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Impact of Hyaluronic Acid on the Viability of Mesenchymal Cells Derived from Adipose Tissue Grown in Collagen Type I/III Membrane

Impacto do ácido hialurônico na viabilidade das células mesenquimais derivadas do tecido adiposo cultivadas em membrana de colágeno tipo I/III

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Abstract

Objective To evaluate in vitro the viability of mesenchymal stem cells derived from adipose tissue (AD-MSCs) in different commercial solutions of hyaluronic acid (HA) before and after being sowed in collagen I/III membrane.

Methods In the first stage, the interaction between AD-MSCs was analyzed with seven different commercial products of HA, phosphate buffered saline (PBS), and bovine fetal serum (BFS), performed by counting living and dead cells after 24, 48 and 72 hours. Five products with a higher number of living cells were selected and the interaction between HA with AD-MSCs and type I/III collagen membrane was evaluated by counting living and dead cells in the same time interval (24, 48 and 72 hours).

Keywords
- cartilage
- articular
- mesenchymal stem cells
- mesenchymal stem cell transplantation
- hyaluronic acid

Introduction

The management of cartilage defects is challenging due to the limited capacity of tissue repair. Several treatment

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Results In both situations analyzed (HA + AD-MSCs and HA + AD-MSCs + membrane), BFS presented the highest percentage of living cells after 24, 48 and 72 hours, a result higher than that of HA.
Conclusion The association of HA with AD-MSCs, with or without membrane, showed no superiority in cell viability when compared with BFS.

Resumo

Objetivo Avaliar in vitro a viabilidade das células-tronco mesenquimais derivadas do tecido adiposo (AD-CTMs) em diferentes soluções comerciais de ácido hialurônico (AH) antes e após serem semeadas em membrana de colágeno I/III.
Métodos Na primeira etapa, analisou-se a interação entre AD-CTMs com sete diferentes produtos comerciais de AH, salina tamponada com fosfato (PBS, na sigla em inglês) e soro fetal bovino (SFB), realizada pela contagem das células vivas e mortas após 24, 48 e 72 horas. Foram selecionados cinco produtos com maior número de células vivas e avaliou-se a interação entre o AH com AD-CTMs e a membrana de colágeno tipo I/III pela contagem de células vivas e mortas no mesmo intervalo de tempo (24, 48 e 72 horas).
Resultados Em ambas as situações analisadas (AH + AD-CTM e AH + AD-CTM + membrana), o SFB apresentou a maior porcentagem de células vivas após 24, 48 e 72 horas, resultado superior ao do AH.
Conclusão A associação do AH com as AD-CTMs, com ou sem a membrana, não demonstrou superioridade na viabilidade celular quando comparado com SFB.

Palavras-chave
- cartilagem articular
- células-tronco mesenquimais
- transplante de células-tronco mesenquimais
- ácido hialurônico

modalities have been carried out to improve healing and promote regeneration. Biological applications have gained notoriety in recent decades,[1] since the first description of treatment with autologous chondrocyte implantation[2] in the 1990s. Years later, the cell implantation technique was improved with the use of three-dimensional structures (scaffolds), improving the clinical results reported with up to a decade of follow-up.[3]

Mesenchymal stem cells (MSCs) have recently been proposed as a potential option for cartilage restoration. They are known to have unique biological characteristics, including immunomodulatory, anti-inflammatory, and pro-regenerative cytokine release properties.[4–6] They can be isolated from various tissues of the human body,[7–9] including adipose tissue (AD-MSCs), muscle, bone, synovia, dental pulp, and umbilical cord.[10] Adipose tissue-derived mesenchymal stem cells are easily obtained, in addition to having a proliferative profile and in vitro differentiation capacity very similar to bone marrow-derived MSCs.[11] They are considered an ideal cell source due to their availability, nonimmunogenic property, anti-inflammatory action, and to the absence of an effect relationship between donor age and proliferation and differentiation capacity.[12] In order to improve cartilage repair, cells can be cultured in vitro in media with exogenous stimuli (hyaluronic acid [HA] and growth factors) and, once they reach the desired concentration, be implanted in three-dimensional matrices, such as collagen membranes.

