ABSTRACT: Studies on the effects of time and passage on porcine primary muscle cell cultures and methods to purify myoblasts were conducted using flow cytometry and fluorescence-activated cell sorting (FACS). Primary muscle cells cultured on single plates revealed a small cell (<10 mm diameter) population consisting of 90% desmin-positive myoblasts and a large cell (≥10 mm diameter) population containing desmin-positive myoblasts and nonmyoblasts. The small myoblasts were detectable up to 28 days but after cell sorting and passage, they became indistinguishable from the large myoblast population. This indicates that pig muscle contains small self-renewing myoblasts similar to humans, that become larger when induced to proliferate. A human myoblast-specific monoclonal antibody allows FACS of both large and small myoblasts from primary cells within 2 days of culture and independent of passage. These characteristics of porcine myoblasts indicate that the pig may be a suitable large animal model for myoblast-mediated gene transfer.
 Muscle Nerve **22:** 43–50, 1999

ISOLATION OF TWO POPULATIONS OF MYOBLASTS FROM PORCINE SKELETAL MUSCLE

JOHN R. BLANTON, Jr, MS,[1] ALAN L. GRANT, PhD,[1] DOUGLAS C. MCFARLAND, PhD,[2] J. PAUL ROBINSON, PhD,[3] and CHRISTOPHER A. BIDWELL, PhD[1]

[1] Department of Animal Sciences, Purdue University, 1026 Poultry Science Building, West Lafayette, Indiana 47907, USA
[2] Department of Animal and Range Sciences, South Dakota State University, Brookings, South Dakota 57007, USA
[3] Department of Basic Medical Sciences, Purdue University, West Lafayette, Indiana 47907, USA

Accepted 24 July 1998

Swine are a suitable large animal model for human biomedical research because their organs and tissues are similar in size and function to adult humans. Pigs have been used to evaluate gene therapy for repair of vascular smooth muscle using adenoviral vectors[9] and for myocardial repair by myoblast transplantation.[13] A transgenic pig model has recently been developed for retinitis pigmentosa.[11] An additional advantage to using pigs in biomedical research is that contemporary pigs can grow to greater than 100 kg within 10 months from conception. This phenomenal growth rate and the accompanying skeletal muscle hypertrophy is supported by a population of muscle satellite cells (myoblasts) located between the basal lamina and the sarcolemma. These myoblasts will fuse and donate nuclei to existing muscle fibers during hypertrophy and muscle repair.[10] This study was initiated to develop the pig as a large animal model for myoblast-mediated gene transfer to study growth and development.

A major limitation in developing a porcine model is the isolation and purification of myoblasts. Primary muscle cell preparations are often contaminated with a significant number of nonmyogenic cells, such as fibroblasts.[1,15] Myoblasts are capable of finite proliferation in vitro,[17] and traditional clonal isolation techniques will expend much of the pig myoblasts' limited proliferative capacity (unpublished observations). Webster et al.[17] utilized a muscle-specific antibody[15] for fluorescence-activated cell sorting (FACS) of myoblasts from primary human muscle cell after in vitro culture. Barrafio et al.[2] developed a technique to isolate human satellite cells from mixed primary human muscle cell prepa-

Abbreviations: EDTA, edetic acid; FACS, fluorescence-activated cell sorting; FALS, forward angle light scatter; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; MEM, minimal essential medium; mRNA, messenger RNA; 90°LS, 90° light scatter; PBS, phosphate-buffered saline; RT-PCR, reverse transcriptase-polymerase chain reaction

Key words: pig; skeletal muscle; myoblasts; flow cytometry; desmin

Correspondence to: Dr. Christopher A. Bidwell

CCC 0148-639X/99/010043-08

rations without cell culture using the forward angle light scatter (FALS) and 90° light scatter (90°LS) characteristics detected by a flow cytometer.

We investigated the effects of time in cell culture and passage on myoblast yield and morphology in mixed primary muscle cell cultures using flow cytometry. The results from these experiments indicated the presence of two populations of myoblasts in pig skeletal muscle and led to the development of methods to isolate porcine myoblasts using FACS based on either cell size or myoblast-specific antibodies. The ability to purify pig myoblasts that retain the majority of their proliferative capacity will enable development of the pig as a large animal model for myoblast-mediated gene transfer.

MATERIALS AND METHODS

Mixed Primary Cell Preparation. Primary muscle cells were prepared by pronase digestion (Calbiochem, San Diego, CA) of the semimembranosus from 2–4-week-old male pigs as described by Doumit and Merkel[4] in accordance with Purdue University animal care and use guidelines. Isolated cell pellets were resuspended in growth medium consisting of minimal essential medium (MEM; Lifetechnologies Inc., Gaithersburg, MD) containing 10% fetal bovine serum (FBS; Harlan Bioproducts, Indianapolis, IN), 1% antibiotic/antimycotic (Sigma Chemical Co., St. Louis, MO), and 0.1% gentamycin (Calbiochem, San Diego, CA). Cell suspensions were filtered through a 250-μm mesh prior to plating on culture dishes coated with 0.1% Type A porcine gelatin (Sigma Chemical Co.).

