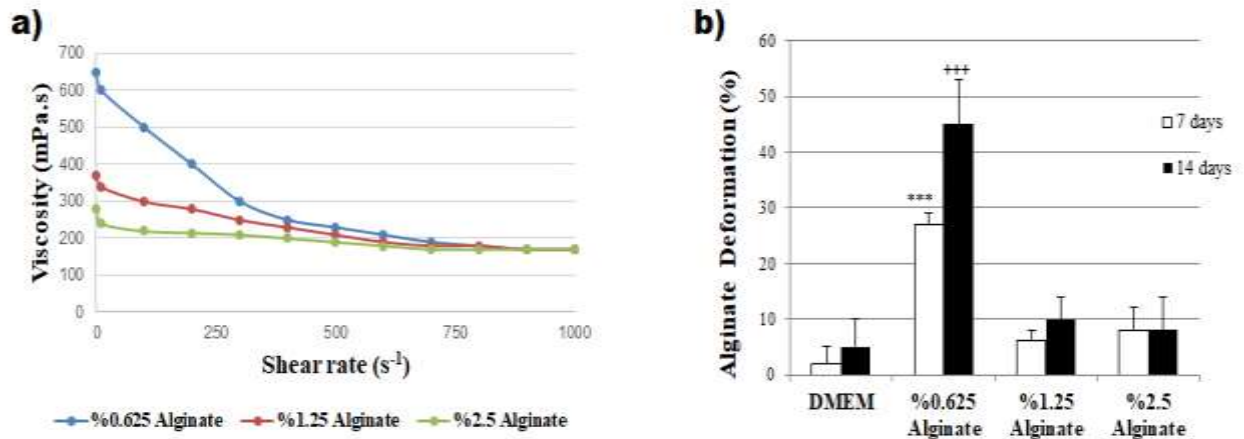


Figure 2. Alginate viscosity and deformation.

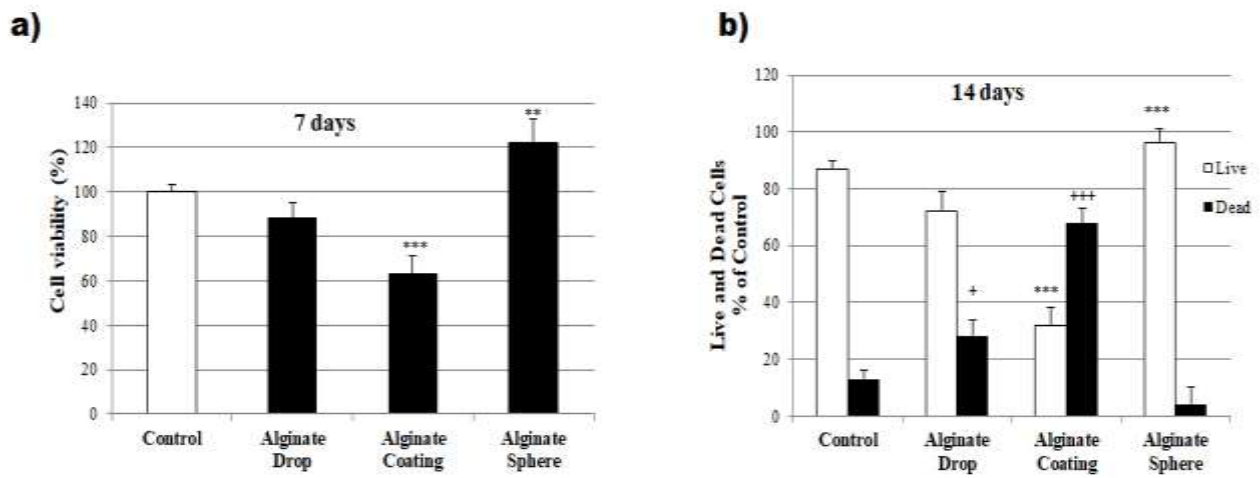


Figure 3. Effects of alginate encapsulation models in cell viability and live/dead cell ratio on UC-MSC.

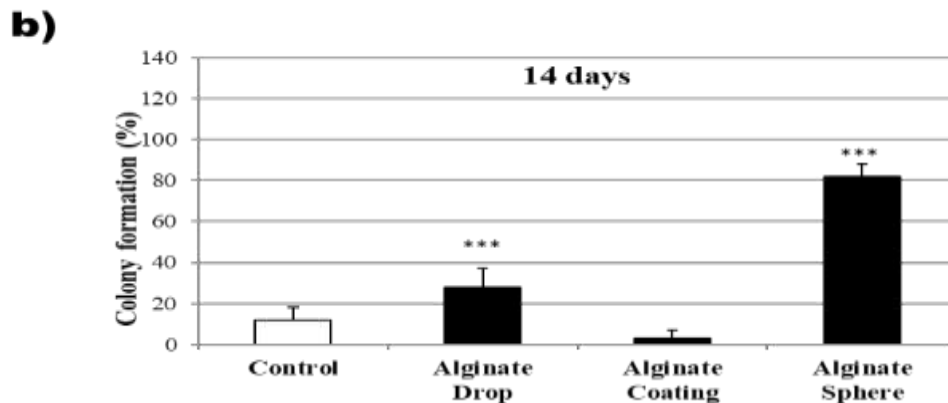
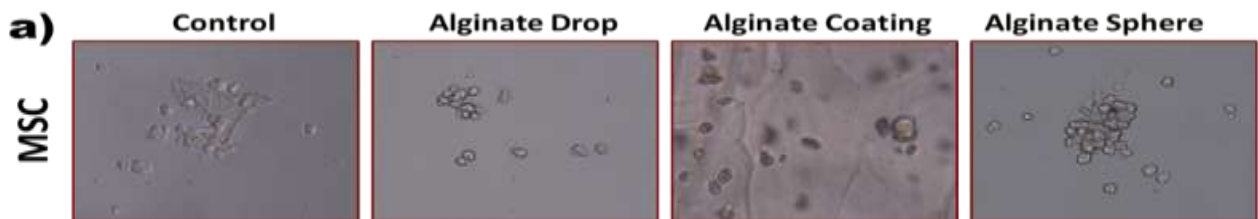