The ideal protocol for the cultivation of AD-MSCs for clinical application is yet to be determined. Hyaluronic acid has been considered an excellent vehicle for the administration of MSCs in tissue repair.[13] It is a biopolymer formed by glucuronic acid and N-acetylglucosamine. There are, however, several HA products commercially available, which differ in factors such as origin (animal or synthetic) and physical-chemical properties (concentration, molecular weights, viscosity, elasticity).

To improve the interaction between scaffolds and cells and, therefore, improve the cartilage repair process, the present study analyzed the interaction between AD-MSCs, HA, and collagen membrane. There is lack of information about the response of AD-MSCs when exposed to HA, as well as whether commercial products with different characteristics influence cell activity. There are few reports of HA interference as substrate for in vitro AD-MSCs prior to or after placement in the biosynthetic membrane of type I/III collagen.

The main objectives of the present study are:

1. To evaluate in vitro the viability of human AD-MSCs when in contact with different commercial formulations of HAs;
2. To analyze the use of HA as a vehicle of AD-MSCs in type I/III collagen membrane.

Materials and Methods

To perform the present in vitro study, five samples of human adipose tissue were obtained for the isolation of AD-MSCs, after signing of the free and informed consent term by the donor, approved by the Research Ethics Committee (CAAE 60075616.5.0000.0071). The procedures related to the transfer of biological material, isolation, and manipulation of cells in vitro was carried out in partnership with the company StemCorp Serviços Biomédicos Ltda., licensed by the National Health Surveillance Agency (ANVISA, in the Portuguese

acronym) as a Cellular Processing Center according to the criteria of Resolution No. 214 of the Collegiate Board.

The collection of between 10 and 20 mL of adipose tissue from the subcutaneous abdominal region was performed by the plastic surgeon in the operating room during a liposuction procedure. After collection, the samples were transferred to a sterile vial containing 10 mL of phosphate-buffered saline solution (PBS) 1X pH 7.4, 200 U/mL of penicillin, 200 ug/mL of streptomycin, 0.5 ug/mL of amphotericin, and 50 ug/mL of gentamicin (Gibco). The samples were transferred to a thermal box with temperature monitoring (4 to 8°C) and were transported to the laboratory for cell isolation.

The AD-MSCs were isolated using the methods previously described by Vieira et al.[14,15] For in vitro cell cultivation, the proliferation medium Dulbecco's Modified Eagle Medium low glucose (DMEM-LG) (Gibco) supplemented with 10% bovine fetal serum (BFS), penicillin 100U/mL, streptomycin 100ug/mL, and amphotericin 0.25/mL (Gibco) was used. The exchange of means was carried out every 3 days. Each shard was kept incubated at 37°C in a humid atmosphere containing 5% CO_2 until 80% confluence was reached. At this point, the cells were transferred to new culture vials, in the area proportion of 1:3, using the TrypLE reagent (Gibco). This procedure was repeated until enough cells were reached to perform the experiments described below.

For the cryopreservation procedure, the cells were taken out of the culture vials using TrypLE (Gibco) and were centrifuged and suspended in StemPro freezing medium (Gibco) containing 10% DMSO. Cell freezing was gradually performed in cryotubes, transferred later to liquid nitrogen tank.

Characterization of Isolated Cells

The three criteria of the International Society of Cell Therapy for the characterization of mesenchymal stem cell were established: 1–adherence to the plastic surface; 2–capacity of in vitro differentiation into adipocytes, chondrocytes, and osteocytes; 3–expression of specific surface markers (immunophenotyping).[16]

Immunophenotyping

For the immunophenotyping test, the cells were cultured until passage 8, removed from the culture vials and centrifuged at 200 g for 5 minutes at room temperature. Then, the supernatant was discarded and the cells were homogenized and suspended in PBS. The cells were then marked with monoclonal antibodies: CD29-PerCP-Cy5, CD31-PE, CD45-FITC, CD73-PE, CD90-PE, CD105-PE, HLA-ABC-FITC, and HLA-DR-PE (BD Biosciences, Franklin Lakes, NJ, USA), incubated at room temperature, and protected from light for 30 minutes. After this period, the cells were washed with PBS, centrifuged at 500 g for 5 minutes, resuspended in buffer solution and placed in a BD FACS Canto II flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). The data obtained were analyzed in the FLOWJO software (TreeStar, Ashland, OR, USA).

