



Methods for animal satellite cell culture under a variety of conditions

Nycole M. Burton, Janet L. Vierck, Lyssa Krabbenhoft, Katherine Byrne & Michael V. Dodson

Washington State University, Department of Animal Science, Pullman, WA 99164, USA

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Abstract. Primary and clonal culture systems have been devised and refined for animal-derived satellite cells. Satellite cell (SC) culture development includes efficient cell isolation techniques, establishment of effective plating and growth conditions, formulation of media requirements and thorough evaluation of experimental limitations. As the field

of muscle cell culture has expanded, the number of animal species from which satellite cells have been isolated has increased. The focus of this paper is to compare and contrast SC culture conditions presently used by a variety of researchers and to introduce a new source of SC from wapiti (elk).

Key words: Cell culture comparisons, Satellite cells, Wapiti

Abbreviations: CS = chick serum; DMEM = Dulbecco's Modified Eagle Medium; DMSO = dimethyl sulfoxide; EGF = epidermal growth factor; EMEM = Eagle Minimal Essential Medium; FBS = fetal bovine serum; FGF = fibroblast growth factor; FN = fibronectin; GH = growth hormone; HS = horse serum; IGF (I & II) = insulin-like growth factors; ITT = insulin, T₃, transferrin; ITT-CF = insulin, T₃, transferrin, hydrocortisone, FGF; PBS = phosphate buffered saline, pH 7.08; PDGF = platelet-derived growth factor; PS = pig serum; PSG = pignskin gelatin; P/S = penicillin/streptomycin; SC = satellite cell; TGF- β = transforming growth factor- β

1. Introduction

Mauro and Katz independently reported the existence of SC [53, 58], leading to numerous electron microscopic studies to elucidate characteristics of these cells [69, 70]. Satellite cells lie juxtaposed to myofibers and facilitate postnatal skeletal muscle growth [19] and regeneration [78]. After initial postnatal muscle growth, SC remain in a quiescent state [4, 14, 22] until they are stimulated to proliferate, either by muscle injury or after being induced by regulatory factors [8, 15, 49, 82]. Following proliferation, SC either contribute their DNA to existing myofibers or fuse together to form new myofibers [4, 13]. Several excellent papers have been published on animal-derived SC structure, function, regulation and culture (Table 1). The development and use of SC culture systems has increased our knowledge of the mechanisms involved in postnatal skeletal muscle growth.

Animal models. Rat SC were first isolated and grown in primary cell culture by Bischoff [11]. Isolation and culture methods were subsequently defined for numerous animals, and resulting cultures were used to investigate mechanisms involved in SC activation, proliferation, differentiation and regeneration [18,

Table 1. Summary of animal satellite cell research with reference to different areas of study

Research area	Reference
Review papers	[7, 8, 14, 19, 28, 29, 49, 79, 80, 82]
Animal species	
Sheep	[23, 25, 26, 66]
Cattle	[24, 42, 43]
Turkey	[33, 59–61]
Chicken	[37, 62, 90]
Horse	[38, 44, 45, 82]
Pig	[30–32]
Fish	[55, 57, 71, 73, 87]
Rat	[5, 17, 69, 70]
<i>In vitro</i> characteristic	
b. Activation	[5, 22, 50–52]
c. Proliferation	[3, 6, 12, 16, 21, 31, 34, 41, 63, 74]
d. Differentiation	[26, 32, 41, 54, 73, 88]
e. Cell to cell interactions	
satellite cell to satellite cell	[57, 62, 67, 74]
satellite cells from different animals	[6, 25]
satellite cells to other cells	[16, 27, 86]
<i>In vivo</i> manipulations	[9, 10, 17, 56]

74]. *In vitro* growth, viability [47, 57] and response to growth regulators [25, 41, 60] varies among SC from different species or sources (Table 2).

Culture methods. Regardless of which animal SC culture system is employed, vigilance is required in order to use the system in the correct manner [89]. Animal type [6, 25], muscle source [62], methods used to harvest SC [12, 23, 24, 30, 73, 90], culture physiology [1, 9, 16, 27, 56] and treatment conditions [3, 26, 51, 63] must be thoroughly designed and evaluated with an appropriate end point in mind. For example, the rat SC system has been useful for some studies of SC activation [5], but rodent SC systems may not be the best choices for examining SC involvement in facilitating all aspects of postnatal muscle growth. Metabolism is different in ruminant animals in comparison to rats, and these differences appear to extend to the SC level [25]. This paper provides a comparative description of steps in SC isolation and culture for specific types of *in vitro* experimentation.

