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Original Article

Evaluation of different commercial hyaluronic acids as a vehicle for injection of human adipose-derived mesenchymal stem cells☆

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ABSTRACT

Objective: The main purpose of this study is to evaluate, *in vitro*, the cytotoxicity of different commercial brands of hyaluronic acids to be used as a vehicle for injection of human adipose-derived mesenchymal stem cells (AD-MSCs).
Methods: AD-MSCs were divided into seven groups: one control group where AD-MSCs were cultivated with phosphate-buffered saline (PBS) and six other groups where AD-MSCs were cultivated with different commercial brands of hyaluronic acid. AD-MSC viability analysis was performed after 4, 24, and 48 h in contact with each treatment, using the trypan staining method on a Countess automated cell counter (Thermo Fisher Scientific).
Results: The results clearly demonstrated a significant difference in cell viability when AD-MSCs were exposed to different hyaluronic acids when compared with the control group.
Conclusion: These data suggest that hyaluronic acid can be used as a vehicle for injection of human AD-MSCs, but caution is needed to choose the best product, aiming at its future therapeutic application.

☆ Study conducted at Laboratório StemCorp de Tecnologia em células-tronco, together with the Instituto Cohen de Ortopedia, Reabilitação e Medicina do Esporte, São Paulo, SP, Brazil.

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Avaliação de diferentes ácidos hialurônicos comerciais como veículo de injeção para células mesenquimais humanas derivadas do tecido adiposo

Palavras-chave:
Doenças da cartilagem
Joelho
Artroscopia
Cartilagem articular
Células-tronco mesenquimais
Transplante de células-tronco mesenquimais

RESUMO

Objetivo: Avaliar *in vitro*, de forma direta, a citotoxicidade de ácidos hialurônicos como veículo de injeção para linhagens de células-tronco mesenquimais (CTMs) obtidas de tecido adiposo humano.

Métodos: As CTMs foram divididas em sete grupos, os quais foram expostos ao ácido hialurônico de seis marcas comerciais, além do contato com tampão fosfato-salino PBS (grupo controle). Após quatro, 24 e 48 horas, foi feita a análise da viabilidade celular através do contador *Countess* pelo método de coloração com *Trypan Blue* (*Thermo Fisher Scientific*).

Resultados: Os resultados demonstraram uma diferença significativa na viabilidade celular quando essas linhagens de CTMs foram expostas aos diferentes ácidos hialurônicos em comparação com o grupo controle.

Conclusão: Os dados sugerem que o ácido hialurônico pode ser usado como veículo de injeção para CTMs, porém é necessária cautela na escolha do melhor produto para aplicação terapêutica futura.

Introduction

Articular cartilage lesions are among the most frequent musculoskeletal pathologies, and the most difficult to treat. This is due to the fact that the chondral tissue is subject to constant stimuli; moreover, it has a low potential for repair due to its vascular and lymphatic circulation deficiency.[1,2]

Among the currently available surgical treatment options, one that presents encouraging results is the autologous chondrocyte implant described in 1994 by Brittberg et al., in which, after collection of healthy cartilage, cell expansion and culture are performed and, at a second time, the chondrocytes are implanted in the chondral lesion.[3,4] Due to the limitations for this procedure, such as unavailability and degeneration of donor cartilage, problems with *in vitro* chondrocyte expansion, and its high cost, viable optional forms have been sought to treat this important joint lesion.[1,5] Thus, mesenchymal stem cells (MSCs) have been identified as a good treatment option. Among the various sources of production, adipose-tissue derived MSCs (AD-MSCs) are highlighted, as they present potentiality for regeneration and differentiation in cartilaginous tissue, are available in large amounts in the body and can be obtained with easy and non-invasive techniques.[6–8] Hyaluronic acid is considered an excellent vehicle for MSCs in tissue repair, due to its viscosity and physicochemical properties, and it has gained prominence in tissue bioengineering[9,10] (Table 1). Nonetheless, no data assessing the potential of MSCs when exposed to different hyaluronate molecular weights, brands, and viscosities are available.

This study is aimed at evaluating *in vitro*, in a direct way, the cytotoxicity of different brands of hyaluronic acid on MSC lineages obtained from human adipose tissue.

Material and methods

Ethical considerations

All human tissue samples for MSC isolation were obtained after the informed consent form was signed by the donor or guardian, in agreement with the Research in Human Beings Ethics Committee of the University Hospital and Institute of Biosciences of the University of São Paulo (protocol no. 040/2005).

Collection, isolation, and expansion of MSCs

AD-MSCs were isolated using methods previously described by the present group.[11–14] Adipose tissue was collected in cesarean sections from the abdominal subcutaneous region of the patient. After collection, the samples were stored in a sterile flask and transported to the laboratory in thermal boxes with temperature control between 4 °C and 24 °C. All samples were processed within 48 h.

Briefly, to isolate the AD-MSCs, the adipose tissue samples were washed in phosphate-buffered saline (PBS) 1x, pH 7.4, with 100 U/ml penicillin and 100 μg/ml streptomycin (Life Technologies), and then dissociated with 0.075% GMP (Serva) collagenase at 37 °C for 30 min. Then, the infranatant was centrifuged at 300 × g for 5 min, and the cell pellet formed was plated on culture bottles, following a ratio of 1000–3500 cells per cm^2.

For *in vitro* expansion of the AD-MSCs, the animal serum-free MSC culture system StemPro MSC SFM (Thermo Fisher Scientific) was used according to the supplier's recommendations (proliferation medium). Each lineage was maintained at 37 °C in a humidified atmosphere with 5% CO_2 until reaching at least 1×10^6 cells to be cryopreserved in liquid nitrogen at −196 °C with the StemPro MSC SFM kit. To achieve the