Differentiation test

The analysis of the differentiation potential of the AD-MSCs was performed with means for the differentiation of adipocytes, chondrocytes, and osteoblasts. Each cell group was cultivated in the presence of the specific medium for 14 days. After this period, the cells were stained with Oil Red O (adipogenic), Alcian Blue (chondrogenic) or Alizarin Red S (osteogenic). The differentiation capacity was evaluated by staining.

Feasibility Test

Cell/Hyaluronic Acid Interaction

Mesenchymal stem cells from adipose tissue lineage, in passage six, were taken from the culture vials using TrypLE solution (Gibco) and centrifuged at 300 g for 5 minutes. At the end of the process, the cells were counted in the Neubauer chamber, 3×10^5 cells were separated and added in 1.5mL microtubes, centrifuged again at 300 g for 5 minutes, and suspended in 20 µl of each solution. Nine groups were analyzed: 7 HA products (►**Table 1**), PBS 1X pH 7.4 (negative control), and proliferation medium (BFS). The cells were incubated with their solutions for 24, 48 and 72 hours at room temperature.

Table 1 Physical-chemical properties of the different hyaluronic acids analyzed

Number	Product	Molecule	Concentration	Molecular weight (MDa)	Elasticity (Pa 2.5 hz)	Viscosity (Pa 2.5 hz)	Source	Crosslink molecule
1	**Opus Joint**	Sodium hyaluronate	1.5%	5.4	145	100	Bacterial	No
2	**SupraHyal Duo**	Sodium hyaluronate	1%	0.75	1.2		Bacterial	No
3	**Fermathron**	Sodium hyaluronate	1%	1.19–2.03	0.84		Bacterial	No
4	**Orthovisc**	Sodium hyaluronate	1.5%	1–2.9	60	46	Bacterial	No
5	**Synolis VA**	Sodium Hyaluronate + sorbitol	2% + 4%	2	329	143	Bacterial	No
6	**Synvisc**	Hilano GF-20	0.8%	6	111	25	Avian	yes
7	**Osteonil**	Sodium hyaluronate	1%	1–2	–	210	Bacterial	No

Characteristic of synovial fluid:
Elasticity = 117 Pa 2.5 Hz; Viscosity = 45 Pa 2.5 Hz; Molecular Weight = 3–4 MDa.[1]
Source: product leaflets

Cell viability was estimated by the number of live cells by the LIVE/DEAD kit Viability/Cytotoxicity Assay Kit (Thermo) and the Countess II FL (Thermo) equipment. The images of the marked cells, obtained in Countess, were counted with the aid of Image J software version 1.8.0_172 (National Institutes of Health, Bethesda, MD USA). Every experimental part was performed in a blind test and each HA product received a number. At the end of the analyses, the trade names were revealed for comparative purposes.

Cell/Hyaluronic Acid/Type I/III Collagen Membrane Interaction

For the analysis of the interaction between AD-MSCs, HA and type I/III collagen membrane, the five HAs that presented the best viability rate in the previous experiment were selected.

Mesenchymal stem cells from adipose tissue lines, in passage 7, were taken from the culture vials using TrypLE solution (Gibco) and centrifuged at 300 g for 5 minutes. At the end of the process, the cells were counted in the Neubauer chamber and 2×10^5 cells were added in 1.5mL microtubes, centrifuged again at 300 g for 5 minutes, and suspended in 40 µl of HA product (numbers 1, 2, 4, 5 and 7; ►**Table 1**), PBS 1X pH 7.4, and BFS. The cell mixture plus solution (HA, PBS, BFS) was then added to the surface of type I/III collagen membrane (Chondro-Gide, Geistlich), packed in 48-well plates. The cells were incubated for 1 hour in an atmosphere of 5% CO_2 at 37°C, the time of cell support in the membrane, then 400uL of proliferation medium was added. The cells were kept in the same atmosphere for 24, 48 and 72 hours, with feasibility analysis in these periods using the methodology described with the LIVE/DEAD kit (Thermo).