Figure 4. Effects of alginate encapsulation models in cell colony formation on UC-MSC.

apoptotic, anti-fibrotic effects on other cells. To benefit from these potentials MSC cells, are being developed, and new therapeutic dosing studies with encapsulation are carried out in this area for cellular therapy methods [15,16]. For MSC cells to be used therapeutically, there is a need for an encapsulation system that can maintain their viability for a long time. Especially in MSCs, UC-derived cells that are small amount but have the most incredible growth ability and contain important biofactors that are essential. UC-MSCs are isolated from umbilical cord blood or cord tissue. MSC isolation from UC tissue requires a lengthy dissection step, and opening the umbilical cord and manual removal of the vessels before mincing Wharton jelly is time-consuming and increases the risk of contamination. UC-MSCs are essential to be isolated under GMP conditions and stored for long periods [17]. UC-MSCs have significant enhancements, have high proliferation potentials, vast differentiation potentials, and enhanced immune modulation properties [18,19]. For these reasons, UC-MSCs have high therapeutic potential and have an important place in clinical trials worldwide [20,21]. Encapsulation of cells is a technique that enables living cells to be confined with unique biopolymeric materials and use them in the potential treatment of various human diseases [22]. There are many studies on low immunity for cell encapsulation and the development of biomaterials to protect stem cells [23]. It is crucial to develop a method for both the isolation of stem cells and their long-term storage. The properties and shapes of biomaterials during production are essential for clinical applications of cell encapsulation of stem cells [24,25]. Many *in vivo* studies showed that the encapsulation and administration of stem cells produced from different sources (such as bone marrow,

adipose tissue) with alginate [26]. For example, alginate-encapsulated human BM-MSCs have demonstrated a therapeutically curative effect at the infarct site in the rat myocardial infarction model [27]. Another study showed that alginate-encapsulated MSCs remained viable for 30 days and did not lose their therapeutic effect when administered subcutaneously to mice [28]. Similarly, in our study, we found that the alginate sphere group's vitality was high on the 14th day. On the other hand, some suggestions would be beneficial to use a single cell microgel encapsulation approach for MSC. In systemic applications of MSC, it provides diffusion limitations in protecting stem cells against hypoxic effects and facilitating molecules released from the cell [29]. There are still restrictive studies regarding colony formation in stem cells in single-cell encapsulations [30]. Colony formation of mesenchymal stem cells (maybe called spheroidization) exhibits improved therapeutic potential *in vitro*, but if spheroids fail to attach in the environment, they leave control of cell function to the extracellular matrix and potentially limit development time. Using biomaterials is supported by spheroid cell transmission, cell retention and stem cells' functions [31]. In the study using the osteoarthritis model, it was reported that allogeneic rat MSC cells survived longer when encapsulated in alginate and showed metabolic activity for at least eight weeks *in vivo* [32]. In this study, high and low weight alginate and allogeneic MSC cells were first mixed in the form of beads and marked with gadolinium for monitoring. It has a similar structure to the drop alginate complexes in our study and is formed with different cells. The production of cell-alginate microcapsules containing MSC cells in a standardized, safe manner must certainly establish the "ready-to-use" potential in clinical

practice [29]. Some researchers, MSC cells isolated from the umbilical cord Wharton jelly were encapsulated with alginate, and differentiation into neuron-like cells was examined. The study showed that alginate could effectively induce neuronal differentiation in a three-dimensional cell culture system by protecting cells [33]. In a different study, MSC cells isolated from Wharton jelly were encapsulated with alginate, and the interleukins, chemokines, growth factors, and soluble forms of adhesion molecules released into the environment were investigated. It has been reported that alginate does not change cells' morphological properties and can provide protein circulation in the general life cycle [34].

Conclusion

For bio fabrication studies, UC-MSC provides dissemination of research on encapsulation. The alginate sphere in our study was created with the logic of microencapsulation. It has been determined that the cells placed in the capsules can form a better colony. When the biodegradable alginate system is opened in the tissues, these colonies' potential to form new cells there will be higher. This method may be more useful for biofabrication studies. For smaller microencapsulation studies, these data will shed light on future studies.

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Conflict of Interest: *The authors declare that they have no conflict of interest.*

Ethical statement: *Since it is a cell culture study and commercial line is used, ethics committee permission is not required.*

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