Porcine skeletal muscle culture: a basic method for physiological and biochemical examinations on the cellular level

M.J. SEEWALD (1), H. BRINKMEIER (1), H.M. EICHINGER (2) and R. RÜDEL (1)

(1) Abt. für Allgemeine Physiologie, Universität Ulm, 7900 Ulm, Germany;
(2) Versuchsstation Thalhausen, TU München, 8051 Kranzberg, Germany

SUMMARY

We have developed a method for establishing porcine skeletal muscle cultures from muscle specimens collected shortly after slaughtering. The samples were stored overnight, then the tissue was mechanically and enzymatically dissociated for the isolation of the satellite cells. A culture medium consisting of 83% Ham's F-12 medium, 15% FCS and 2% CEE was found to be most suitable for cell proliferation. Both MEM or HME medium with little amounts of serum (4-5%) were effective in supporting the differentiation of myoblasts to multinucleated myotubes. In general, this method provides porcine skeletal muscle cells that are suitable for the electrophysiological and biochemical investigation of cellular mechanisms, such as genetic alterations or growth characteristics in pigs. Since the necessary muscle specimens are easily and economically obtained *post mortem*, the method is a very suitable one with respect to animal conservation.

INTRODUCTION

Cell culture has been a useful tool for the investigation of differentiation and growth of mammalian skeletal muscle. Basic biochemical and physiological properties of developing muscle, including myoblast fusion, appearence of muscle-specific proteins, development of the electrical properties and even innervation, have been succesfully investigated with cultured muscle cells from mouse (HAUSCHKA et al. 1979) rat (GARRELS, 1979) and man (BLAU and WEBSTER, 1981). In addition, muscle culture has been used to clarify the pathomechanisms in hereditary muscle disorders (BLAU and WEBSTER, 1981; MEHRKE et al. 1985; RÜDEL et al. 1989). The effects of growth factors on muscle development have been studied with a rat muscle cell line (ALLEN and BOXHORN 1987) and with embryonic pig muscle (PAMPUSH et al. 1990). The aim of the present study was the establishment of skeletal muscle cultures with adult pig muscle as the source of myogenic precursor cells.

MATERIAL AND METHODS

Muscle samples were obtained from 5 German Landrace pigs ranging in age from 1 week to 5 months. Shortly after stunning and exsanguination approximately 2 g of the m. supraspinatus were removed and stored overnight in Hank's salt solution with 2 mM HEPES. The tissue was mechanically dissected into small pieces and further dissociated by different enzymatic treatments: i) fragments were incubated 4 times for 15 min at 36 °C in a shaking water bath with 1.12 mg/ml trypsin (DIFCO LABORATORIES, Detroit, Mi, U.S.A.) and 1 mg/ml collagenase (Type 1, SIGMA, St. Louis, Mo, U.S.A.), and after each incubation the cell suspension was harvested; ii) alternatively, an enzyme composition of 2mg/ml protease (Type X, SIGMA) and 1.5 mg/ml collagenase (Type V, SIGMA) in Hank's salt solution

was used for about 1 hour of incubation at 36 °C. The cell suspension was filtered (pore size 20 m) for the removal of the remaining tissue fragments, and subsequently centrifuged for the harvesting of the isolated satellite cells.

The cells were seeded in plastic tissue culture dishes (GREINER, # 690160 or # 658170, Frickenhausen, F.R.G.) at a density of about 1000/cm^2 and cultured in three different conditions:

1) 83% Ham's F12 medium, 15% FCS (fetal calf serum, GIBCO) and 2% CEE (chick embryo extract, GIBCO);
2) HME with 2% HS (horse serum, GIBCO) and 2% FCS;
3) a special medium with D-valin instead of L-valin for the inhibiton of fibroblast growth, containing 83% MEM, 15% dialysed FCS and 2% CEE.

The media were renewed every 3 days. When confluency was reached, the media were changed to MEM supplemented with 5% HS (condition 1 and 3) or left unchanged (condition 2).

RESULTS

The enzymatic dissociation of adult muscle tissue yielded 2-3 times 10^5 satellite cells/g fresh weight. There was no significant difference in the yield when the dissociation solution was made up of either trypsin and collagenase or protease and collagenase. After the cells were seeded in the different growth media they proliferated and reached confluency at a time dependent on the individual experiment and the culture conditions. On average, the cultures reached confluency within 7 d in Ham's F12 medium and within 10 d in MEM and in HME medium (Figure 1a).