Results

Isolation of AD-MSCs from Adipose Tissue

There was 100% success in obtaining adherent cells from the 5 adipose tissue samples to perform the in vitro study. The amount collected to isolate the cells of each patient were: A1 (11.27 g); A2 (18.57 g); A3 (12.16 g); A4 (20.99 g); A5 (18.88 g). The samples were obtained from female patients with a mean age of 30.2 years old. All strains presented morphology compatible with mesenchymal cells (long and fusiform, similar to fibroblasts), with an expected proliferation rate until the evaluated passages.

Characterization of Isolated Cells

Immunophenotyping

The five strains presented specific characteristics of mesenchymal stem cells. We observed a positive percentage (> 80%) for markers related to CD29 and CD90 adhesion; related to the characterization of CD73 and CD105 mesenchymal cells; and Hla-ABC Class I MHC marker. Negative percentages (< 1%) were observed for hematopoietic marker CD45; endothelial marker CD31; and Class II HLA-DR MHC marker (►**Figure 1**).

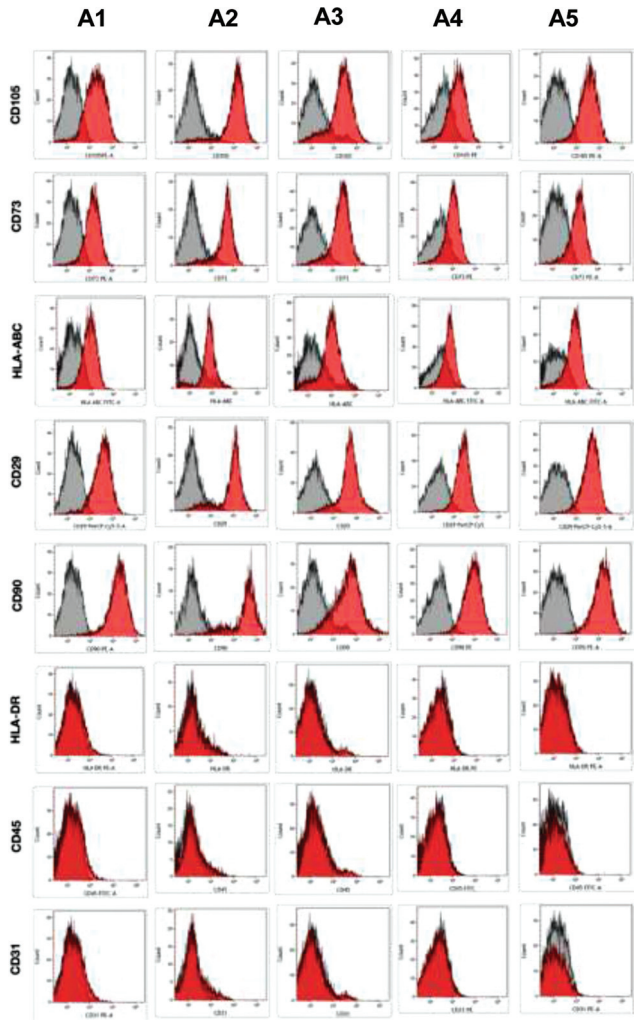

Fig. 1 Immunophenotyping of adherent cells obtained from adipose tissue. Gray histograms represent the population of cells not marked with antibodies (negative control). Histograms in red represent the population of cells marked with the respective antibodies described in each row. The graphs show the number of cells versus fluorescence intensity in the five cases.