Clonal Line Isolation and Characterization. Myoblast and fibroblast clonal lines were isolated to validate myoblast-specific antibody binding and to determine flow cytometry parameters for purification of myoblasts from mixed primary cultures. Single cells were isolated from mixed primary porcine muscle cell preparations by a Quixell robotics cell manipulator (Stoelting Co., Wood Dale, IL) and transferred to a 96-well plate. Once clonal lines had established growth, they were transferred to 10-cm tissue culture plates and allowed to proliferate until 70–80% confluent. Clonal cells were grown and split into three culture aliquots for use in fusion assays (first aliquot) and reverse transcriptase-polymerase chain reaction (RT-PCR, second aliquot); the third aliquot was allowed to proliferate in culture and frozen in liquid nitrogen for future flow cytometric analysis. The first aliquot was cultured in the presence of growth medium until 60–80% confluent, at which time medium was replaced with myotube fusion medium consisting of MEM containing 10^{-5} mol/L insulin for 4 days. Monolayers were stained with Geimsa (Sigma Chemical Co.) and myotubes were defined as cells containing three or more nuclei. Fusion index was determined in three microscopic fields and calculated by dividing the total number of nuclei in myotubes by the total number of nuclei in the field.

Poly A+ messenger RNA (mRNA) was isolated from the second culture aliquot and used for RT-PCR to determine the presence of myogenin, α-skeletal actin, and myoD mRNA. Primers for glyceraldehyde-3-phosphate dehydrogenase were used as a positive control for mRNA isolation and complementary DNA synthesis.

Flow Cytometric Analysis. For analysis of live cells by flow cytometry, adherent cells were removed from plates via a 15-min incubation in a 0.5 mol/L edetic acid (EDTA)–phosphate-buffered saline (PBS) solution. To reduce cell clumping, cells were incubated in a 0.53 mmol/L EDTA-PBS solution for 30 min, centrifuged for 5 min at 500 *g*, and finally resuspended in MEM prior to analysis. Some muscle cell preparations were analyzed using a myoblast/myotube-specific monoclonal antibody 5.1H11[14,15] obtained from the Developmental Studies Hybridoma Bank (The University of Iowa, Iowa City, IA). Tissue culture plates containing primary preparations were blocked with 5% bovine serum albumin in PBS for 30 min, followed by a 30-min incubation with 5.1H11. This was followed by a 30-min incubation with a phycoerythrin-conjugated goat antimouse second antibody. To remove cells from the tissue culture plate without disrupting antibody binding, plates were incubated for 15 min in a calcium phosphate-free PBS solution containing 0.53 mmol/L EDTA. Cells were then centrifuged for 5 min at 300 *g* and resuspended in MEM prior to FACS analysis. Propidium iodide was added to all cell suspensions to detect nonviable cells.

A Coulter EPICS Elite flow cytometer (Coulter Corp., Hileah, FL) with a 488-nm argon laser was used to detect FALS, 90°LS, and phycoerythrin emissions at 590 nm of antibody-labeled porcine cells. Fluorescent beads of 10-, 20-, and 40-μm diameters (Fluorospheres Fullbright, Coulter Corp.) were used to calibrate FALS for cell size measurements. Electronic compensation was used to decrease background interference. Following sorting, cells were plated on 10-cm gelatin-coated tissue culture plates for 24 h. The presence of myoblasts in sorted populations was determined by the myotube fusion assay as described above and the presence of the muscle-

specific intermediate filament protein, desmin.[3] Cell monolayers were fixed with 1:1 methanol:acetone and incubated with a desmin monoclonal antibody (ICN Biomedical, Costa Mesa, CA) for 1 h, washed extensively in PBS, and then incubated with a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse secondary antibody. Fluorescent cells and total cells were counted in five microscope fields to determine percentage of desmin-positive cells.

RESULTS

Clonal myoblast lines were identified by the presence of muscle-specific mRNA for myoD, myogenin, and α-skeletal actin, as well as their ability to form myotubes. Nonmyoblast clonal lines were not further characterized. Once clonal myoblasts and nonmyoblasts had been established, fourth and sixth passage cells were analyzed by flow cytometry. The parameters of cell size and nuclear/cytoplasmic ratio were determined by FALS and 90°LS, respectively. When clonal myoblasts and nonmyoblasts were examined individually they produced similar distributions of FALS and 90°LS (Fig. 1A and B). When clonal myoblasts and nonmyoblasts were mixed together, the two separate cell types could not be distinguished (Fig. 1C).