In order to isolate sufficient numbers of SC from rats for experimentation, muscles from the back and hind limbs of many animals must be processed [6]. This time-consuming effort may be viewed statistically as being reasonable, especially as experimental units (animal numbers) are high. However, satellite cells isolated from different muscles possess different capabilities to proliferate [6, 55, 73], differentiate [25, 62, 66] or be regulated by growth modifiers [29]. During isolation, the satellite cell fraction from certain muscles may overwhelm those from others.

Alternatively, there may be a loss of some SC populations [2], which may interject variability into the types and numbers of SC isolated [7, 14]. In contrast to the rat system, only one muscle is required for SC isolation if larger animals are used as the SC source [6, 23, 24].

Regardless of animal and muscle source, enzymes are used to liberate SC from cleaned [23, 24, 59, 73] and minced [11, 23] muscle. Pronase [11, 23, 24, 30, 44, 59, 73] and trypsin [11, 71] are effective enzymes for this purpose, because they are assumed to easily destroy components of the basal lamina and sarcolemma, allowing the liberation of SC and other intact cells of muscle. However, because these enzymes are also destructive to SC [11], temperature [55, 57, 71, 73] and length of exposure of the minced muscle to the enzymes [30] must be monitored during each isolation procedure (Table 3).

Once the enzyme treatment liberates SC from their encapsulated position juxtaposed to the myofiber [58], enrichment of the SC fraction may be accomplished through a variety of techniques. Differential centrifugation and preplating the total cell suspension on uncoated plastic dishes for short time intervals allow nonmyogenic cells to attach, thereby increasing the relative numbers of SC that are available for subsequent uses [23]. Another technique for SC enrichment is to use Percoll gradients to separate myogenic cells from other cell types [99]. A variety of other SC enrichment methods are available including use of a fluorescent activated cell sorter or differential lifting of (lightly) attached cells from culture plates and replating these cells to other

Table 2. Differences of animal models used in satellite cell cultures

SC source	Characteristics	References
Rat	Easy to obtain and maintain SC from several muscles are commonly pooled for experiments	[3, 5, 7, 11, 25]
Turkey and chicken	Feathers must be plucked, increasing the chance of contamination The pectoralis muscle has small amounts of connective tissue Selective breeding may have changed the physiology of these animals May possess different subclasses of satellite cells Pig serum is toxic to SC	[29, 33, 36, 59–63, 90]
Sheep and cattle	Hard to maintain in general academic setting Only one muscle is used Ruminant physiology may have an effect on SC activity Depreciation of carcass value should be considered	[23–26, 29, 43, 66–68, 88]
Horse	Difficult to obtain Only one muscle is used	[38, 44, 45, 82, 83]
Fish	Glycoprotein covering and scales must be removed White, epaxial muscle may have different subpopulations of SC Has a unique nervous system that may affect SC activity	[55, 57, 71, 73]
Pig	Live animal is hard to maintain in general academic setting Only one muscle is used Chicken serum is toxic to SC	[21, 30–32, 34]

Table 3. Comparisons of animal satellite cell culture conditions

Animals	Muscle location	Isolation enzyme ^a			Culture conditions substratum	Defined media	Serum source	Defined media		References
		Collagenase	Trypsin	Pronase				Proliferation	Differentiation	
Rat	Back and leg	X	X	X ^b	Fibronectin	DMEM/EMEM	HS, FBS	X		[2, 4–7, 11–13]
Cattle	Sternomandibularis		X	X	PSG	DMEM	FBS	X		[24, 43]
Pig	Semimembranosus			X ^c	PSG	MEM	FBS	X	X	[29–32]
Sheep	Semimembranosus, Semitendinosus			X	PSG	McCoy's 5A, DMEM	FBS, HS	X	X	[23, 25, 26, 66, 67]
Chicken	Pectoralis major	X	X	X ^d	PSG	HAMS F-10, DMEM	HS			[36, 37, 90]
Turkey	Pectoralis major			X	PSG	McCoy's 5A, DMEM	HS, FBS, CS	X		[59–62]
Horse	Semimembranosus			X	PSG	DMEM	HS			[38, 44, 45]
Fish	White epaxial	X	X	X ^e	Matrigel	Leibovitz's L-15, DMEM	FBS, HS			[57, 71, 87]
Wapiti	Sternomandibularis			X ^f	PSG	DMEM	HS			

^a Enzymatic digestion was for 60 minutes at 37 °C, and cellular isolation was by differential centrifugation except where indicated.