Feasibility Tests

Cell Interaction with Hyaluronic Acid

We observed that PBS presented the best viability rate in the analyzed times, being, therefore, considered the reference solution for comparisons with the other solutions (►**Table 2**). At 24 hours, acid 5 presented an average value of percentages of living cells (96.2% ± 1.7) similar to the rate observed with PBS. We did not observe evidence of differences between PBS and acid 1 ($p = 0.075$), acid 2 ($p = 0.169$), acid 3 ($p = 0.090$), acid 5 ($p = 0.704$) and acid 6 ($p = 0.084$). At 48 hours, acid treatment 5 also presented rates similar to that of PBS (95.8% ± 2.8). However, we did not observe evidence of differences between PBS and acid 1 ($p = 0.097$), acid 4 ($p = 0.059$) and acid 5 ($p = 0.089$). At 72 hours, all treatments presented lower rates of living cells compared with PBS.

Cell/Hyaluronic Acid/Collagen Membrane Interaction (I/III)

After evaluating the results of the first stage (AD-MSCs + AH interaction), five HAs with a better viability rate were

Table 2 Estimated mean values and comparisons for means and standard deviations of percentages of living cells

Moment	Treatment	Average % of living cells		SD of % of living cells	
		Mean (95%CI)	*p-value*	Mean (95%CI)	*p-value*
24h	HA 1	93.7 (90.3–97.3)	0.075	2.7 (1.4–5.3)	0.956
	HA 2	94.4 (91.5–97.5)	0.169	2.9 (1.4–6.0)	0.839
	HA 3	93.4 (91.4–95.4)	0.090	2.1 (1.0–4.2)	0.707
	HA 4	92.9 (88.4–97.6)	< 0.001	3.7 (2.1–6.5)	0.470
	HA 5	**96.2 (93.3–99.3)**	0.704	**1.7 (0.8–3.5)**	0.669
	HA 6	95.3 (91.0–99.8)	0.084	2.2 (1.0–5.1)	0.854
	HA 7	94.7 (92.2–97.4)	0.041	2.5 (1.1–5.6)	0.908
	Middle	88.2 (84.1–92.5)	< 0.001	3.8 (2.2–6.5)	0.276
	PBS	97.3 (93.2–100.0)	Reference	2.6 (0.6–11.4)	Reference
48h	HA 1	95.7 (91.7–99.9)	0.097	2.4 (0.7–7.5)	0.095
	HA 2	94.5 (91.2–97.8)	0.001	1.2 (0.8–1.9)	0.865
	HA 3	94.1 (91.6–96.6)	< 0.001	2.6 (1.7–4.0)	0.272
	HA 4	95.0 (90.6–99.7)	0.059	3.0 (1.6–5.5)	< 0.001
	HA 5	**95.8 (93.4–98.3)**	0.089	**2.8 (1.5–5.3)**	< 0.001
	HA 6	93.5 (88.7–98.5)	0.003	3.4 (1.7–6.7)	< 0.001
	HA 7	92.7 (87.1–98.6)	0.011	2.3 (1.8–2.9)	0.173
	Middle	86.1 (81.9–90.4)	< 0.001	5.7 (2.8–11.5)	0.002
	PBS	98.1 (96.2–100.0)	Reference	1.3 (0.4–4.6)	Reference
72h	HA 1	93.9 (88.5–99.8)	0.049	3.2 (1.3–8.2)	0.105
	HA 2	94.3 (91.8–96.8)	< 0.001	3.7 (2.4–5.8)	0.308
	HA 3	93.6 (91.7–95.4)	< 0.001	5.6 (2.6–12.4)	0.123
	HA 4	91.6 (86.7–96.8)	0.002	3.1 (1.5–6.3)	0.299
	HA 5	88.5 (80.3–97.5)	0.007	7.3 (3.4–15.6)	0.025
	HA 6	93.3 (89.4–97.3)	0.002	4.6 (2.5–8.4)	0.059
	HA 7	92.7 (90.0–95.4)	< 0.001	2.9 (2.3–3.7)	0.534
	Middle	82.6 (70.3–97.2)	0.009	4.4 (2.7–7.2)	0.091
	PBS	98.6 (97.2–100.0)	Reference	2.1 (0.6–7.4)	Reference

Abbreviations: CI, confidence interval; HA, Hyaluronic Acid; PBS, phosphate-buffered saline; SD, standard deviation.