To investigate the possibility that extended growth in cell culture or passage may have changed cell morphology, flow cytometric analysis was conducted on primary porcine preparations prior to first passage and following second passage. The effect of time in cell culture was tested by incubating mixed primary muscle cell preparations on a single plate for 2, 3, 4, 5, 10, 15, and 28 days prior to analysis. Tissue culture plates had become 100% confluent by day 10 and two separate populations were detected by 90°LS and FALS at all time points. The results for days 2, 5, and 28 are shown in Figure 2 (A–C). The small cell population was composed of cells less than 10 μm in diameter, whereas the large cell population was composed of cells greater than 10 μm and less than 20 μm diameter as determined by presized fluorescent beads. Over time, the proportion of cells in the small cell population decreased and the large cell population increased. To determine if passage would affect cell morphology, mixed primary muscle preparations were split and replated two times at a density of 10^4 cells per square

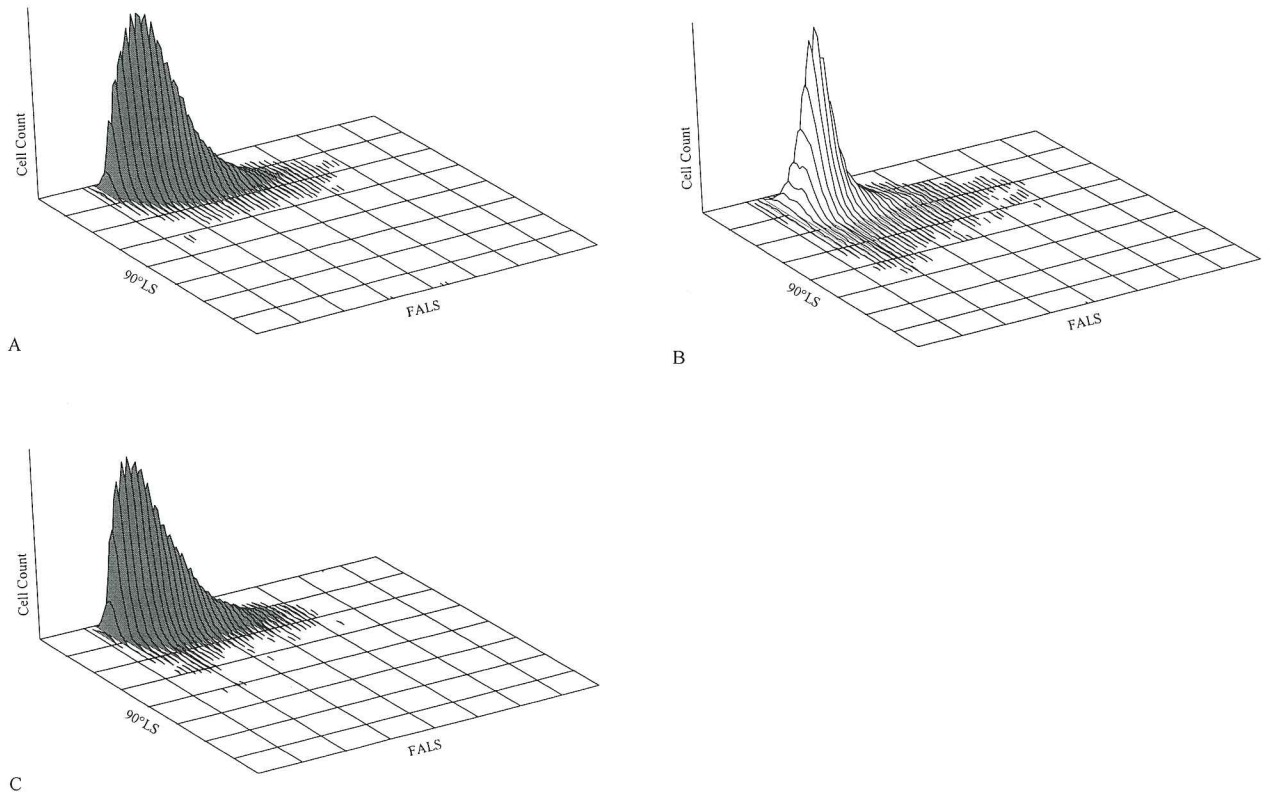

FIGURE 1. Three-dimensional contour plot of porcine clonal muscle cell lines using 90°LS, FALS, and cell count parameters for **(A)** fourth passage clonal myoblasts, **(B)** fourth passage clonal nonmyoblasts, and **(C)** mixed fourth passage clonal myoblasts and nonmyoblasts.