^b Differential centrifugation and sedimentation.

^c 40 minutes at 37 °C.

^d 10–60 minutes at 37 °C, differential centrifugation and percoll gradients.

^e 30–90 minutes at 11 °C–35.5 °C, differential centrifugation and percoll gradients.

^f 40 minutes at 37 °C.

cultures. All of these methods reduce nonmyogenic cell contamination and, therefore, increase the number of SC in the initial isolate.

Isolated SC are either plated onto cultureware with a substratum coating [23, 24, 59, 73, 90] or cryopreserved [67]. While selection of substratum type is dependent on the animal source [26, 87], commonly used substrata are Matrigel® [87], FN [24, 26] and PSG [26, 30, 44, 59]. Matrigel®, a solubilized basement membrane preparation produced from mouse sarcomas, has been used to facilitate myoblast attachment [87]. However, Matrigel® preparations contain other factors that stimulate the growth of SC, and cells attached to Matrigel® are hard to release for counting purposes. Although expensive, fibronectin is commonly used as a substratum coating, because it is a component of the basal lamina associated with muscle fibers [24] and is monodispersed in solution. PSG (denatured collagen) has been used as a substratum support in studies on SC proliferation [26, 30, 45] and differentiation [59, 88].

If SC are to be cryopreserved, the following general conditions should be employed: SC are first placed into a preservation medium containing DMEM + 20% HS and 10% DMSO and then aliquoted into cryovials with an average of 1.0×10^6 cells per vial. The vials are placed into a -80°C biofreezer overnight, then transferred to liquid nitrogen [67]. When needed for experimental use, SC are thawed in a 37°C waterbath.

After the SC have attached to a substratum coating, their viability [26, 87] and activity are influenced [59] by the use of a synthetic medium designed to supply the requisite vitamins, minerals, amino acids and inorganic salts [7]. Such media furnish an artificial environment to SC that not only provides nutrients to the cells but also allows waste products of the SC to be removed from the immediate area around the cells. The importance of using the correct medium cannot be over emphasized, because cell function and survival depend on this critical fluid.

There are two major types of media: serum-containing media [2, 14, 80] and defined media [29, 85]. Serum is commonly added to a basal medium as a source of growth factors and other metabolic agents [24, 26, 30, 59]. Horse [26, 30, 44, 73] and fetal bovine sera [24] are commonly used, although certain other sera have had beneficial effects on specific SC systems [59]. Although serum-containing media are useful for stock plates [11, 23, 24, 44, 59, 73, 90], serum contains numerous unknown mitogenic factors, which make it unsuitable for use in most mechanistic studies [25, 27, 43, 47, 60, 67, 88]. Defined treatment media have been developed for these latter applications [3, 25]. Florini and Roberts [39] formulated an initial defined medium that supported L6 myoblast growth. Since that time, defined media have been designed that sustain SC

proliferation [3, 31, 43, 63] and differentiation [32, 38, 61, 88]. These defined media have many constituents in common [reviewed in 29], but the concentration of components varies between species [25].

Application of culture methods. Primary [11, 23, 24, 30, 44, 55, 59, 73, 90] and clonal [3, 25, 32, 45, 47, 61, 63, 90] cultures have been used to define growth characteristics of SC. Primary SC cultures are derived directly from muscle tissue. As such, the *in vitro* properties exhibited by primary cultures of SC more closely reflect their *in vivo* properties than those exhibited by cloned SC [2], even though both cell systems lack nerve signals and the myriad of other factors that would influence the cells *in vivo*. Primary SC cultures, however, do possess non-myogenic cells [11, 23]. Even if enrichment techniques are used to decrease the presence of nonmyogenic cells in primary cultures, remaining nonmyogenic cell contamination in these SC culture systems makes it difficult to interpret regulatory influences of specific treatments. Mechanisms and regulation of SC attachment [26, 71, 73], proliferation [26, 30, 55, 59, 71, 73] and differentiation [24, 26, 30, 41, 55, 59, 71, 73] were originally obtained from primary SC culture systems.

