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Isolation, culture and biological characteristics of multipotent porcine skeletal muscle satellite cells

Cell and Tissue Banking

December 2017, Volume 18, Issue 4, pp 513–525 | Cite as

- Jinjuan Yang (1) (2)
- Hao Liu (1)
- Kunfu Wang (1)
- Lu Li (1)
- Hongyi Yuan (1)
- Xueting Liu (1)
- Yingjie Liu (3) Email author (yangxiaonuodexiao@163.com)
- Weijun Guan (1) Email author (weijunguan301@gmail.com)

1. Department of Animal Genetic Resources (AnGR), Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, People's Republic of China
2. Sports Science College, Beijing Sports University, Beijing, People's Republic of China
3. Institute of Physical Education, University of JiMei, Xiamen, People's Republic of China

Article
First Online: 02 March 2017
Received: 17 September 2016
Accepted: 23 February 2017

Abstract

Skeletal muscle has a huge regenerative potential for postnatal muscle growth and repair, which mainly depends on a kind of muscle progenitor cell population, called satellite cell. Nowadays, the majority of satellite cells were obtained from human, mouse, rat and other animals but rarely from pig. In this article, the porcine skeletal muscle satellite cells were isolated and cultured in vitro. The expression of surface markers of satellite cells was detected by immunofluorescence and RT-PCR assays. The differentiation capacity was assessed by inducing satellite cells into adipocytes, myoblasts and osteoblasts. The results showed that satellite cells isolated from porcine tibialis anterior were subcultured up to 12 passages and were positive for Pax7, Myod, c-Met, desmin, PCNA and NANOG but were negative for Myogenin. Satellite cells were also induced to differentiate into adipocytes, osteoblasts and myoblasts, respectively. These findings indicated that porcine satellite cells possess similar biological characteristics of stem cells, which may provide theoretical basis and experimental evidence for potential therapeutic application in the treatment of dystrophic muscle and other muscle injuries.

Keywords

Satellite cells Self-renewal Multi-differentiation potential
Biological characteristics Porcine

Introduction

Skeletal muscle is the most significant and abundant tissue composed of multinucleated fibers with strong ability to contraction and relaxation which contract to generate body movement. Skeletal muscle has a huge regenerative potential upon vigorous physical activities or muscle injury before adulthood, which primarily depend on satellite cells, a mitotically quiescent stem cell that resides between the basal lamina and sarcolemma of the muscle fiber (Schultz et al. 1978) and is responsible for the development, maintenance and regeneration in postnatal skeletal muscles. Satellite cells possess common properties with other stem cell populations, such as, self-renewal capacity, multi-potential differentiation and proliferative potential. Besides, several studies have suggested that satellite cells are not homogeneous pool of committed myogenic stem cells, but exist as a heterogeneous population (Motohashi and Asakura 2014). When activated, satellite cells are driven out of quiescent states and enter the cell cycle to expand their progeny, called myogenic precursor cells. Proliferating cells stop their mitosis, differentiate into myocytes and fuse with each other to be mature muscle fibers or existing myofibers so as to repair injured muscle. This results in a continuous increase in the number of nuclei in muscle fibers (White et al. 2010).

Satellite cells are essential for the growth and maintenance of stem pool in postnatal skeletal muscles. However, there exist some inherited and non-inherited muscle disorders which may give rise to the loss of mechanical strength and muscle mass, such as, aging (Carlson and Conboy 2007), diseases and toxins, leading to progressive muscle weakness with chronic degeneration. Skeletal muscle has remarkable functional and structural decline in many aging individuals and organisms (Augustin and Partridge 2009) with a decrease in satellite cell numbers and regenerative potential, which mainly attributed to the changes of the microenvironment cells rely on. Sarcopenia, known as an age-related muscle syndrome, shows a downward trend in muscle strength, function and mass in late adulthood (Kim and Choi 2013). Duchenne muscular dystrophy (DMD) (Wilschut et al. 2012), the most prevalent progressive muscular dystrophy, strongly impair structure and function of muscles and is the most serious genetic disorder in skeletal muscle (Leung and Wagner 2013). In addition to these muscle-specific diseases, there are some pathologies that originate from other tissues can consequently affect skeletal muscle, for example, spinal muscular atrophy, which is a typical muscle injury related to neural degeneration. Currently, several clinical strategies and rehabilitation methods have been used in regenerative medicine to improve muscle regeneration, but the therapeutic effect remains poor.

Among various therapeutic approaches for muscle diseases, one area that has attracted close attention is the area of stem cell-based therapy. Satellite cells were thought to be an ideal choice to promote muscle regeneration and avoid the negative consequences. However, there still exist some problems that should be taken into consideration, among these problems, to identify the optimal cell types which could be used to treat the variable skeletal muscle diseases seems to be most critical and troublesome. Researches on muscle satellite cells and muscle regeneration has been a long time and the achievement is exciting. However, currently, studies of satellite cells were mainly focus on mice, rat, chicken, rabbit and other animals and rarely performed on pig. In addition, the difficulty of isolating pure populations of satellite cell in sufficient number has precluded their use in cell-based tissue repair. In this article, we chose pig as experimental animal which is an important economic animal and make a significant contribution towards meeting human nutritional requires. We explored optimized culture medium for satellite cells cultures in vitro and detected their biological characteristics and multi-lineage differentiation, which may provide a better understanding of their properties in vitro and render an ideal candidate for cellular transplantation therapy.