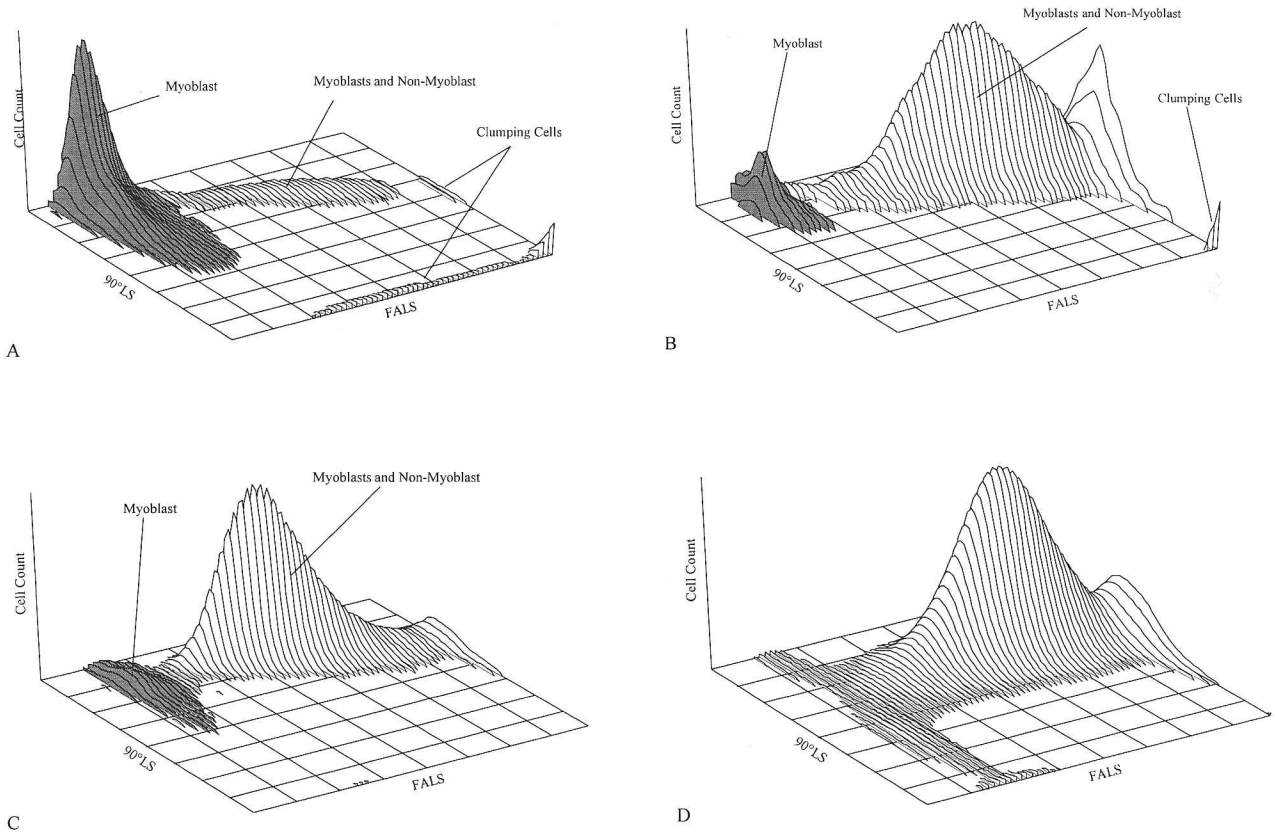

FIGURE 2. Three-dimensional contour plot of mixed primary porcine muscle cell preparations using 90°LS, FALS, and cell count for analysis of cells cultured on a single plate for **(A)** 2 days, **(B)** 5 days, and **(C)** 28 days and **(D)** second passage mixed muscle cell cultures. Forward angle light scatter and 90°LS indicate a small cell (<10 μm: dark shading) population whose numbers decrease relative to the large (≥10μm: no shading) population. After passage, the small cell population was not detected.

centimeter. After the second passage, only the large cell population could be detected (Fig. 2D).

An antidesmin antibody assay and myotube fusion assay were used to determine the proportion of cells in each population that were myogenic. The large and small cell populations were sorted into separate 10-cm tissue culture plates on days 2, 4, 5, and 28. After all incubation periods, the small cell population formed large multinucleated myotubes. Representative myotubes from days 2 and 28 are shown in Figure 3 (A–B). The proportion of the small cell population that fused into myotubes was greatest on day 2 and progressively decreased through day 28 (Table 1). Desmin staining of sorted cell populations confirmed the myotube fusion assay data and indicated that greater than 90% of the cells within the small cell population were myoblasts (Table 2). The majority of the cells in the large cell population detached from the plate or died when cultured in fusion medium, which is indicative of nonmyoblast cell types. Some cells from the large cell population did survive and formed a few multinucleated myotubes, indicating that the large cell population did contain some myoblasts. More than half of the cells in the large cell population expressed desmin, indicating that these cells were a mixture of myoblast and nonmyoblast cell types (Table 2). The proportion of desmin-positive cells was higher in the prefusion large cell population than the postfusion large cell population. This may be the result of losing some myoblasts along with nonmyoblasts in fusion medium.