selected, in addition to the mean (BFS) and PBS, and we observed a variability in the number of living cells compared with the different treatments and times analyzed. Treatment with BFS showed better stability between the analyzed times, with excellent rates of living cells, > 95%. At time zero, we observed a variation in the percentage of living cells (between 87 and 99.96%). After 24 hours, we observed a variation of between 41 and 100%, and acids 5, 2, and 1 presented a higher variability index (►**Figure 2A**). At 48 hours, the percentage of living cells varied between 65.8 and 100%, and the greatest variability occurred with acids 4, 5 and 7 (►**Figure 2B**). At 72 hours, the variation of living cells was of between 60 to 100%, and the greatest variability was observed with acids 1 and 5 (►**Figure 2C**).

In the analysis of the estimated means of living cells, the treatment with BFS presented the best rate of living cells at 24, 48, and 72 hours, with a statistically significant difference at 24 hours (95.8%) when compared with PBS (►**Table 3**). At 48 hours, the treatments with acids 1 and 5 were inferior, with statistical significance (►**Table 3**). After 72 hours, however, we did not observe differences in comparison with PBS. When comparing all treatments, in the 3 times, we observed that acids 1 and 5 presented inferiority when compare with BFS, with statistical relevance (►**Table 4**).

Discussion

The present study demonstrated that HA is a product that is biocompatible with AD-MSCs. It was not possible to establish a relationship between the feasibility and type of commercial preparation of the product. Cell manipulation also suffered interference according to the viscosity of the formulation. The medium with BFS resulted in a better viability rate at all times analyzed (24, 48, and 72 hours) which was ~ 95%, higher than those of the products tested. There was a statistical difference only at 24 hours (BFS versus PBS), and