Materials and methods

Experimental animal

The Institutional Animal Care and Use Committee of Chinese Academy of Agricultural Sciences approved all animal procedure. 9 bama miniature pigs which were 1-day old were provided by the farm of The Chinese Academy of Agricultural Sciences. All experimental procedures involving pig were conducted in accordance with the protocols and guidelines for agricultural animal research codified by the Committee for Ethics of Beijing, China.

Isolation and culture of porcine satellite cells in vitro

Satellite cell isolation was performed as previously described (Fukada et al. 2013; Montarras et al. 2005). Skeletal muscles were isolated from tibialis anterior of neonatal pig, muscle samples were washed 6–8 times with phosphate buffer solution (PBS, Gibco, Carslbad, CA, USA), connective tissue, blood vessels, nerve bundles and adipose tissue were removed and the sterile muscle tissue were cut into small pieces, the comminuted tissues were disaggregated by combinational digestion with 0.3 mg/mL collagenase type I (Sigma, Aldrich, St. Louis, MO, USA) at 37 °C for 35 min and 0.25% of trypsin–EDTA (Gibco, Carlsbad, CA, USA) for 60 min, orderly. DMEM/F12 (Gibco, Carslbad, CA, USA) containing 15% fetal bovine serum (FBS, Invitrogen Corporation, Carlsbad, CA) was added to terminate the digestion process. The suspension was filtered through a 70 μm mesh sieve and centrifuged at 1200 r/min for 8 min at room temperature with the supernatant discarded. Cells were resuspended in complete culture medium (DMEM/F12) supplemented with 15% FBS, 10 ng/mL basic fibroblast growth factor (bFGF), 10 ng/mL epidermal growth factor (EGF), 2 mM l-glutamine, 1%

penicillin–streptomycin mixture (all from Invitrogen Corporation, Carlsbad, CA). Cells suspension was seeded into 60 m Petri dish and incubated at 37 °C, 5% CO_2 atmosphere.

In order to separate and purify satellite cells were isolated, the suspension were transferred to new plates 2 h later to get rid of the fibroblasts, cells were labelled as P0 generation. Continued to culture until the confluence reached about 80%, washed cells with sterile PBS for 2 times and dissociated with 0.25% trypsin–EDTA to subculture onto new plates at the ratio of 1:2. Cells went on proliferating as generation increased.

Optimization of cell culture systems for porcine satellite cells

To explore the optimal culture system for porcine satellite cells, cells at passage 2 were collected and reseeded at a density of 1 × 10^5 cell/well in four different culture system whose components were shown in Table 1. The generation time in every culture system was counted three times: culture system A (DMEM/F12 +15% FBS + 2 mM l-glutamine + 100 IU/mL penicillin + 100 IU/mL streptomycin); culture system B (DMEM/F12 + 15% FBS +2 mM l-glutamine + 100 U/mL penicillin + 100 IU/mL streptomycin + 10 ng/mL bFGF); culture system C (DMEM/F12 + 15% FBS + 2 mM l-glutamine + 100 U/mL penicillin + 100 IU/mL streptomycin + 10 ng/mL bFGF + 10 ng/mL EGF); culture system D (Ham's F10-Medium +15% FBS + 2 mM l-glutamine + 100 U/mL penicillin + 100 IU/mL streptomycin + 10 ng/mL bFGF +10 ng/mL EGF).

Table 1

Option culture condition for porcine satellite cells

Culture system			
A	**B**	**C**	**D**
Components			
DMEM/F12	DMEM/F12	DMEM/F12	Ham's F10
15% FBS	15% FBS	15% FBS	15% FBS
2 mM l-glutamine	2 mM l-glutamine	2 mM l-glutamine	2 mM l-glutamine
	10 ng/mL bFGF	10 ng/mL bFGF	10 ng/mL bFGF
		10 ng/mL EGF	10 ng/mL EGF

Cell population growth dynamics

To evaluate the growth dynamics of porcine satellite cells, cells at P2, P5 and P10 were digested and planted in a 24-well microplates at a density of 1.0×10^4/well. Cells were cultured for 8 days and counted three wells randomly each day. The mean cell counts at each time point were used to plot growth curves of porcine satellite cells, and the PDT calculated by the formula PDT = $(t - t_0)\ lg_2/(lgNt - lgN_0)$, where t_0 = starting time of culture; t = termination time of culture; N_0 = initial cell number of culture; N_t = ultimate cell number of culture.

Karyotype analysis

Cells at P5 were collected for Karyotype analysis assay. After culturing in Colchicine solution for 24 h, cells were harvested to hypotonic treatment, fixed and stained with Giemsa. The chromosome numbers were counted from 100 metaphase spreads under an oil immersion objective.

Self-renewal and colony-forming cell assay

Satellite cells from P3, P5, P8 and P11 were collected and seeded in 60 mm plates at a density of 1.0×10^3 cells/well. After culturing for 10 days, the numbers of colony-forming units of each passage were counted and the cloning efficiencies were calculated as: colony forming unit number/starting cell number × 100% (Gao et al. 2012).