The 5.1H11 monoclonal antibody was tested for cross-species reactivity to porcine cells using clonal myoblasts and nonmyoblast cells. The antibody bound to four different clonal myoblast cell lines (Fig. 3D) but did not bind to three different clonal nonmyoblasts cell lines (Fig. 3F). The 5.1H11 antibody was subsequently tested in conjunction with FALS and 90°LS for isolation of myoblasts from primary, second passage, and fourth passage muscle cell cultures. Fluorescence-activated cell sorting of 4-day primary cultures showed that both the large and small cell populations were 5.1H11-positive (Fig. 4A) and greater than 80% of all cells were selected (Table 3). Greater than 95% of the two sorted popu-

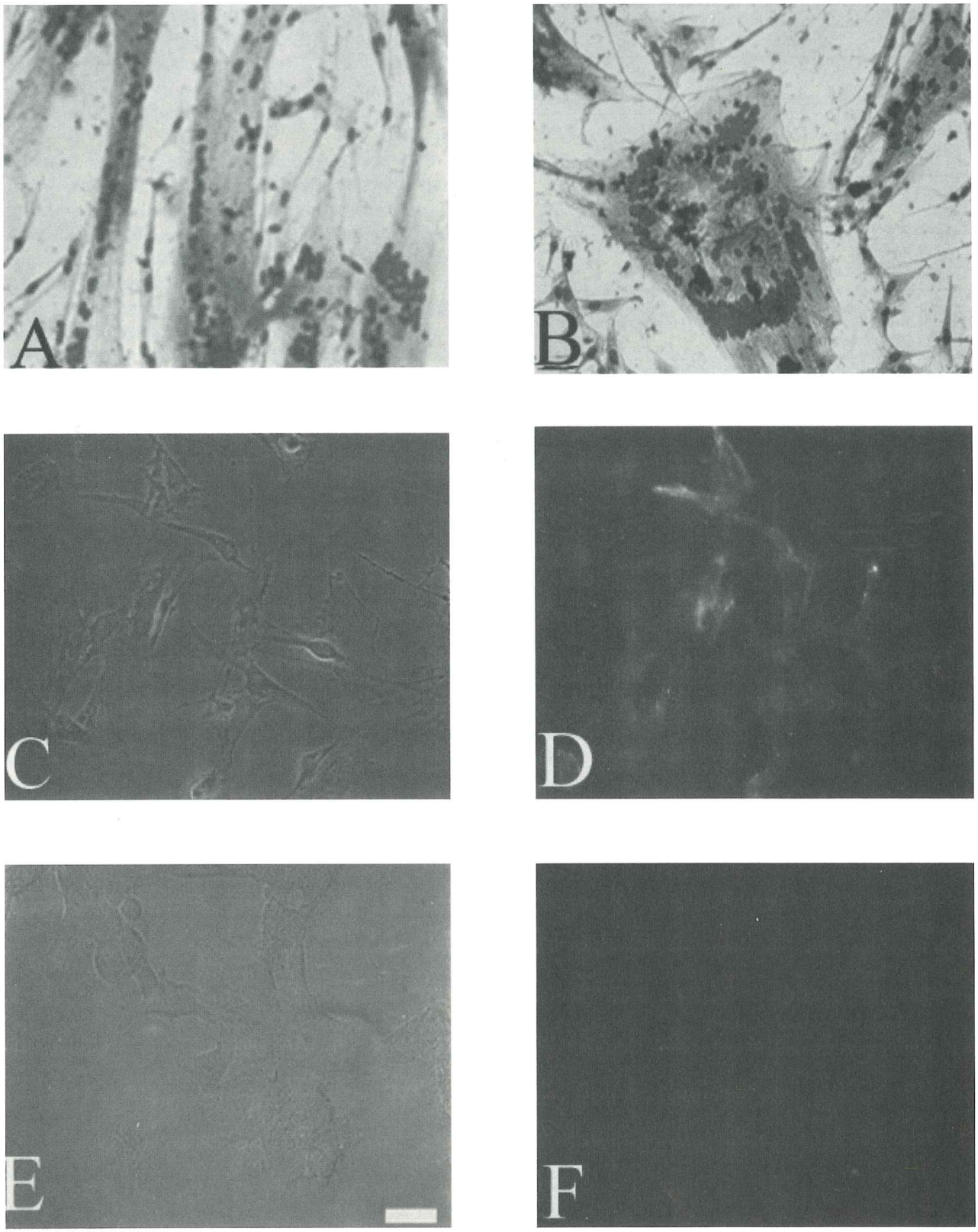

FIGURE 3. Myotube fusion assay of sorted small cell populations isolated from **(A)** 2-day and **(B)** 28-day primary porcine muscle cell preparations. Following sorting, cells were allowed to attach and grow until cultures became 70% confluent at which time fusion medium was added. To detect myotubes, cells were fixed and stained with giemsa. **(C)** Light field view and **(D)** fluorescent detection of clonal myoblasts incubated with 5.1H11 and FITC-conjugated second antibody. **(E)** Light field view and **(F)** fluorescent detection of clonal nonmyoblasts incubated with 5.1H11 and FITC-conjugated second antibody.