Approximately two days after the medium was changed to MEM with 5% HS or HME with 2% FCS and 2% HS, some of the cells fused to multinucleated tubes (Figure 1b) indicating the presence of muscle precursor cells in the culture. Most of the cells (about 90%) remained unfused although they continued to proliferate slightly for some days. This resulted finally in a densely packed lawn of fibroblast-like cells.

Unexpectedly, the special MEM-medium (condition 3) designed to suppress fibroblast growth did not prevent the proliferation of mononucleated fibroblast-like cells.

DISCUSSION

This study demonstrates that it is possible to obtain muscle cultures from adult swine skeletal muscle. We chose pigs with a wide variation of age and weight for sampling. Surprisingly, the yield of isolated satellite cells was always the same. One possible explanation might be that swine are already pretty mature at birth and leaving the nest. This implies that muscle differentiation and development have to be completed to a certain level which would explain the similarities in our findings. A major problem, obvious in all our experiments, was the high amount of non-fusing fibroblast-like cells contaminating the cultures. The introduction of the L-valin-free MEM medium for the selective inhibition of fibroblast growth had not the expected success. Since this medium was originally developed for mice and rats, differences between these two species and swine in amino acid metabolism may explain this result. The aim of future experiments should be the decrease of the fibroblast content in the cultures. It may be achieved by selective inhibition of fibroblast growth via a cell-sorting procedure after tissue dissociation or by cloning of muscle cells (HAUSCHKA et al. 1979; MEHRKE et al. 1985).

CONCLUSION

The aim of this study was to make use of muscle collected *post mortem* from adult animals for the isolation of satellite cells. With some practice the samples were obtained in less than 3 minutes. In general, this procedure can be organized and adapted to almost every slaughterline which means that the material is easily and economically obtained. Another important reason for *post mortem* sampling is the fact that this procedure is the best with respect to animal conservation.

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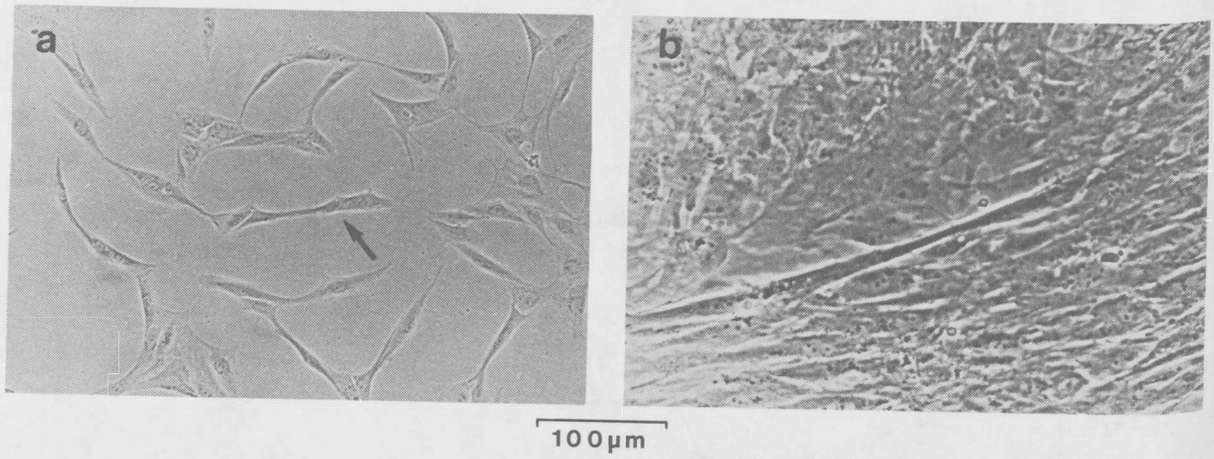

Figure 1. a) proliferating cells 4d after plating in Ham's F-12 medium, mainely myoblasts and fibroblasts. First fusion of cells occur (arrow). b) a multinucleated myotube in a dense layer of non-fusing cells 14d after plating and 4d after switching the medium to MEM with 5% HS.