Immunofluorescent detection of cell surface markers

The procedure for immunofluorescent detection was described previously (Bentzinger et al. 2013; Cerletti et al. 2008). Cells were seeded in 6-well microplates. When reached approximately 60% confluence, cells were washed with PBS three times, fixed with 4% paraformaldehyde for 20 min at room temperature, then permeabilized with 0.25% Triton X-100 for 15 min. After rinsing three times in PBS, cells were blocked with PBS containing 1% goat serum for 1 h at ambient temperature, and subsequently incubated in 1% bovine serum albumin (BSA) containing the primary monoclonal antibodies, including Pax7, MyoD, desmin, PCNA and NANGO, at 4 °C overnight, washed with PBS three times, then incubated in PBS containing fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit secondary antibody for 1 h at room temperature in dark. After washing with PBS, cells were counterstained with 1 μg/mL DAPI (Sigma) for 15 min then washed with PBS. Images were taken using Nikon TE-2000-E confocal microscope with an attached Nikon ZE-1-C1 3.70 digital camera system (Nikon, Tokyo, Japan), then quantified by a video densitometric analysis using image software (Yokohama, Kanagawa Japan).

RT-PCR assays

Porcine muscle satellite cells of passage 5 were collected and Total RNA was extracted using Trizol reagent (Invitrogen) (Bentzinger et al. 2013) RNA concentrations were reverse transcribed followed by 35 PCR cycles using RNA PCR kit ver 3.0 (TARAKA, China). The RT-PCR was continued for 35 cycles after an initial denaturation at 94 °C for 10 min. Each cycle of PCR consisted of 94 °C for 30 s, annealing temperature for 30 s and 72 °C for 30 s, as well as a final extension of 10 min at 72 °C. PCR products were visualized with ethidium bromide on a 2% agarose gel electrophoresis. The primer sequences were listed in Table 2.

Table 2

The primer sequences for RT-PCR

Gene name	Primer sequences	Temperature (°C)	Length (bp)
NANOG	F: 5′-GCAGAGAAGAGCACAGAGAAGGAG-3′ R: 5′-CAGAAGCGTTCACCAGGCATCC-3′	64	300
PAX7	F: 5′-CAACCACATCCGCCACAAGATAGTA-3′ R: 5′-GATCACAGTGCCCGTCCTTCAG-3′	63	284
PCNA	F: 5′-GTCGTTGTGATTCCACCACCATGT-3′ R: 5′-TCCATTTCCGAGTTCTCCACTTGC-3′	63	554
desmin	F: 5′-GATCCAGTCCTACACCTGCGAGAT-3′ R: 5′-AGGCTCACTCACTTCCAACATCCA-3′	63	676
MYOG	F: 5′-CGCAGGCTCAAGAAGGTGAATGA-3′ R: 5′-ATCCTCCACTGTGATGCTGTCCA-3′	63	363
c-Met	F: 5′-GCCAGATTCTGCCGAACCAATGA-3′ R: 5′-AGGTTAGCGATGGTGAGGTCTCC-3′	63	315
MyoD1	F: 5′-GCAACAGCGGACGACTTCTATGAT-3′ R: 5′-CATGCCGTCGGAACAGTTGGAG-3′	63	561

Gene name	Primer sequences	Temperature (℃)	Length (bp)
β-actin	F: 5′-GCAAGGACCTCTACGCCAACAC-3′ R: 5′-TGCTGTCACCTTCACCGTTCCA-3′	64	468
LPL	F: 5′-ATGGCTGGACGGTGACAGGAAT-3′ R: 5′-CACGGATGGCTTCTCCAATGTTACA-3′	63.8	513
PPARr	F: 5′-ACCACTCCCACTCCTTTGACATCA-3′ R: 5′-TCTCTGCCAACAGCTTCTCCTTCT-3′	63.6	510
OPN	F: 5′-GGAGGAAACGGACGACTTCAAACA-3′ R: 5′-GGCTTCGGATCTGCGGAACTTC-3′	63.6	454
Runx2	F: 5′-CTACCAGCCACCGAGACCAACA-3′ R: 5′-GCAGCACAGAGCACAGGAAGTT-3′	64	470

Multiple differentiation potential of porcine muscle satellite cells

Adipogenic differentiation of porcine muscle satellite cells

To test adipogenic potential, satellite cells of passage 3 were plated at a density of 2.0×10^4 cell/well and then divided into the control group and the inducted group when reached about 60% confluence. The adipogenic medium consisted of DMEM/F12, 5% FBS, 50 mg/L vitamin C, 0.5 mM isobutyl methyl xanthine (IBMX), 10 mg/L insulin and 200 μM indomethacin. Cells control group were cultured in complete medium. The medium was refreshed every 2 days. 10 days later, intracellular lipid accumulation in two groups detected by oil red O dye and adipocyte specific genes were tested by RT-PCR assay.

Osteogenic differentiation of porcine muscle satellite cells

As a test of osteogenic potential of satellite cells, cells were seeded at a density of 2.0 × 104 cell/well and divided into the control group and the induced group when reached about 60% confluence as above. Cells in induced group were incubated in osteogenic medium which consisting of DMEM/F12 supplemented with 5% FBS, 10 mM-glycerophosphate, 50 ng/mL dexamethasone and 50 mg/L vitamin C. Cells in control group were cultured in complete medium. The medium was changed every 2 days. 3 weeks later, the capacity of calcium node formation of cells was detected by alizarin red staining and specific genes were tested by RT-PCR assay.

Myogenic differentiation of porcine muscle satellite cells

In order to test the myogenic differential potential, passage 3 cells were seeded at a density of 2.0×10^4 cell/well and divided into the inducted group and control group as described (Conboy and Rando 2006). Myogenic induction medium consisted of DMEM/F12 and 2% horse sera. The control group cells were cultured in complete medium. The medium was changed every 2 days. After 2 weeks, the cells of each group were harvested and the specific marker of myoblasts MHC (Smolina et al. 2015) were detected by Immunofluorescence.