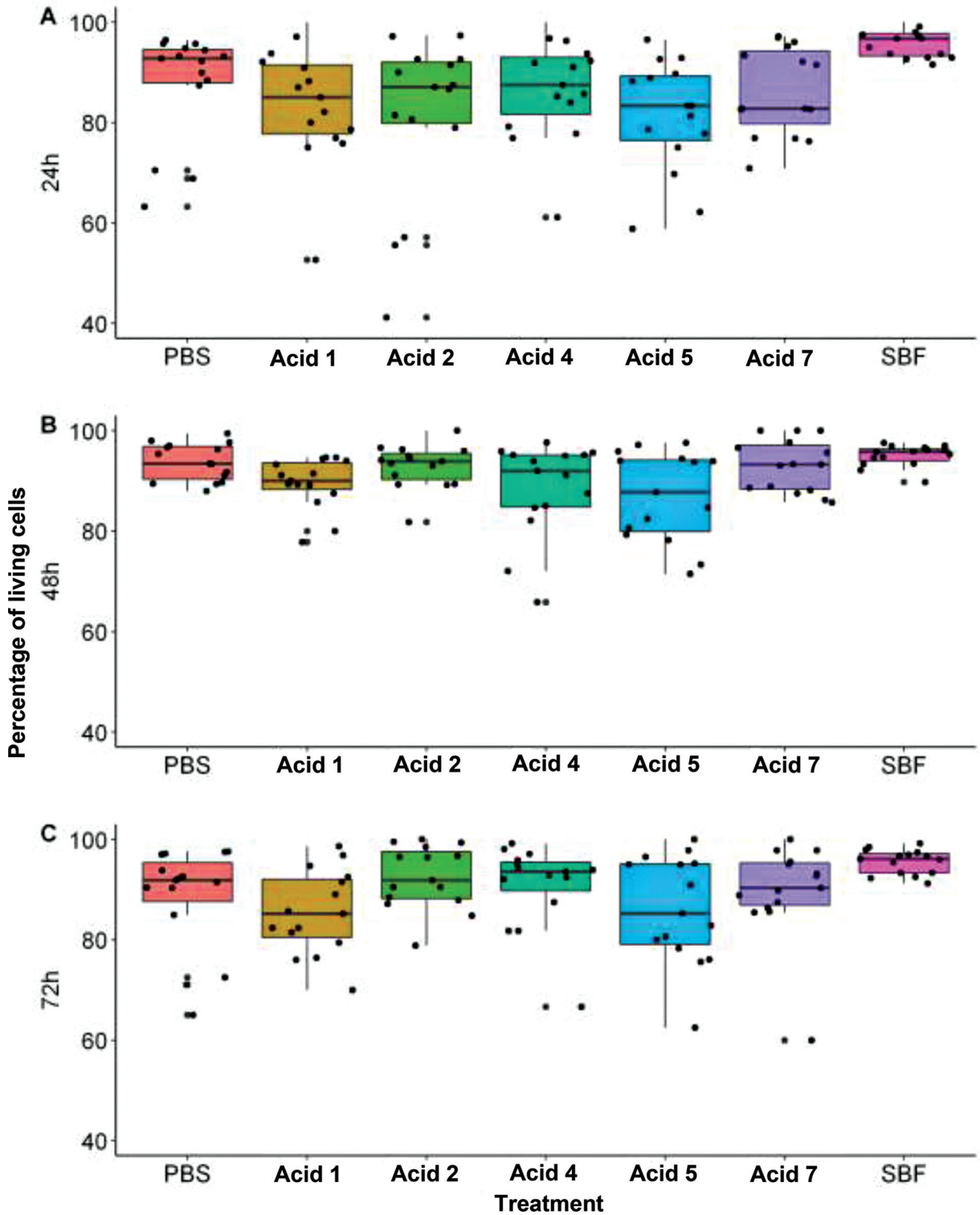

Fig. 2 Percentage of living cells per treatment in (A) 24 hours, (B) 48 hours, and (C) 72 hours.

at this time the HA products presented a similar performance to PBS. In the other times, considering PBS as a reference, there was no difference.

Hyaluronic acid has been widely used as a biomaterial for its biocompatibility and biodegradability characteristics. However, there is few data in the literature regarding its in vitro effect on MSCs.[13,17,18] Ding et al.[17] evaluated the effect of HA on AD-MSCs from Hoffa fat, concluding that a concentration of between 25 and 75% of HA does not affect cell proliferation. They concluded that the presence of HA is not toxic, did not alter the expression of the CD44 marker (HA receptor), nor did it induce the chondrogenic differentiation of the cells in the short interval of 7 days, characterizing HA as an appropriate vehicle for the injection of AD-MSCs.[17]

Table 3 Percentages of living cells estimated at 24, 48, and 72 hours

Moment	Treatment	Estimated mean (95%CI)	*p-value*
24h	BFS	**95.8 (93.4–97.4)**	< **0.001**
	Acid 7	86.3 (77.7–91.5)	0.826
	Acid 5	81.3 (72.3–87.3)	0.350
	Acid 4	86.6 (81.8–90.2)	0.801
	Acid 2	81.2 (61.8–90.7)	0.429
	Acid 1	83.7 (74.4–89.6)	0.555
	PBS	87.8 (74.5–94.1)	Reference
	BFS	95.0 (93.3–96.3)	0.395
	Acid 7	93.0 (88.9–95.5)	0.755
	Acid 5	86.9 (79.3–91.8)	**0.017**
48h	Acid 4	88.6 (78.6–93.9)	0.222
	Acid 2	92.9 (89.8–95.0)	0.699
	Acid 1	89.5 (86.7–91.7)	**0.033**
	PBS	93.8 (90.9–95.7)	Reference
	BFS	95.6 (93.3–97.0)	0.063
	Acid 7	89.7 (84.8–93.0)	0.765
	Acid 5	86.1 (78.2–91.1)	0.749
72h	Acid 4	90.8 (81.7–95.4)	0.674
	Acid 2	92.5 (87.7–95.4)	0.298
	Acid1	85.5 (79.7–89.6)	0.671
	PBS	88.4 (75.9–94.4)	Reference

Abbreviations: BFS, bovine fetal serum; CI, confidence interval; PBS, phosphate-buffered saline.

