



Aneural Culture of Rat Myoblasts for Myocardial Transplant

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ABSTRACT

Due to the peculiar characteristics of skeletal muscle, myoblast transplants have emerged as a therapy for cardiomyopathy, particularly after myocardial infarction. The objectives of this study were to define the mean time of cultivation necessary to obtain a cellular concentration of 10^6 to expand the mass for transplant, and to identify the proliferation phase of myoblasts. Ten myoblast cultures were performed using newborn Wistar rats. The isolation method used enzymatic dissociation in culture medium (HAM-F12 and 199) supplement with basic-fibroblast growth factor (b-FGF) and insulin growth factor (IGF-I). The mean cultivation time to obtain the desired concentration of 10^6 was 7 days, with expansion of up to 10^8 /g. When b-FGF was used, the cellular yield was approximately 10^7 , with IGF-I the cellular yield was approximately 10^8 , independent of the medium. We concluded that IGF-I is the better option for mass cellular expansion of myoblasts for application in myocardial transplants.

STRIATED skeletal muscle originating from mesoderm regenerates from myogenic mononuclear cells that exist in a satellite position in the muscle fibers. The satellite cells attach themselves without fusing to the basal membrane.¹ They may be isolated by explantation-re-explantation and enzymatic dissociation techniques.² Once in culture, viable myogenic cells proliferate and then fuse, differentiating in vitro into multinuclear, excitable structures—myotubes—and subsequently into muscle fibers.³ Two types of cells have been isolated in muscle culture: myoblasts with a fusiform aspect and fibroblasts with a widened aspect.

The greater the number of cells in the initial dissociation, the greater the number of myoblasts, which are the viable elements to fuse and form myotubes and, later, muscular syncytium. A culture is considered rich in myoblasts when fusion is observed among 80% of cells. In contrast, fibroblasts occasionally align in a pre-fusion phenomenon but do not fuse. The fusion index is a criterion used for cellular differentiation. The fusion index is a percentage ratio between the total number of nuclei in the myotubes versus the sum of nuclei in the myotubes and myoblast nuclei. A percentage greater than 80% indicates a good fusion index.

The following factors can influence both proliferation and differentiation: epidermal growth factor, basic-fibroblast growth factor (b-FGF), platelet derived growth factor, insulin growth factor (IGF-I),⁴ and transforming growth factor.⁵ Corticoids may stimulate (or inhibit) proliferation in different cell types, accelerate maturation,⁶ upregulate

the expression of acetylcholine receptors,⁷ and also increase dystrophin levels “in vitro”.⁸ Autologous myoblast transplants have emerged as an option to recover injured areas considering that adult cardiomyocytes show a low rate of cell division and are incapable of regenerating tissue, and that fetal cardiomyocytes show ethical restrictions for use in clinical trials.⁹ Therefore, it is necessary to optimize mass production by finding the best growth factor for these cells.

Our objectives were to define the mean cultivation time to obtain a desired cellular concentration of 10^6 with mass expansion aiming at myocardial injections. Furthermore, we sought to identify the proliferation phase of this specific cell type.

METHODS

Ten myoblast cultivation procedures were performed. Muscle isolation was performed according to the Delaporte technique.¹⁰ The culture was maintained for 14 days. Culture media were changed every 48 hours. HAM F-12 or 199 medium were supplemented with 15% fetal calf serum, 1% antibiotic, and 10 ng/mL b-FGF or IGF-I. Cultures were trypsinized when they reached cellular confluence.

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Table 1. Myoblast Culture: Newbauer Chamber Count

| Group | No. of Rats | Age of Rats | Culture Medium | Growth Factor | Total Passages | Total Cells in 01 Week |
|-------|-------------|-------------|----------------|---------------|----------------|------------------------|
| I | 2 | 6 d | 199 | b-FGF | P3 | 2.1×10^7 |
| II | 2 | 3 d | 199 | b-FGF | P2 | 4.6×10^7 |
| III | 2 | 8 d | 199 | b-FGF | P2 | 2.5×10^7 |
| IV | 2 | 6 d | F12 | b-FGF | P2 | 8.8×10^7 |
| V | 2 | 3 d | F12 | IGF-I | P2 | 1.3×10^8 |
| VI | 2 | 2 d | F12 | IGF-I | P2 | 5.5×10^8 |
| VII | 2 | 2 d | F12 | IGF-I | P2 | 1.85×10^8 |
| VIII | 2 | 4 d | 199 | IGF-I | P2 | 1.13×10^8 |
| IX | 2 | 9 d | F12 | IGF-I | P2 | 4.2×10^8 |

Note: In cultures using b-FGF, the number of cells was approximately 10^7 . In cultures using IGF-I, the number of cells was approximately 10^8 , regardless of the medium. Cultivation was performed for 14 days. One culture (group X) was contaminated.

RESULTS

Our results showed a mean cultivation time to obtain the desired cellular concentration of 10^6 for transplant, with mass expansion of up to $10^8/g$ of muscle. Table 1 shows the obtained results. The mean time of cultivation is 1 week to obtain 10^6 cells. The proliferation phase occurred between the 3rd and 11th day of cultivation.

DISCUSSION

Cell death remains a major limitation of cell transplantations and up to 90% of cells may die after injections. Various factors contribute to death, including physical strain during injection, inflammation, apoptosis, and hypoxic environment.¹¹ Many authors have demonstrated the action of both b-FGF and IGF-I and agree with their capacity of proliferation effects and also their mechanisms,¹² but do not emphasize the aspect of the optimization of cell survival if we could offer to enhance the number of cells for transplantation.

CONCLUSION

IGF-I is the best option for mass cellular expansion of myoblasts for use in myocardial cellular transplant. The proliferation phase occurs between the third and the 11th day of cultivation.

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