Results

Morphology observation of porcine satellite cells

Primary cells isolated from porcine tibialis anterior muscle of lower limb began to adhere to petri dish 2 h later. Approximately 2 days later, cells were about 70% confluence with a few other cell types mixed, and most of cells exhibited as typical spindle-shaped fibroblasts or triangle-shaped (Fig. 1a). The morphology of cells stayed stable after passage 3 and there were no obvious morphological differences among the following passages (Fig. 1b, c). Muscle satellite cells were subcultured up to passage 12 in this research, after which cells almost stopped growing and showed senescent appearance as karyopyknosis in most cells (Fig. 1d).

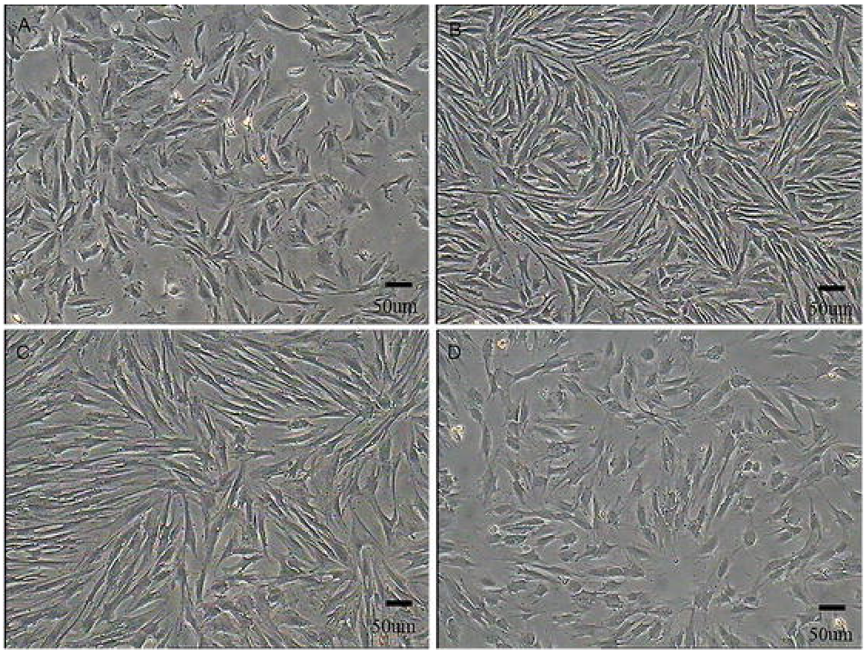

Fig. 1

Morphology of porcine skeletal muscle satellite cells under inverted microscope. **a** After 2 days of primary culture, cells adhered to plates showing spindle-shaped morphology. **b** Passage 3, cells were purified; **c** cells at passage 5 displayed typical fibroblast fusiform. **d** Porcine satellite cells at passage 12 showed senescent appearance and the proliferation capability declined (*scale bar* 50 μm)

Optimization of cell culture systems for porcine satellite cells

As is known to all, cells reduced their proliferation potential as continuous differentiation during cultured in vitro. As different sample sources of skeletal muscle satellite cells, the proliferation medium differed. Through a series of trial, in the culture system C, the number of cells increased significantly during 10 days culture period and population doubling time decreased apparently compared with others. As a consequence, we considered culture system C as an optimal proliferation medium for porcine muscle satellite cells' culture in vitro, and culture system C was applied in following experiments (Fig. 2).

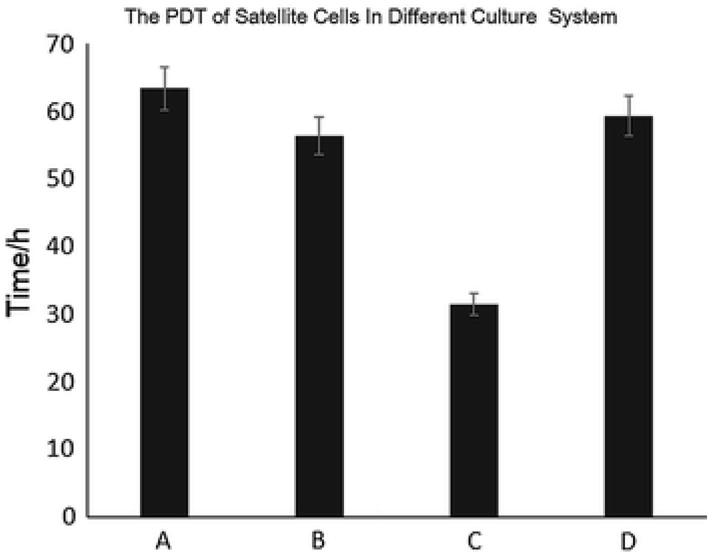

Fig. 2

Optimization of porcine satellite cells culture system. Comparison of cell proliferation in different culture systems, the PDT were 63.37, 56.4, 31,47 and 59.3 h for system A, system B, system C and system D respectively and culture system C was suitable for porcine satellite cells proliferation in vitro. Data are expressed as the mean ± SD of triplicates

Cell population growth dynamics

The growth kinetics of porcine satellite cells at passage 2, passage 5 and passage 10 was as shown by the growth curves, which were all typically sigmoidal. Satellite cells entered logarithmic phase after about 48 h later, reached the plateau phase at day 5 and followed by the decline phase about 6–7 days later (Fig. 3). The population doubling time (PDT) was calculated to be 32.81, 36.74 and 51.36 h for passage 2, passage 5 and passage 10, respectively, indicating that porcine satellite cells of different passages had different proliferation ability.

