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Could the Coculture of Skeletal Myoblasts and Mesenchymal Stem Cells Be a Solution for Postinfarction Myocardial Scar?

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ABSTRACT

Currently two lines of research have been proposed for treatment of heart failure in an attempt to address its main cause: skeletal myoblast (SM) transplants, which increase the contractile muscular mass, and mesenchymal stem cell (MSC) transplants, which increase neoangiogenisis. The objective of this study was to establish methods whereby cocultures of SM and MSC proliferate and expand, making possible the interaction of these cell types prior to their transplantation to the myocardium. Seeking to support the survival of these cells after myocardial transplantation and achieve subsequent functional improvement, SM and MSC from 10 rats were isolated and cultivated in DMEM medium supplemented with 15% fetal calf serum, 1% ATB, and growth factors. Following plating in variable proportions of satellite cells/mononuclear cells namely 2:1, 1:1, 1:2, morphological observations were made regarding cell survival, adhesion to substrate, and confluence. After 48 hours nonadherent cells were aspirated from the flasks, leaving the adherent cells, SM, and MSC. The better level of cell proliferation was observed with the proportion 2:1 cocultivated at a concentration of 5×10^5/mL for 14 days. The results were satisfactory; the cell production was up to 10^8, increasing the chances of transplant success after myocardial infarction. Transplants with this model are ongoing.

MANY SURGICAL TECHNIQUES have been used to restore the geometry of the heart without contributing to the primary cause of the disease, that is the loss of myocardial contractile function. The disadvantage of these techniques (with the exception of cardiac transplant) is that they aim to restore ventricular function without treating basic cause, adult myocardiocyte loss. The aim of experimental transplants of skeletal[1] and smooth[2] muscle cells using fetal myocardiocytes[3] or adult cardiac cells,[4,5] in cases of myocardial infarction is to improve the healing in the damaged area, thereby avoiding the development of dilated myocardiopathy, which can lead to congestive heart failure.

The use of MSC has several advantages: these cells are found in the bone marrow (BM) of patients of all ages, are easily collected, and make possible an autologous source, which prevents rejection problems. Experimental studies show that the transfer of BM cells, after cultivation and treatment in the laboratory, induces expression of muscular proteins in the damaged area and consequently improves cardiac function.[6]

According to the literature, skeletal myoblasts transplanted into the myocardium of cryolesion models or myocardial infarcts improve function, and contribute to the treatment of heart failure. However, most of these cells die from lack of nutrition. On the other hand, transplanted MSC are pluripotential and seem to favor angiogenesis, since they have endothelial cell precursors as well myogenics in their pool, thereby improving cardiac function. Both SM and MSC are attractive therapeutic tools.[7] In research models as cited above these cells are transplanted after mass cultivation and expansion in addition to being isolated. These encouraging results suggest the need for continuing research into cellular cocultivation for transplantation for the treatment of myocardial disease.[8]

The objectives of the study were to develop an MSC and SM cocultivation method, using both cell types for proliferation and expansion as well as facilitating interaction of these cells before myocardial transplant.

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Table 1. Mononuclear Cell and Satellite Cell Harvest

Group	Puncture: number of rats	Mononuclear cells	Sacrifice; number of rats	Mononuclear cells	Number of rats (muscle)	Number of satellite cells
I	12	3×10^7	1	2×10^7	3	1.4×10^8
II	12	3.57×10^7	1	1.34×10^7	2	5.12×10^7
III	—	—	1	3.55×10^7	3	2.12×10^8
IV	—	—	2	3.93×10^7	2	1.7×10^7
V	—	—	2	6.78×10^7	3	3.3×10^7
VI	—	—	2	3.28×10^7	2	1.28×10^7
VII	12	3×10^7	3	20.44×10^7	3	4.67×10^7
VIII	10	5.04×10^7	2	11×10^7	2	2×10^7
IX	10	3.9×10^7	2	16.7×10^7	2	1.18×10^8
X	—	—	2	9.6×10^7	4	1×10^7

METHODS

The isolation of SM from sacrificed neonatal rats used the enzymatic dissociation technique reported by Delaporte et al,[9] employing muscular fragments from the four limbs. The isolation of MSC of BM was performed by two different harvesting techniques; a puncture-aspiration and a sacrifice of animal. The collected material was processed using a Ficoll-Hypaque density gradient (d = 1.077) according to Boyüm.[10] The isolated cells were plated as described below. Assays were performed in 25-cm^2 flasks with satellite cells/stem cells initially plated in variable proportions, 2:1; 1:1; 1:2. Morphological observations included cell survival, adhesion to substrate, and confluence. The cells were then cultivated for 14 days in coculture, in a proportion of 2:1, nearly 5×10^5/mL. The DMEM culture medium contained 15% FCS, 1% ATB, and 10 ng/mL IGF-I (insulin growth factor). The cultures were maintained in an 5% CO_2/37°C incubator.

RESULTS

The 2:1 ratio of satellite cells/stem cell was chosen because upon morphological observation the vials were more confluent in this proportion. Ten cocultures were performed, but only four groups (I, II, VII, and IX) were transplanted (Table 1 and 2).

DISCUSSION

It is concluded that cultivation of two distinct cell groups simultaneously may optimize the results already obtained from the transplantation of individually cultivated SM and MSC. We have demonstrated the possibility of coinjection of cultured cells preceeded by cocultivation may facilitate autologous transplantation, and transplants with this model are ongoing.

Table 2. Coculture

Group	Transplantation	Number of cells
I	10 syringes with 7.5×10^6	2.5×10^7
II	2 syringes with 7.5×10^6	7.5×10^7
VII	9 syringes with 1×10^7	5.4×10^8
IX	6 syringes with 5×10^6	3.3×10^7

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