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Original article

# Mesenchymal stromal cells from bone marrow treated with bovine tendon extract acquire the phenotype of mature tenocytes☆

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ABSTRACT

*Objective:* This study evaluated *in vitro* differentiation of mesenchymal stromal cells isolated from bone marrow, in tenocytes after treatment with bovine tendon extract.
*Methods:* Bovine tendons were used for preparation of the extract and were stored at −80 °C. Mesenchymal stromal cells from the bone marrow of three donors were used for cytotoxicity tests by means of MTT and cell differentiation by means of qPCR.
*Results:* The data showed that mesenchymal stromal cells from bone marrow treated for up to 21 days in the presence of bovine tendon extract diluted at diminishing concentrations (1:10, 1:50 and 1:250) promoted activation of biglycan, collagen type I and fibromodulin expression.
*Conclusion:* Our results show that bovine tendon extract is capable of promoting differentiation of bone marrow stromal cells in tenocytes.



## Células mesenquimais do estroma da medula óssea tratadas com extrato de tendão bovino adquirem o fenótipo de tenócitos maduros

*Palavras-chave:*
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RESUMO

*Objetivo:* O estudo avalia a diferenciação *in vitro* das células mesenquimais isoladas do estroma da medula óssea em tenócitos após tratamento com extrato de tendão bovino.
*Métodos:* Tendões bovinos foram usados para confecção do extrato e estocados a −80 °C. Células mesenquimais do estroma da medula óssea (BMSCs) de três doadores foram usadas para os testes de citotoxicidade por MTT e diferenciação celular por qPCR.
*Resultados:* Os dados mostram que células mesenquimais do estroma da medula óssea tratadas por até 21 dias em presença do extrato de tendão bovino diluído em concentrações

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crescentes (1:10, 1:50 e 1:250) promovem a ativação da expressão de biglican, colágeno tipo I e fibromodulina.

*Conclusão:* Nossos resultados mostram que o extrato de tendão bovino é capaz de promover a diferenciação das BMSCs em tenócitos.



## Introduction

Tendons are a specialized type of tissue composed of tenoblasts and tenocytes, which are embedded in an extracellular matrix mostly composed of type I collagen. Tenocytes only have limited potential for proliferation and thus confer low regenerative capacity on tendons.[1,2]

Tendon injuries constitute a serious problem within orthopedic practice and generate high costs for the public healthcare system, as well as having an impact on the quality of life of the patients affected. Although regeneration is the aim of the clinical treatments used, the methods currently available continue to be ineffective. Thus, tendon dysfunctions lead to definitive physical incapacity.[3–5]

Mesenchymal cells isolated from bone marrow stromal cells (BMSCs) are known to be a promising therapeutic option within the field of cell therapy and bioengineering of musculoskeletal tissues.[5–8] Their use in association with synthetic biomaterials has been proposed as an option for modern treatments aiming to toward tendon reconstruction, using an allograft, autograft or xenograft.[9,10] Use of autologous BMSCs biosynthetic grafts has the aims of improving the results from conservative surgery and reducing the time taken for the pre-injury biomechanical properties to be restored.[11] Furthermore, the low immunogenicity of BMSCs makes it possible to use them allogeneically and minimizes the need for immunosuppression of the receptor.[12]

Despite the significant therapeutic potential of BMSCs, little is yet known about the mechanisms and signaling pathways involved in determining that BMSCs will differentiate toward a tenogenic route, or in relation to progression of their differentiation. Considering that BMSCs seem to respond to stimuli that are present in extracts from healthy mature tissues and have specific phenotypic characteristics,[13,14] we developed the hypothesis that tendon extracts might induce differentiation of BMSCs into tenocytes. Thus, the present study had the objective of evaluating the influence of treatment of human BMSCs with different concentrations of bovine tendon extract, on *in vitro* differentiation toward a tenocytic route.

## Material and methods

### *Isolation and expansion of mesenchymal cells from the stroma of human bone marrow*

BMSCs were isolated from surgical waste that originated from hip arthroplasty procedures on five patients (two men and three women) aged 45–60 years, who did not present any comorbidities. Informed consent was obtained from all of these individuals after approval of the study protocol by the institutional ethics committee. After the samples had been collected in the surgical center, they were stored in sterile flasks containing Iscove's modified Dulbecco's medium (IMDM; Sigma–Aldrich, St. Louis, MO, USA), supplemented with 20% bovine fetal serum (BFS; Gibco, Grand Island, NY, USA), at 4 °C for not more than 18 h. To isolate the total cellular fraction, the bone marrow was resuspended in phosphate-buffered saline (PBS) solution and was mechanically dissociated from any bone fragments. The cell suspensions thus obtained were collected in 50 mL tubes and were centrifuged at 836 × *g* and 4 °C for 5 min. The cells were then resuspended in 50 mL of IMDM supplemented with 20% BFS and were counted using a Neubauer chamber. Following this, $6 \times 10^5$ mononuclear cells were distributed in culturing flasks of volume 75 $cm^2$, in 10 mL of IMDM with 20% BFS, and were maintained at 37 °C under 5% $CO_2$. Three days later, the non-adherent fraction was removed by means of lavage with PBS and the culturing medium was changed. After a further 14 days, the cells were removed using a solution of 0.125% trypsin and 0.78 mM EDTA and were expanded.

### *Preparation of bovine tendon extract*

Five bovine calcaneal tendons were obtained. They were macerated mechanically and then were ground up using an electric blender of power 20 W, in the proportions of 1 g of tissue to 2 mL of IMDM, without BFS.[15] The tissue extract was°C centrifuged at 836 × *g* and 8 °C for 5 min and was then stored at −80 °C for a maximum of two months.

### *Analysis of cell viability using the MTT method*

BMSCs were cultured on 24-well plates, at a density of $2.5 \times 10^4$ cells/well and were treated with bovine tendon extract diluted in the proportions of 1:10, 1:50 or 1:250 (v/v) in IMDM supplemented with 10% BFS. Cell viability was assessed 24, 72, 120 and 168 hours after the treatment, in the presence of MTT (thiazolyl blue tetrazolium bromide; Sigma–Aldrich) at a concentration of 25 mg/mL. Equal concentrations of dimethyl sulfoxide (DMSO) were used as a negative control. Colorimetric evaluation was performed at a wavelength of 550 nm, using the SIRIO S SEAC reader (Burladingen, Germany).

### *Analysis of gene expression using qPCR*

BMSCs were cultured under the different experimental conditions described above (1:10, 1:50 or 1:250, v/v), for 7, 14 or 21 days. The cells were then washed and the total RNA