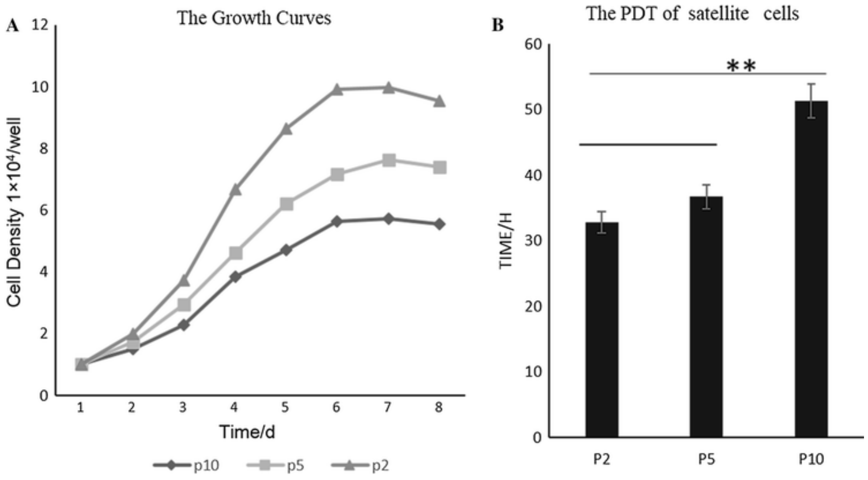

Fig. 3

Cell population growth dynamics of porcine satellite cells at different passages. **a** The growth curves of cells all displayed typically sigmoidal under culture system C. **b** The population doubling time

(PDT) of porcine satellite cells at different passages, the PDT were 32.81, 36.74 and 51.36 h for passage 2, passage 5 and passage 10, respectively. Data are expressed as the mean ± SD of triplicates (**$P < 0.01$)

Karyotype analysis

Karyotype of pig was shown in Fig. 4. Porcine satellite cells were diploid (2n = 38), which contains a pair of sex chromosomes and 18 pairs of autosomes. In this experiment, the chromosome numbers in 100 spreads were counted to calculate the diploid rates which accounted for 95%. The results showed that satellite cells of pig cultured in vitro remained genetic stable.

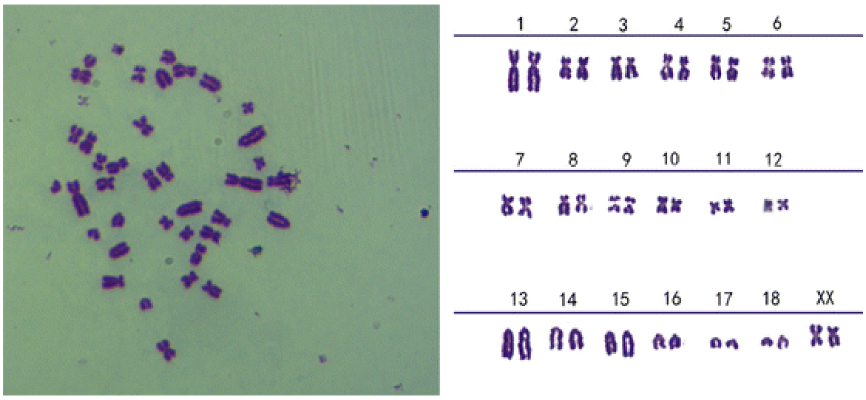

Fig. 4

Karyotype of porcine satellite cells and chromosomes at metaphase. The chromosomes number of pig was 2n = 38, which contains 18 pair of autosomes and a pair of sex chromosomes

Self-renewal and colony-forming cell assay

Porcine satellite cells began to form colonies after culturing for 6 days as observed under inverted microscope. Colonies were stained by Giemsa and displayed heterogeneous in number and in size when cultured for approximately 10 days. As is shown in Fig. 5, cells at P3 and P5 showed large polygonal and star-shaped, while passage 8 and passage 11 cells were sparse in number and irregular in shape. The colony-forming efficiency rates were (33.21 ± 0.27%), (31.69 ± 0.33%), (24.22 ± 0.18%) and (7.48 ± 0.35%) for P3, P5, P8 and P11, respectively. As can be seen from the chart, colony-forming rates showed a downward trend but did not disappear with the increasing passage number. These results showed the self-renewal ability and the proliferation capacity of porcine satellite cells cultured in vitro.

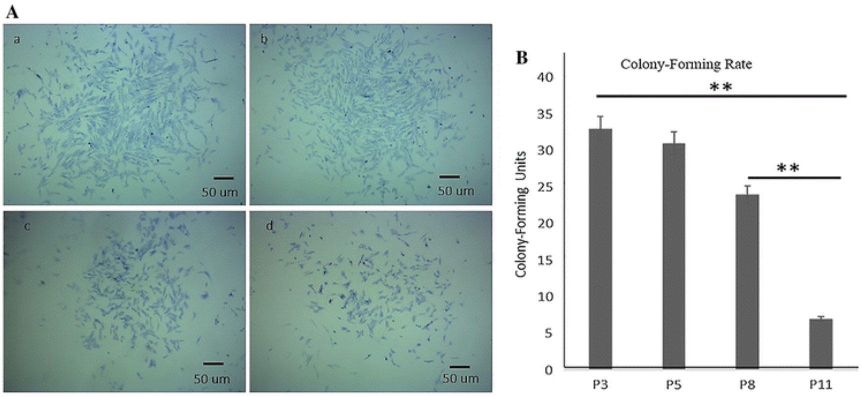

Fig. 5

The colony-forming cell assay. **A** Cell morphology of porcine satellite cells after culturing for 10 days and cells exhibited different sizes and densities of colonies at different passages (*scale bar* 50 μm). **B** Colony-forming rates for different passages of porcine satellite cell. Data are expressed as the mean ± SD of triplicates ($^{**}P < 0.01$)