Clonal culture systems have been produced from isolated SC populations from muscles of a number of different animals [31, 43, 47, 65, 88, 90]. Satellite cell clones are propagated from single cells, and their use eliminates many of the problems associated with primary SC cultures. For example, the paracrine effects between non-myogenic cells and SC [29] are eliminated with the use of cultures of cloned SC. Defined media used with clonal cultures have greatly expanded information on the regulation of SC activity by extrinsic factors [25, 27, 31, 43, 60, 65, 88].

The advent of clonal SC culture systems has opened up new research questions. Data from experiments using sheep SC clones has been interpreted to suggest that heterogeneous populations of SC [67] may exist *in vivo*. Co-culture is another example of a new research focus being addressed by the use of SC clones [16, 27, 48, 86]. Co-culture systems were developed to examine regulatory interactions between myoblasts and adipocytes [27, 48], SC and macrophages [16], and SC and neurons [86]. In many of these co-culture systems the cells are intermixed together in monolayers [16, 86]. However, in the myoblast and adipocyte co-culture system, the cells are physically separated by a microporous insert [27], which allows the detection of cell to cell communication/regulatory factors. Other SC culture systems have also been devised to define SC activity *in vitro*. A single fiber [12, 13, 77] system was developed to determine if the parental myofiber regulates SC activity [12, 13, 77]. This system has

subsequently been modified to allow different myofiber types to be examined and for greater ease in manipulating fibers while in culture [77].

Satellite cell culture systems have also been adapted for the study of SC involvement in muscle regeneration following an injury [40, 50, 64, 83] or due to muscle disease [9, 10]. Muscle regenerative properties seem to be influenced by the nature of the injury [9, 10, 16, 18, 54, 72] and/or the type of myofibers damaged [15, 20, 50, 56, 68, 81]. Rapid regeneration of mixed myofibers can be observed by simply cutting or crushing muscles [20]. More extensive regeneration studies often require whole muscles to be injured by the injection of myotoxic agents [50], freezing with liquid nitrogen [10], or disrupting their vascular [17] or neural [9, 18, 54, 72] supplies either experimentally or by transplantation [17, 56, 81]. These injuries have been inflicted on muscles of known myofiber type to observe how the SC of specific myofibers respond to certain types of injury [15, 19, 85, 91].

Differences and limitations of SC culture systems.

Most SC culture systems have added to our understanding of postnatal skeletal muscle growth [25, 30, 55, 59, 73]. Some SC culture systems are restricted in their adaptability, however [6, 31, 44, 71, 88]. For example, fish display muscle hyperplasia after hatching. Growth of hatched fish is in part correlated to environmental temperature [57]. As such it is reasonable that isolated fish SC activity may also be influenced by incubator temperature [57]. Consequently, trout SC are incubated at 20–22 °C [87],

and SC from catfish are incubated at 37–40 °C [71], and one should not expect exceptional growth of the SC at the alternative temperature. Also, observations of ruminant systems [23, 24] have documented different SC responses to specific substrata types [26] (Table 4).

Satellite cells from different species not only vary in response to culture methods but also to the same treatments [21, 22, 25]. With the use of clonal cultures and defined media formulations, SC from specific species had variable responses to the same regulatory factor [25, 31, 45, 47, 60, 90] (Table 5). Also, responses to specific doses of regulatory factors may differ among animal systems [25]. Similar increases of insulin dosages promote differentiative effects in rat SC and proliferative events in sheep SC [25].

The use of defined media has been valuable in determining extrinsic regulation of SC. Most defined media have been formulated for a specific SC system, but there have been instances in which the SC of one animal actually perform better in the defined media originally formulated for another species. This was observed when rat SC functioned better when exposed to defined media originally formulated for sheep SC [25], and when ITT, a medium designed for non-myogenic cells, enhanced differentiation of sheep SC [88] (Table 4).