Succar et al.[19] evaluated the effect of different concentrations of high molecular weight HA (0.5 to 5.0 mg/mL) on MSCs, measuring adhesion and proliferation on plastic surface and cartilage adhesion assays. One hypothesis is that high viscosity would negatively influence the bond with cells, which was confirmed by verifying that the higher the concentration, the lower the adhesion, and that HA has a dose-dependent effect on the kinetics of cell growth at concentrations > 1mg/mL.[19] During our study, we noticed the negative interference of high viscosity in cell manipulation; however, we could not establish a relationship with the concentration of the products. One limitation is that in cell + HA + membrane interaction analyses, we did not evaluate the products with the lowest concentration, due to the unsatisfactory result observed in cell viability.

Thus, it is believed that molecular weight and viscosity may be responsible for the differences found. Among the brands surveyed, Suprahyal (Meiji Seika Pharma, Tokyo, Japan) presented the highest amount of viable AD-MSCs at the end of the analyzed period, being the product with the lowest molecular weight tested (0.75 Mda). This result may be related to evidence that HA with high molecular weight acts as an inhibitor of angiogenesis and cell proliferation in addition to having an anti-

Table 4 Multiple comparisons between treatments at 24, 48, and 72 hours

Moment	Comparison			Corrected *p-value*
	BFS	X	Acid 7	0.105
	BFS	X	Acid 5	**0.003**
	BFS	X	Acid 4	< **0.001**
	BFS	X	Acid 2	0.497
	BFS	X	Acid1	**0.028**
	Acid 7	X	Acid 5	0.497
24h	Acid 7	X	Acid 4	>0.99
	Acid 7	X	Acid 2	>0.99
	Acid 7	X	Acid1	>0.99
	Acid 5	X	Acid 4	0.497
	Acid 5	X	Acid 2	>0.99
	Acid 5	X	Acid1	>0.99
	Acid 4	X	Acid 2	>0.99
	Acid 4	X	Acid1	>0.99
	Acid 2	X	Acid1	>0.99
48h	BFS	X	Acid 7	>0.99
	BFS	X	Acid 5	**0.017**
	BFS	X	Acid 4	>0.99
	BFS	X	Acid 2	>0.99
	BFS	X	Acid 1	**0.033**
	Acid 7	X	Acid 5	0.197
	Acid 7	X	Acid 4	>0.99
	Acid 7	X	Acid 2	>0.99
	Acid 7	X	Acid 1	>0.99
	Acid 5	X	Acid 4	>0.99
	Acid 5	X	Acid 2	>0.99
	Acid 5	X	Acid 1	>0.99
	Acid 4	X	Acid 2	>0.99
	Acid 4	X	Acid 1	>0.99
	Acid 2	X	Acid 1	0.204
	BFS	X	Acid 7	**0.002**
72h	BFS	X	Acid 5	**0.012**
	BFS	X	Acid 4	>0.99
	BFS	X	Acid 2	>0.99
	BFS	X	Acid1	<**0.001**
	Acid 7	X	Acid 5	>0.99
	Acid 7	X	Acid 4	>0.99
	Acid 7	X	Acid 2	>0.99
	Acid 7	X	Acid1	>0.99
	Acid 5	X	Acid 4	>0.99
	Acid 5	X	Acid 2	>0.99
	Acid 5	X	Acid1	>0.99
	Acid 4	X	Acid 2	>0.99
	Acid 4	X	Acid1	>0.99
	Acid 2	X	Acid1	0.213

Abbreviation: BFS, bovine fetal serum.

inflammatory and immunosuppressive effect.[6] Therefore, it can be suggested that cell viability may be directly related to the concentration and molecular weight of the HA used and that this may be a determining factor in the success of the treatment of chondral lesions.[20] It must be mentioned that the results of the present study are relevant in the context of tissue engineering related to the influence of HA on AD- MSCs and not to its use as viscosupplementation in the treatment of osteoarthritis.

The present study is not exempt from limitations. This is a laboratory study with experimental results that should not be extrapolated to the clinical routine, although they may guide future research. A small number of samples were analyzed despite the statistical range of the sample.

Conclusions

Bovine fetal serum showed better performance to maintain the viability of AD-MSCs compared with commercial HA products, without and with collagen membrane. In addition, HA can be a means for the association of AD-MSCs.

Conflict of Interests
The authors that have no conflict of interests to declare.

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