Identification of porcine satellite cells

The specific surface antigen markers of porcine satellite cell were detected via immunofluorescence staining (Fig. 6) and RT-PCR assays (Fig. 7). Immunofluorescent staining showed that cells were positive for the several markers, such as the porcine satellite cell specific markers Pax7, MyoD and desmin, the proliferating-cell nuclear antigen marker PCNA and the stem cell marker Nanog. RT-PCR results indicated that cells expressed satellite cell specific genes Pax7, MyoD and desmin, c-Met Nanog but did not express muscle-specific gene Myogenin (Fig. 7).

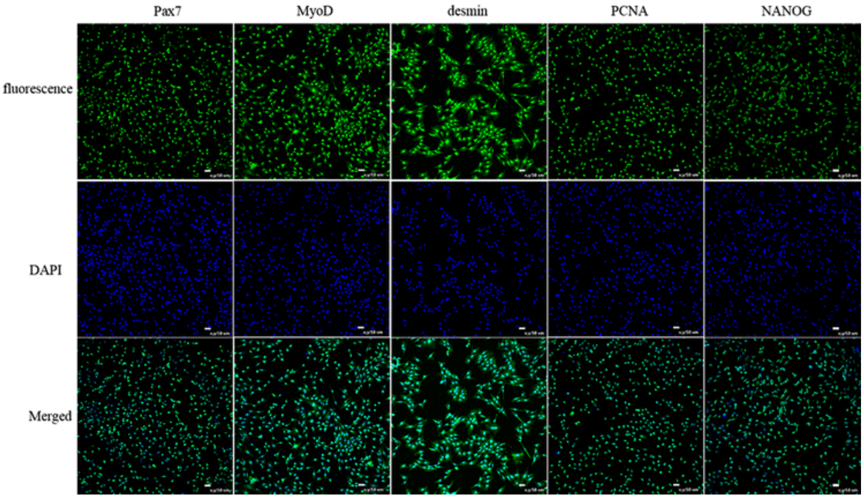

Fig. 6

Surface marker detection of porcine satellite cells by immunofluorescence. Cells expressed Pax7, MyoD, PCNA and NANOG in nucleus and desmin in cytoplasm, showing in *first line*. The nuclei of porcine satellite cells were stained with DAPI displaying in *second line* and the merged images were showing in *last line* (*scale bar* 50 μm)

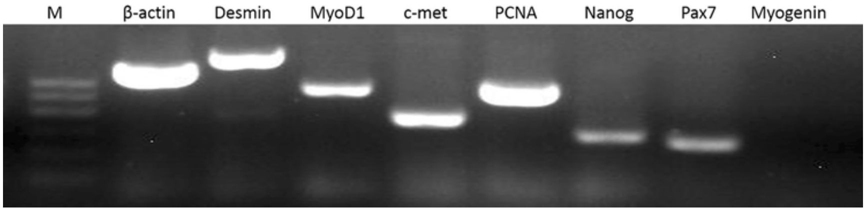

Fig. 7

Detection of c-Met, Pax7, MyoD1, PCNA, Desmin, Myogenin and Nanog in porcine satellite cells by RT-PCR. Except for Myogenin, others were positively expressed. β-actin served as the internal control

Multiple differentiation potential of porcine muscle satellite cells in vitro

Adipogenic differentiation of porcine muscle satellite cells

After inducting in adipogenic medium for 5 days, cells in induction group had obvious change in morphology with many small lipid droplets accumulated (Fig. 8a), as induction progressed, the number of lipid droplets increased and aggregated to form larger ones at approximately day 10 (Fig. 8b). Detected with oil red O dye, lipid droplets were stained red in inducing group (Fig. 8c) while cells cultured in complete medium showed no change in cell morphology and were negative for oil red O staining (Fig. 8d). Cells in both group were harvested for RT-PCR assay. The results showed that cells in adipogenic medium expressed adipocyte-specific genes PPAR-γ and LPL, while the control group did not (Fig. 8B).