Table 1. Myotube fusion percentages for cells sorted by light scatter.*

	Small cell† population	Large cell† population
Day 2‡	92%	14%
Replicates ≈ 10^6 cells	2	1
Day 4‡	90%	12%
Replicates ≈ 10^6 cells	2	2
Day 5‡	88%	5%
Replicates ≈ 10^6 cells	2	1
Day 28‡	78%	—
Replicates ≈ 10^6 cells	1	—

*Seventy- to eighty-percent confluent cultures were incubated in fusion medium for 4 days. Fusion percentage = total number of nuclei count in myotubes/total number of nuclei.
†Small cell population = cells sorted from small cell population; large cell population = cells sorted from large cell population.
‡Number of days cells were incubated in a single plate prior to cell sorting.

lations were positive for desmin (Table 2), confirming that the 5.1H11 antibody can select porcine myoblasts from a mixed population of primary muscle cells. There was a progressive decline in the proportion of 5.1H11-positive cells after the second and fourth passage of mixed cultures (Table 3), but only a 2–3% reduction in the proportion of sorted cells that were desmin-positive. In agreement with light scatter measurements (Fig. 2), the relative cell numbers of the small cell population decreased with increased passage number (Table 3). However, in fourth passage mixed cultures, a small amount of material was detected in the size range of the small cell population. After sorting and replating, cells failed to grow from this fraction, indicating that this was cell debris rather than the small cell population (Fig. 4B).

Our previous experiments had shown that the small myoblast population declined as a proportion of total cells with time in culture and disappeared after passage of cells to new plates. To determine if the small cells had a longer cell cycle time, or were not dividing, the large and small myoblast populations were sorted and replated separately. Both myoblast populations were capable of proliferation and after three passages the cells were reexamined using 90°LS and FALS parameters. The cells derived from the small myoblast population had become greater than 10 μm in diameter and indistinguishable from the large cell population (Fig. 4C). Furthermore, greater than 90% of the cells were desmin-positive. This indicated that the decline of the small myoblasts over time or passage was due to differentiation of the cells into the large myoblast phenotype. However, when the large cell population was examined after three passages it had maintained the original size distribution (data not shown).

DISCUSSION

In this study we demonstrate that clonal myoblasts have essentially the same distribution of cell size and cytoplasmic/nuclear ratio as other clonal cell types isolated from primary muscle cell preparations.

Table 2. Percent desmin-positive cells following sorting.[a]

Cell sorting parameters[b]	Small cell < 10 μm	Replicates ≈ 10^6 cells	Large cell ≥ 10 μm	Replicates ≈ 10^6 cells
Light scatter				
Prefusion[c]	90.7 ± 1.0	2	63.5 ± 3.8	2
Postfusion[d]	92.0 ± 0.0	1	48.0 ± 0.0	1
Fluorescence and light scatter				
Prefusion[c]				
Primary cultures[e]	95.7 ± 0.9	2	95.5 ± 0.2	2
Second passage[f]	93.2 ± 0.3	2	91.2 ± 4.5	2
Fourth passage[g]	—	—	93.9 ± 2.4	3
Sorted small cells[h]	—	—	92.0 ± 1.8	2
Postfusion[d]				
Primary cultures[e]	91.0 ± 0.0	1	93.0 ± 0.0	1
Second passage[f]	94.0 ± 0.0	1	92.0 ± 0.0	1

[a]Percentage of cells positive for desmin, mean ± standard error.
[b]Light scatter = 90°LS and FALS. Fluorescence and light scatter = 5.1H11, 90°LS and FALS.
[c]Prefusion = cells analyzed for presence of desmin 24 h following sorting.
[d]Postfusion = cells analyzed for presence of desmin following a 4-day incubation period in fusion medium.
[e]Primary cultures = cells analyzed by flow cytometry 3 days following isolation.
[f]Second passage = cells analyzed by flow cytometry following a growth period which required replating one time.
[g]Fourth passage = cells analyzed and sorted by flow cytometry following a growth period that required replating 3 times.
[h]Sorted small cells = cells sorted from small cell population at zero passage, allowed to grow through four passages, and then reexamined.