Though variations between systems exist, SC cultures are powerful tools in addressing questions about muscle growth. Use of SC cultures has increased our understanding of the differences between embryonic and postnatal myogenesis [60,

Table 4. Similarities and differences between SC systems in their responses to culture treatments

Treatments	Effects				References
	Proliferation		Differentiation		
	Increase	Decrease	Increase	Decrease	
Substrata type					
PSG	S ^b , P ^e		S, P		[26, 30]
FN	B ^a				[24]
Matrigel [®]	F ^f		F		[87]
Media formulations					
DMEM	T ^d , P		B, S, T, P		[24, 30, 59]
McCoy's 5A	S, T				[26, 59]
Serum type					
HS	S, T		B, S, T		[24, 59]
FBS	P		P		[30]
PS		C ^c , T		C, T	[59, 90]
CS	T	P	T	P	[30, 59]
Defined media					
ITT			S		[88]
ITT-CF			H ^g		[38]

^a B = Cattle; ^b S = Sheep; ^c C = Chicken; ^d T = Turkey; ^e P = Pig; ^f F = Fish; ^g H = Horse.

Table 5. Similarities and differences in response of SC culture systems to regulatory factors^k

Treatments	Effects				References	
	Proliferation		Differentiation			Additional effects
	Increase	Decrease	Increase	Decrease		
Factors						
Testosterone		P ^c			[32]	
Insulin	B ^a , S ^b , C ^c , T, P		C, T ^d , P		S = ↓PD ⁱ C, T = ↑GU ^j [25, 75, 84]	
IGF-I	B, C, T, P, F ^f , H ^g	H	B, C, T,		S, P = ↑PS ^h S = ↓PD C, T = ↑GU [31, 34, 36, 37, 43, 45, 48, 55, 65]	
FGF	B, S, C, T, P, H	H			[21, 31, 42 43, 45, 63]	
GH		H		H		
TGF-β		B, T, P, H		B, S, C, T, H	[21, 43, 45, 47]	
EGF	P		S		S = ↑PS S = ↓PD [76]	
PDGF	C, T, P				[21, 31]	
Glucocorticoids	S, T, P				[25, 63, 35]	
Vitamin D ₃	C		C		C = ↑PS [35]	

^a B = Cattle; ^b S = Sheep; ^c C = Chicken; ^d T = Turkey; ^e P = Pig; ^f F = Fish; ^g H = Horse; ^h PS = Protein synthesis; ⁱ PD = Protein degradation; ^j GU = Glucose uptake; ^k Adapted from [29].

61, 75]. Application of SC cultures has also led to explanations of how regulatory factors influence muscle growth [5, 21, 31, 36, 40, 42, 47, 65, 84] and how SC activity may be altered by other cell types [16, 27, 48, 86]. Data generated from SC cultures have helped broaden the study of muscle regeneration [9, 17, 50, 74, 79].

2. A new SC system

Many wild animals [46] and most fish undergo seasonal bouts of growth throughout their lifespans. This phenomenon offers an opportunity to examine a variety of SC properties at markedly different stages of development. In this paper we report methods for SC isolation from adult male wapiti (*Cervus elaphus*) using adaptations from previous techniques [23, 24].

Materials

A. Equipment

1. Laminar flow hood, Model EG-4252.¹
2. Small meat grinder (1.5 mm plate), Cat. No. 793.83.²
3. Balance (top loading), Cat. No. A-160.³
4. Vortex.
5. 37 °C waterbath, Cat. No. 185.⁴

6. Centrifuge, tabletop, Model TJ-6, Cat. No. 14400.⁵
7. Pipette Aid, Cat. No. 174.⁶
8. 37 °C incubator (5% CO₂), Model 6000, Cat. No. 35909-750.⁷
9. Diaphot phase-inverted microscope.⁸

B. Supplies

1. 15 cm plastic culture plates, Cat. No. 168381.⁹
2. Sterile, 50 ml conical, centrifuge tubes, Cat. No. 2098.¹⁰
3. Surgery pans (size as needed).
4. Surgical instruments
 - a. 13 cm straight dissecting scissors, Cat. No. 25878-048.¹¹
 - b. Specimen hemostats, Cat. No. 25729-627.¹¹
 - c. Tissue forceps, Cat. No. 25729-864.¹¹
5. Buckets, Polypropylene, 250 ml and 1000 ml, Cat. No. 1393, 1396.¹²
6. Surgical gloves, disposable, Cat. No. 7825.¹³
7. Assorted bottles (one or two liter as needed).