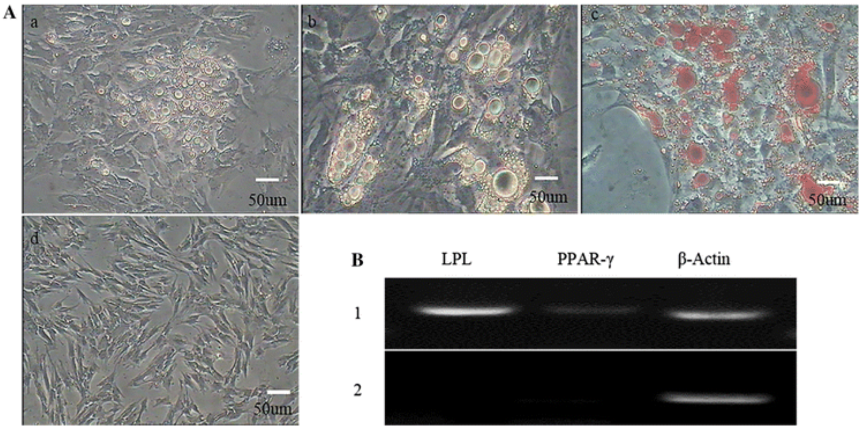

Fig. 8

Adipogenic differentiation of satellite cells. **A** Cells incubated in induction medium metamorphosed from fibroblast-like to oblate and formed a few tiny lipid droplets in the first few days (*a*). Lipid droplets increased obviously in number and in size after 10 days' induction (*b*). Cells in both group were stained by oil red O dye, and the induction group were positive (*c*) while the control group were negative for oil red O dye (*d*) (*scale bar* 50 μm). **B** Detection of adipocyte-specific genes by RT-PCR assays. RT-PCR showed that the induction group expressed the adipocyte-specific genes PPAR-γ and LPL (*lane 1*) while the control group did not expressed these genes (*lane 2*). β-actin served as the internal control

Osteogenic differentiation of porcine muscle satellite cells

After incubation in osteogenic medium for 8 days, porcine muscle satellite cells showed obvious change in shape with long spindle cells began to contract and became triangular or round (Fig. 9a). As induction progressed, cells incubated in osteogenic medium formed some mineralized nodules, the number and size of nodules increased after about 4 weeks (Fig. 9b, c) and were positive for Alizarin red dye (Fig. 9c). Cells in control group were negative for Alizarin red staining (Fig. 9d). The RT-PCR results showed that the induction group expressed osteoblast-specific genes OPN and Runx2, however, these genes were negatively expressed in control group (Fig. 9B).

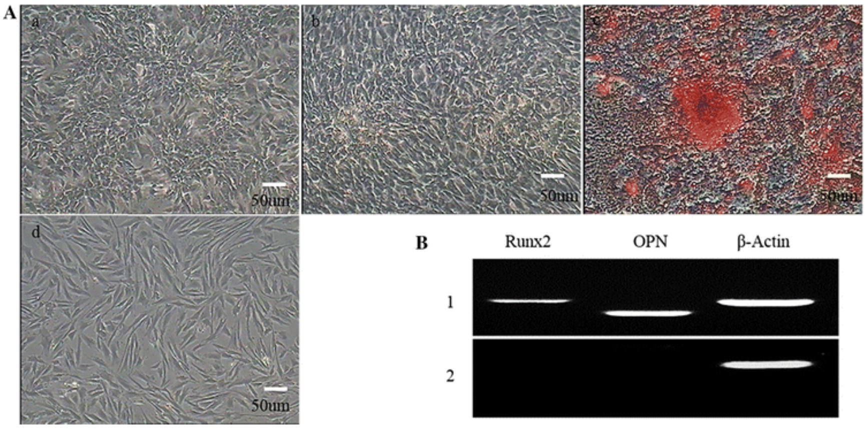

Fig. 9

Osteogenic differentiation of porcine muscle satellite cells. **A** Cell morphological changes, Cells began to contract and changed from long spindle or fibroblast-like to round or triangular-shape (*a*). Cells began to contract and formed some nodules (*b*, *c*). After stained by Alizarin red dye, the induction group were positive while the control group were negative (*d*) (*scale bar* 50 μm). **B** The RT-PCR showed that the induction group expressed osteogenic-specific genes OPN and Runx2 (*lane 1*), these genes were not expressed in control group (*lane 2*). β-actin served as the internal control

Myogenic differentiation of porcine muscle satellite cells

Cells cultured in myogenic induction medium began to stretch in length and grew slowly in the first few days. After inducting for approximately 7 days, cells started to fuse with each other and some multinucleated myotubes were found in inducted group (Fig. 10a, c). Cultured in complete medium, the control group showed no significant morphological changes during culturing (Fig. 10d). Immunofluorescence staining indicated that myosin heavy chain (MHC), the myogenic marker was positively expressed in the differentiated cells while cells in control group were not (Fig. 10c, f).

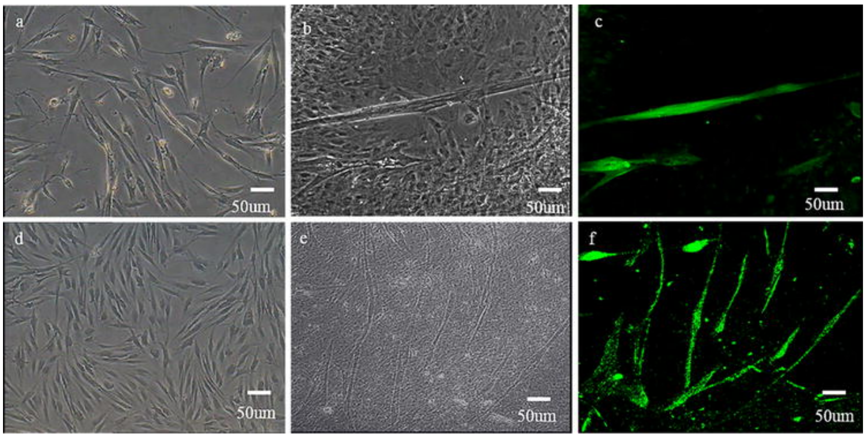

Fig. 10

Myogenic differentiation of satellite cells. Satellite cells changed in shape with multinucleated myotubes formed (**a**, **b**, **e**); Immunofluorescence staining indicated that the differentiated satellite cell expressed MHC (**c**, **f**); Control group cells (**d**) (*scale bar* 50 μm)