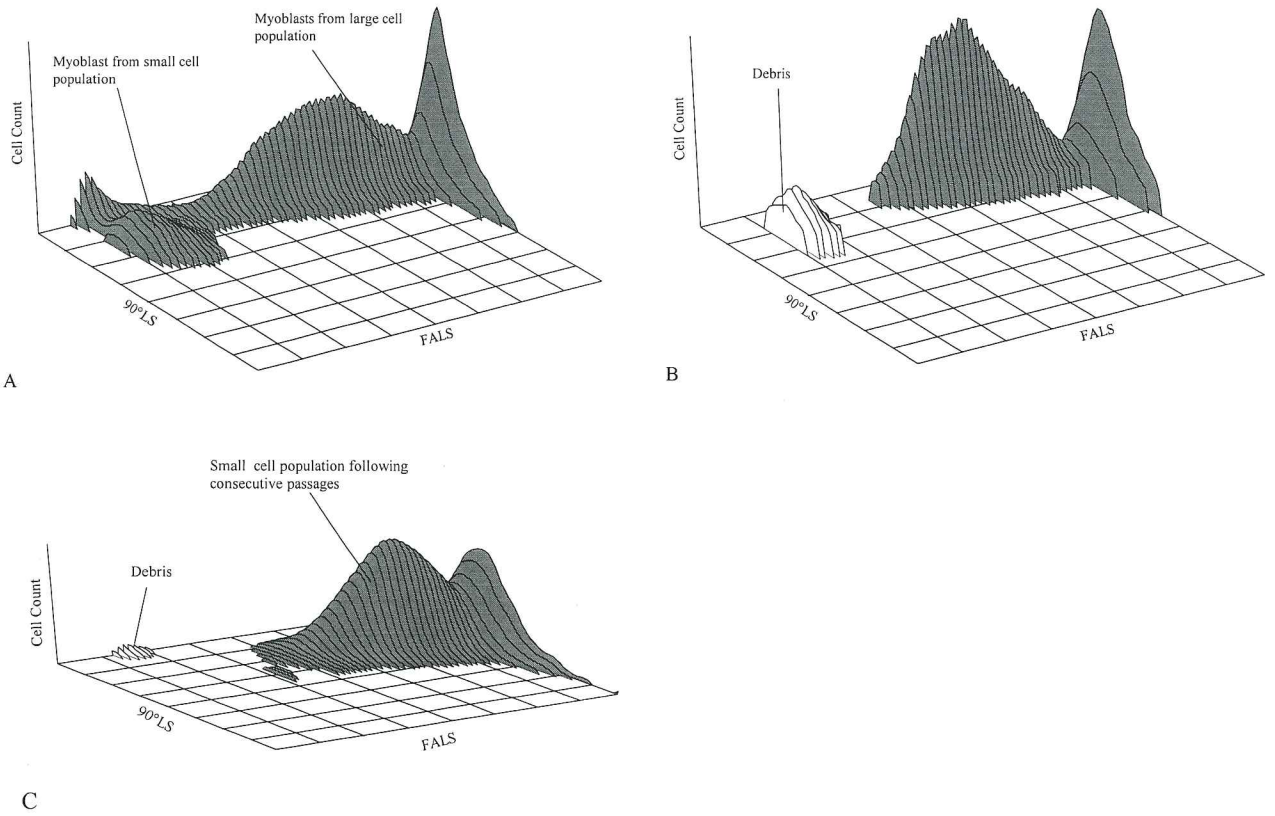

FIGURE 4. Three-dimensional contour plot of porcine muscle cell isolations using the muscle-specific monoclonal antibody 5.1H11 on **(A)** zero passage, **(B)** fourth passage, and **(C)** fourth passage cells derived from the small cell population.

However, when primary porcine muscle cell preparations were maintained on a single plate, two separate populations were found. The small cell population consisted of mostly myoblasts whereas the large cell population was comprised of both myoblasts and nonmyoblasts. Interestingly, if the primary cell preparation was passaged to a new cell culture plate, the small cell population was lost and myoblasts and nonmyoblasts were indistinguishable by cell size and cytoplasmic/nuclear ratio.

The use of the 5.1H11 antibody allows selection of myoblasts independent of passage and indicates that about 80% of the total mononucleated cells in a muscle preparation are myoblasts. Both methods produce myoblasts with greater than 90% purity after a single sorting step. The neural cell adhesion molecule is the reactive antigen for the 5.1H11 antibody[16] and labels human fetal myoblasts and myotubes but not fibroblasts.[15] The antigen is weakly expressed on proliferating human fetal myoblasts and signal amplification was required for FACS.[17] The 5.1H11 antigen was not expressed in freshly dissociated human muscle biopsies and required 2 weeks of culture time for maximum expression.[2] However, we found that the 5.1H11 antigen was readily detectable on porcine myoblasts as early as 2 days following primary isolations, and it was expressed on both the large and small myoblast populations.

The behavior of the small myoblasts on a single plate over time and their morphological change after replating at low density suggests that these cells are self-renewing myoblasts as described by Barrafio et al.[3] Self-renewing myoblasts maintain a minimal cell size and remain relatively quiescent until surrounding densities become extremely low, at which time they reenter the cell cycle and become larger,

Table 3. Percentage of cells sorted by 5.1H11 antibody.*

	Small cell population†	Large cell population†	Combined populations
Replicates ≈ 10^6 cells	2	2	2
Primary cultures	86.3 ± 0.9	86.3 ± 2.4	84.5 ± 2.3
Second passage	64.5 ± 0.1	69.1 ± 0.9	67.9 ± 0.6
Fourth passage	—	57.8 ± 2.3	57.8 ± 2.3

**Percentage of cells isolated from a mixed primary porcine preparation using muscle-specific antibody 5.1H11, mean ± standard error.*
†Small cells = cells sorted from small cell population; large cell = sorted from large cell population.

presumably due to the increase in protein accretion associated with cell activity. In the pig mixed primary cultures, the small myoblast population was the predominant cell type at 2 days after isolation. As the cells proliferate in culture, most of the small myoblasts become larger proliferating myoblasts but a population of small myoblasts remain as long as the cells are cultured on a single plate. After passage of mixed cells or sorting of small myoblasts and replating at low density, the quiescent small myoblasts reenter the cell cycle and become large proliferating myoblasts. After a second passage and proliferation, the small myoblasts become undetectable. Barrafio et al.[3] showed that the percentage of self-renewing myoblasts decreased with each passage but that they could be recovered from clonal cell lines by inducing myotube fusion and reisolation of the nonfusing myoblasts. Our clonal cell lines were replated either two or four times and extensively proliferated without myotube fusion so the small myoblasts became undetectable.