C. Reagents

1. PBS + Antibiotics
 - 10.0 g Sodium chloride, Cat. No. S-5886.¹⁴
 - 0.25 g Potassium chloride, Cat. No. P-5405.¹⁴
 - 1.44 g Sodium phosphate, monobasic, Cat. No. S-5136.¹⁴
 - 0.25 g Potassium phosphate, monobasic, Cat. No. P-5655.¹⁴

- Distilled water, bring to volume (1 liter), pH to 7.08.
- Antibiotic-antimycotic mix (Penicillin, 10,000 U/ml; streptomycin, 10 mg/ml; Fungizone, 25 µg/ml), Cat. No. 15140-122, 15240-013.¹⁵
- 2. PSG
 - 0.1 g PSG/500 ml distilled water, Cat. No. G-1890.¹⁴
- 3. DMEM + 10% HS + Antibiotics
 - DMEM, 13.5 g, Cat. No. 12800-017.¹⁵
 - 3.7 g Sodium bicarbonate, Cat. No. S-5761.¹⁴
 - Distilled water, bring to volume (1 liter), pH = 7.08.
 - HS, 100 ml/885 ml DMEM, Cat. No. 16050-122.¹⁵
 - Penicillin/streptomycin mix, 10 ml/985 ml DMEM + HS, Cat. No. P-0781.¹⁴
 - Gentamicin, 5 ml/995 ml DMEM + HS + P/S, Cat. No. G-1272.¹⁴
- 4. Protease: type XIV, bacterial
 - 1 mg Pronase/1 ml PBS, Cat. No. P-51470.¹⁴
- 5. Ethanol (70%)

Procedures

A. Preparation of muscle (aseptic techniques are required for all steps).

1. Sternomandibular muscles (200 to 400 g) are surgically removed [24] and placed into sterile PBS (22 °C) for transport to the cell culture laboratory.
2. Muscle is quickly washed numerous times in sterile PBS (37 °C) + antibiotics.
3. Large pieces of connective tissue are removed.
4. Cleaned muscle (100 g) is ground in a small, sterile meat grinder.

B. Isolation of satellite cells

1. Ground muscle is distributed into 15 ml/50-ml centrifuge tubes, and pronase (1 mg/ml PBS) is added to a total volume of 35 ml per tube.
2. Tubes are placed in a 37 °C water bath for 40 min, with agitation every 10 min.
3. Tubes are centrifuged for 4 min at 1500 ×g (2700 rpm). The supernatant (enzyme) is discarded and replaced with PBS to a total volume of 35 ml per tube. Each tube is vortexed briefly.
4. Tubes are then centrifuged for 10 minutes at 400 ×g (1000 rpm) three times, and the supernatants from each cycle are saved and pooled into sterile 50 ml centrifuge tubes.
5. Supernatant tubes are centrifuged for 6 minutes at 1500 ×g (2700 rpm), and the resulting supernatants are discarded.
6. 12 ml of medium (DMEM + 10 % HS + antibiotics) are added to each tube containing

a cell pellet, and the tubes are vortexed for 0.5 min.

7. The cell suspensions from the initial muscle sample (step 6) are pooled into a single centrifuge tube.

C. Plating of cells

1. 10–12 ml of the pooled cell suspensions are aliquoted into PSG-coated 15 cm culture plates. Media (DMEM + 10 % HS + antibiotic) is added to each plate to bring the total volume to 35 ml per plate.
2. Plates are incubated for 24 hours in a humidified 37 °C, 5 % CO₂ incubator.
3. Cultures are washed with DMEM until the majority of nonmyogenic debris has been removed.
4. DMEM 10 % HS-containing medium is applied for the specific times required to allow proliferation of the cells to the point of use in an experiment. Alternatively, cells can be lifted off the plate with trypsin, counted with a hemocytometer and plated into treatment wells for use in an experiment (Figure 1).