Discussion

Injuries to the musculoskeletal system are common, debilitating and expensive. Currently, the healing is imperfect and often leads to chronic impairment. In last few years, with a good application perspective, the therapeutic application of stem cell has attracted great attention and has being a research hotspot in regenerative medicine and clinical medicine (Meregalli et al. 2014). Previous research has manifested that skeletal muscle stem cells, namely satellite cells, play an indispensable role in postnatal muscle growth and muscle regeneration when injured (Collins et al. 2005; Tedesco et al. 2010). However, the proliferation potential of satellite cells isolated from many species have proven insufficient to promote muscle regenerative. In this study, we isolated porcine skeletal muscle satellite cells by using combined enzymatic digestion, as described previously. To purify satellite cells population, we transferred the suspension to a new petri dish after primary culturing for 2 h, during which most fibroblasts adhered to the bottom of the dish while satellite cells still remained suspended state. Cells purified by this method have shown superior cell morphology, however, the population doubling time of isolated cells were comparatively long and the generations was inferior during cultured in culture medium system A in vitro.

Culture medium is selected to provide an environment for cell growth and to give higher proliferation maintaining cell immaturity in vitro. Actually, the complete culture medium differed as the different species of sample source. In this article, in order to improve the ability of proliferation and expansion potential, we tried other three kinds of culture system which were all considered useful for satellite cells proliferation in vitro. Finally, by calculating the PDT of different culture systems we concluded that culture system C was an optimal choice for porcine skeletal muscle satellite cell cultures. We speculated that DMEM/F12 provide better nutritive constituents and cytokines for mammalian cells (Megeney et al. 1996; Powell et al. 2013), bFGF and EGF have been proved to promote proliferation in vitro. Ham's F10 medium (culture system D) was mainly used for human and mouse's cell types. In our research, satellite cell cultured in system C were finally subcultured up to 12 passage. We integrated growth factor and proliferative potential to provide a better microenvironment for direct expansion of satellite cells, which representing advance in understanding of molecular proliferation mechanism of satellite cells (Brack and Rando 2007). Culture medium C were used for the following experiments. During this process, we detected the proliferative potential and self-renewal capacity by growth dynamics and colony-forming unit assays which indicated that, compared with other species (Gilbert et al. 2010; Montarras et al. 2005), satellite cells isolated from porcine proliferate slower and easier to differentiation.

By analyzing the karyotype of cells, we found that satellite cells were stable in chromosome number and structure, the diploid rates accounted for 95%, indicating that the genetic property of satellite cells remained stable when cultured in vitro.

Cell surface markers of satellite cells were detected by immunofluorescence and RT-PCR assays. Pax7 (Bentzinger et al. 2010; Bosnakovski et al. 2008; Lagha et al. 2008; Seale et al. 2000), the paired type homeo box transcription factor, plays key roles in maintaining the proliferation of progenitors and preventing early myogenic differentiation. Myod (Zammit et al. 2004; Kim et al. 2013; Kitzmann et al. 1998), the myogenic differentiation antigen, is essential for myogenic differentiation and maintenance during muscle regeneration and is partly expressed (Kanisicak et al. 2009). c-Met is hepatocyte growth factor receptor involved in cell information regulation, proliferation and differentiation (Charge and Rudnicki 2004). PCNA, a proliferating-cell nuclear antigen marker, has significant effect on cell proliferation. Nanog is a protein coding gene, which imposes pluripotency and prevents differentiation towards extraembryonic endoderm. Myogenin is a member of the MyoD family of transcription factors, and it usually expressed during the process of differentiation in myogenesis. In this experiment, satellite cells expressed Pax7, MyoD, desmin, c-Met, PCNA and Nanog, but did not express myogenin, which is consistent with previous studies.

Studies have revealed that extrinsic alterations in the microenvironment and intrinsic changes in cell-autonomous regulatory mechanism would be responsible for the fate of stem cells (Brack et al. 2007; Price et al. 2015). In addition, the nutritional status (Harthan et al. 2014) was also considered as important factor which affects the potential for satellite cell proliferation and differentiation. In our experiments, we explored the multipotency of satellite cell in vitro by incubating cells in induction medium that supplemented with several regulatory factors. Finally, cells were differentiated into osteoblasts, adipocytes and myoblasts, respectively. The results indicated that inducting factors play a considerably significant role in determining the differential direction of stem cells, which made them an optional choice for cell-based therapies in musculoskeletal disease, tissue regeneration and repair.

Conclusion

In summary this study provided an integrated method of isolating satellite cells from pig and established an optimized method for cell culture in vitro. Moreover, their proliferative capacity and differential potential were detected in vitro. These findings may not only provide a better understanding of biological characteristic of satellite cells but also lay a foundation for satellite cells as an alternative cell source for cell-based therapies for tissue-engineering and regeneration.

Notes

Acknowledgements

This research was supported by The Agricultural Science and Technology Innovation Program (ASTIP) (cxgc-ias-01), the China Postdoctoral Science Foundation funded Project (2015M571182), the project National Infrastructure of Animal Germplasm Resources (2014 year).

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Cite this article as:

Yang, J., Liu, H., Wang, K. et al. Cell Tissue Bank (2017) 18: 513. https://doi.org/10.1007/s10561-017-9614-9

- DOI (Digital Object Identifier) https://doi.org/10.1007/s10561-017-9614-9
- Publisher Name Springer Netherlands
- Print ISSN 1389-9333
- Online ISSN 1573-6814

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