Mouse models have been employed extensively to examine muscle development and disease, but the mouse model is limited by size and muscle development patterns.[5–8,10,12] Our present results show that the pig has two distinct myoblast populations, similar to humans, so the role of both the small and large myoblast populations in muscle hypertrophy or muscle repair can be studied from the fetus to the adult. Pig myoblasts can be isolated by FACS at greater than 90% purity without clonal analysis and extensive in vitro proliferation. This will allow myoblast-mediated gene transfer studies to be more readily extended to the pig. Finally, the relatively short development time and the mature size of pigs make them a suitable model for development of myoblast-mediated gene therapies for human medicine.

This work was supported by NRI Competitive Grants Program/USDA award 95-37205-2215. The 5.1H11 antibody was obtained from the Developmental Studies Hybridoma Bank maintained by the University of Iowa under contract N01-HD-7-3263 from the NICHD. The authors thank Kathy E. Ragheb, Jane E. Pesall, and Kysa K. Gilkerson for their excellent technical assistance. This is journal paper no. 15,681 from the Purdue University Agricultural Research Programs.

REFERENCES

1. Barrafio A, Bochaton-Piallet ML, Gabbiani G, Bader CR. Heterogeneity in the progeny of single human muscle satellite cells. Differentiation 1995;59:259–268.
2. Barrafio A, Hamann M, Bernheim L, Bochaton-Piallat ML, Gabbiani G, Bader C. Identification of self-renewing myoblasts in the progeny of single human muscle satellite cells. Differentiation 1996;60:47–57.
3. Barrafio A, Aubry JP, Kaelin S, Krause RM, Hamann M, Bader CR. Purification of human muscle satellite cells by flow cytometry. Muscle Nerve 1993;16:498–505.
4. Doumit M, Merkel R. Conditions for isolation and culture of porcine myogenic satellite cells. Tissue Cell 1992;24:253–262.
5. Huard J, Acsadi G, Jani A, Massie B, Karpati G. Gene transfer in skeletal muscles by isogenic myoblasts. Hum Gene Ther 1994;5:949–958.
6. Irintchev A, Langer M, Zweyer M, Theisen R, Wernig A. Functional improvement of damaged adult mouse muscle by implantation of primary myoblasts. J Physiol 1997;500:775–785.
7. Kurachi K, Yao SN. Gene therapy by myoblast mediated gene transfer. In: Chang PL, editor. Somatic gene therapy. Ann Arbor, MI: CRC Press; 1995. p 121–133.
8. Murray CE, Wiseman RW, Schwartz SM, Hauschka SD. Skeletal myoblast transplantation for repair of myocardial necrosis. J Clin Invest 1996;98:2515–2523.
9. Ohno T, Gordon D, San H, Pompili VJ, Imperiale MJ, Nabel GJ, Nabel EG. Gene therapy for vascular smooth muscle cell proliferation after arterial injury. Science 1994;265:781–784.
10. Partridge T. Myoblast transfer; a possible therapy for inherited myopathies. Muscle Nerve 1991;14:197–202.
11. Petters RM, Alexander CA, Wells KD, Collins EB, Sommer JR, Blanton MR, Rojas G, Hao Y, Flowers WL, Banin E, Cideciyan AV, Jacobson SG, Wong F. Genetically engineered large animal model for studying cone photoreceptor survival and degeneration in retinitis pigmentosa. Nat Biotechnol 1997;15:965–970.
12. Robinson SW, Cho PW,Levitsky HI, Olson JL, Hruban RH, Acker MA, Kessler PD. Arrterial delivery of genetically labeled skeletal myoblasts to the murine heart: long term survival and phenotypic modification of implanted myoblasts. Cell Transplantation 1996;5:77–91.
13. Van Meter CH Jr, Claycomb WC, Delcarpio JB, Smith DM, deGruiter H, Smart F, Ochsner JL. Myoblast transplantation in the porcine model: a potential technique for myocardial repair. J Thorac Cardiovasc Surg 1995;110:1442–1448.
14. Walsh F. Identification and characterization of plasma membrane antigens of neurons and muscle cells using monoclonal antibodies. Eur Soc Neurochem Meetings 1980;3:285–319.
15. Walsh FS, Ritter MA. Surface antigen differentiation during human myogenesis in culture. Nature 1981;289:60–64.
16. Walsh FS, Dickson G, Moore SE, Barton CH. Unmasking NCAM. Nature 1989;339:516.
17. Webster C, Pavlath GK, Parks DR, Walsh FS, Blau HM. Isolation of human myoblasts with the fluorescent-activated cell sorter. Exp Cell Res 1988;174:252–265.