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COMPARATIVE STUDY OF TWO MUSCLE DERIVED STEM CELL ISOLATION PROTOCOLS IN RATS TO STRESS URINARY TREATMENT

Hypothesis / aims of study

In the last few years, several reports of MDSC urethral injection has been described as an alternative to stress urinary incontinence treatment. There are different protocols described to cell isolation. However, it has not been evaluated the difference between these cells isolation methods. The aim of the present study is to compare two different protocols to isolate smooth muscle derived stem cell (MDSC).

Study design, materials and methods

Tissue procurement: The methodology and the animals used are all in accordance with the research ethics committee of Universidade Federal de São Paulo. A section of approximately 0,5 cm^3 was harvested from the quadriceps muscle of male adult Wistar rats. The piece was transported in HBSS solution (Hank's Balanced Salt Solution) with 2% of antibiotic.

Muscle derived stem cell isolation:

Potocol 1 – Fragments of the muscle were treated with 0,046% of collagenase type IA for 2 hours at 37°C. Then, the cell culture was maintained for 72 hours. After this period, the cells were washed with HBSS and incubated with Dulbecco's Modified Eagle Medium (DMEM) containing 10% of Fetal Bovine Serum (FBS) for 2 hours. The cells were then washed and incubated again in DMEM with 10% FBS for cellular growth.

Protocol 2 – Muscle segments were treated first with a solution of 0,2% collagenase type IA for 1 hour and 30 minutes, then treated with a 0,1% trypsin solution for 30 minutes at 37°C. The muscle cells were extracted and cultured in DMEM with 10% FBS and 10% Horse Serum (HS) for 1 hour. All non-adherent cells were preplated to another cell culture flask for one hour. After 24, 48 and 96 hours, non-adherent cells were preplated to a new flask and incubated at 37°C.

After isolation, we determined the growing rate, morphology and the number of cells obtained in each protocol. The MDSCs were tested by means of flow cytometry to identify the following markers: CD90, CD29, CD44, CD45 and smooth muscle alpha actin (alpha actin).

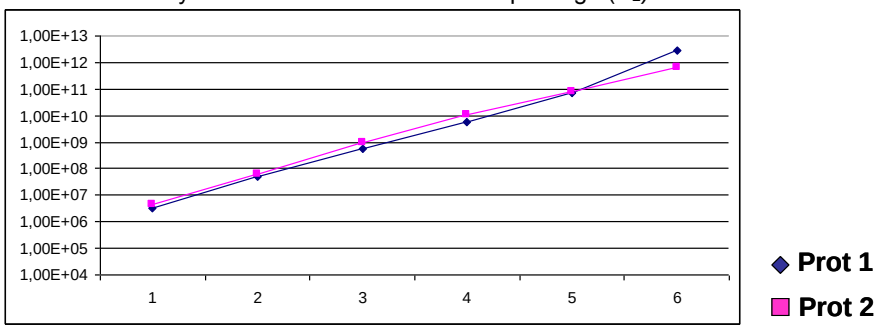
To demonstrate the potential to differentiation, the MDSC at second passage (P2) were induced to differentiation using osteogenic and adipogenic media. Cellular differentiation were analysed by histochemistry and immunofluorescence.

Results

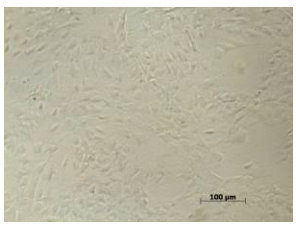
The growing rate and the number of cells obtained in each protocol was similar, as shown in Figure 1. However, we observed different morphology between the two cells isolation protocol, as shown in fig 2.

Both MDSC isolation protocol provide a cell population with potential to adipogenic (fig 3) and osteogenic (Fig 4).

The flow citometry for rat's MDSC in the second passage (P_2) is demonstrated in Table 1.



Tab1 - comparison of the 2 growing rates during the cell culture.


A

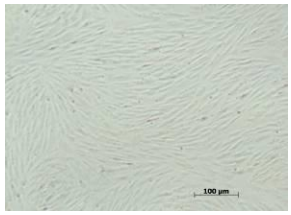

B

Fig 2 - Differences in cell morphology between two methods for MDSC (A = protocol 1, B = protocol 2). demonstrated that protocol 1's cells are more fusiforms and they don't aggregate into colonies as the cells of protocol 2.

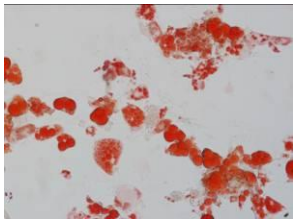
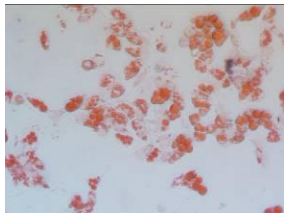
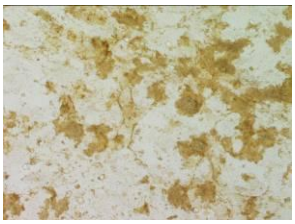
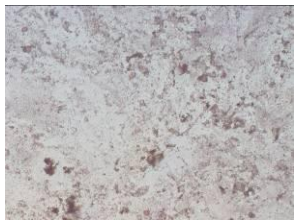
 A  B

Fig 3 - Adipogenic differentiation demonstrated by Oil Red O staining (A = protocol 1, B = protocol 2).

 A  B

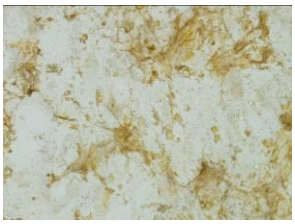
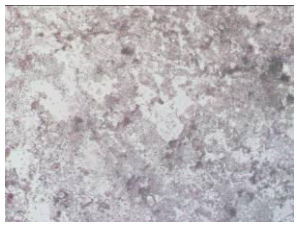
 C  D

Fig 4 - Osteogenic differentiation demonstrated by Von Kossa and Alizarin Red S staining (A, B= protocol 1, C,D= protocol 2)

	CD 90	CD 29	CD 44	ASMA	CD 45
Protocolo 1	+	+	+	+	-
Protocolo 2	+	+	+	+	-

Table 1. Flow cytometry comparative results. Protocol 1 were positive for CD90 (99%), CD29 (99%), CD44 (29%) and ASMA (14%); and negative for CD45. The cells of protocol 2 were positive for CD90 (96%), CD29 (97%), CD44 (21%) and ASMA (68%); and negative for CD45.

Interpretation of results

Different protocols provide a multipotent cells population which present specific stem cell makers. This study also demonstrated that the cells from both protocols can be differentiated into different types of cells.

Concluding message

Our study confirms that MDSC can be easily isolated from muscle biopsy. There were no substantial differences between two different MDSC isolation protocols.

Specify source of funding or grant	**FAPESP and CAPES**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Research Ethics Committee of Universidade Federal de São Paulo**