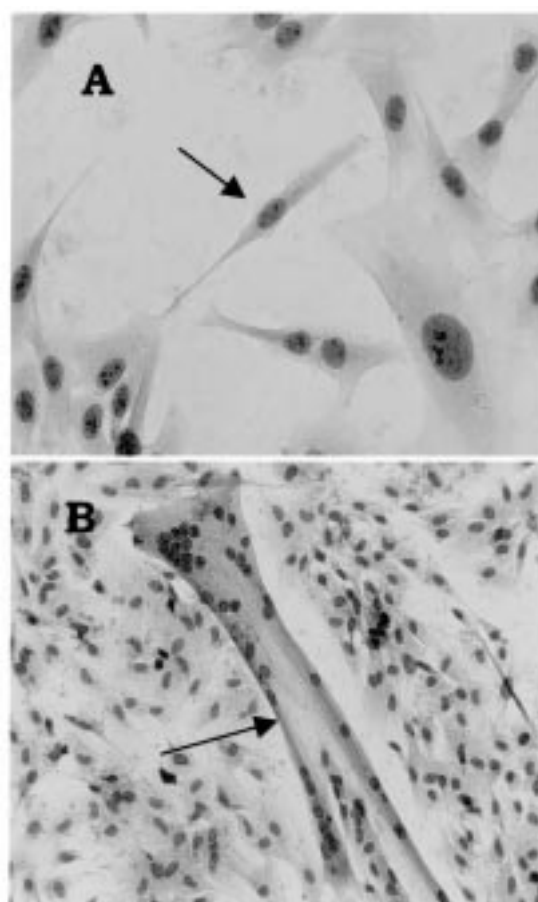


Figure 1. Wapiti satellite cells were isolated, cultured and allowed to proliferate and differentiate *in vitro*. (a) Newly isolated wapiti satellite cells (arrow; 40× magnification). (b) Wapiti SC-derived myotubes (arrow) at 120 hours *in vitro* (20× magnification). Pictures were taken with a Nikon Diaphot phase contrast microscope.

3. Summary

After SC were identified within muscle tissue, numerous microscopic studies confirmed their function in association with skeletal myofibers. Satellite cells are isolated from muscle tissue through the use of enzymes, differential centrifugation and other pre-culture techniques and are maintained in basal media plus serum. Certain levels of sera have been observed to facilitate SC proliferation and differentiation. However, because serum contains a number of unknown factors, defined media that are permissive to SC proliferation and differentiation were developed for a number of SC culture systems. Animal and muscle type, isolation procedures, serum concentrations and defined media formulations must be individually tailored when developing specific SC culture systems. Primary SC cultures exposed to serum-containing media provide a system to gauge initial proliferation and differentiation dynamics of SC, but the use of defined media with clonal SC cultures has greatly expanded the understanding of SC regulation. Modifications to the primary and clonal SC cultures have led to the development of other SC culture systems, such as co-culture and the single fiber system for use in evaluating SC involvement in muscle growth and regeneration. To date, research on agricultural animals and traditional laboratory animals such as mice and rats has been emphasized. The development of new systems, like the wapiti system, can further illuminate developmental differences in muscle growth, as mediated by SC.

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Notes on suppliers

1. Baker Co., Inc., Sanford, MN, USA
2. Sears and Roebuck, Chicago, IL, USA
3. Denville Scientific, Inc., Denville, NJ, USA
4. Precision Scientific, Chicago, IL, USA
5. Beckman Instruments, Inc., Palo Alto, CA, USA
6. Drummond Scientific Co., Brownhill, PA, USA
7. Sheldon Man. Inc., Cornelius, OR, USA
8. Nikon, Nippon K. K., Tokyo, Japan
9. Nunc Co., Denmark
10. Falcon Co., Onard, CA, USA
11. VWR Scientific, Seattle, WA, USA
12. Nalgene Co., Rochester, NY, USA
13. Ansell Incorporated, Dothan, AL, USA
14. Sigma Chemical Co., St. Louis, MO, USA
15. GIBCO Laboratories, Grand Island, NY, USA

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Address for correspondence: M. V. Dodson, Ph.D., Muscle Biology Laboratory, Department of Animal Sciences, Washington State University, Pullman, WA, 99164-6351, USA
Phone: 509-335-9644; Fax: 509/335-1082
E-mail: dodson@wsu.edu