Histol Histopathol (2005) 20: 891-899

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Muscle-derived stem cells in tissue engineering: defining cell properties suitable for construct design

J. Buján[1], G. Pascual[1], C. Corrales[1], V. Gómez-Gil[1], M. Rodríguez[2] and J.M. Bellón[2]

[1]Department of Medical specialities and [2]Department of Surgery, Faculty of Medicine, University of Alcalá, Madrid, Spain

Summary. The terms construct or tissue equivalent refer to neotissue produced by tissue engineering techniques. The elements forming the construct are scaffolds on which cells are "recreated" to form an enginnered-tissue sensitive to certain cell signals. The ability of the cells to expand and differentiate on the scaffold is determined by properties such as fixation, adhesion, proliferation and migration. Among the cell types that seem to be most promising for designing constructs are tissue-residing, or adult, stem cells, which show two main features: a capacity to differentiate into many cell lineages and the power of self-renewal. These features make them good candidates for cell replacement therapies. Here, we report the identification, isolation and culture of muscle stem cells aimed at establishing the ideal culture in terms of defining when the cultured cell population would show optimal characteristics for transfer to the scaffold to obtain a particular construct. Stem cells harvested from the dorsal muscle of white New Zealand rabbits were cultured in vitro and characterized 5 to 14 days after the start of culture. Fibroblasts obtained from the same experimental animal served as controls. The stem cells were examined by light and scanning electron microscopy. For stem cell identification, we used the antibodies anti-m-cadherin, anti-CD34 and anti-Myf-5. The markers of muscle differentiation used were: anti-vimentin, anti-a-actin, anti-desmin and anti-myosin. The expression profiles of the different markers of muscle differentiation and TGFß1 in the cell cultures were confirmed by Western blotting. Proliferation rates were determined by monitoring tritiated thymidine incorporation.

The thymidine incorporation rate was substantially higher for the population of undifferentiated cells than for control fibroblasts obtained from the same animal. During the first five days of culture, most cells were negative for all the markers examined, with the exception of m-cadherin, CD34 and Myf-5, although discrete signs of vimentin expression started to emerge. After 14 days of culture, the adult stem cells showed vimentin (94.2%) and desmin (33.8%) expression yet scarce labeling for myosin (16.2%) and α-actin (8.3%). Control fibroblasts showed intense labeling for vimentin (99.3%) and α-actin (62.2%), while less than 2% of the population expressed myosin (0.9%) and desmin (1.6%).

After two weeks of culture, muscle-derived stem cells show good proliferative and adhesion properties as they initiate differentiation. These conditions seem ideal for obtaining the desired construct.

Key words: Muscle-derived stem cells, Cell differentiation, Tissue engineering, Regenerative therapy

Offprint requests to: Dra. J. Buján, Departamento de Especialidades Médicas, Facultad de Medicina, Universidad de Alcalá, Ctra N-II, Km 33.600, 28871 Alcalá de Henares, Madrid, Spain. e-mail: mjulia.bujan@uah.es

Introduction

The appearance of stem cells on the research front and their potential for clinical applications has provided a new work tool: that of undifferentiated cells that will differentiate in the environment we wish to repair.

Tissue engineering is a multidisciplinary area of research aimed at regenerating tissue and restoring organ function by the transplant of tissues or adult differentiated cells stimulated to grow on implanted matrices (Vacanti and Langer, 1999; Stock and Vacanti, 2001) to create substitute tissue (Bellón et al., 1993; Hernando et al., 1994).

Adult stem cells occur in specific niches, or tissue compartments, and their role is to preserve tissue integrity by repairing any damage it may suffer. Their cell cycle is generally short and they are capable of responding to specific signals to generate new stem cells by self-renewal or selecting a specific differentiation program (Fuch and Segre, 2000). The number of stem cells and the differentiation process are highly regulated according to the tissue's needs at a given moment, and cell signals have been identified that maintain an appropriate balance between undifferentiated precursor cells and their differentiated progeny (Rao and Mattson,

2001).

Adult stem cells are considered multipotent since they give rise to a small number of cells or tissues restricted to a given germ line, as opposed to embryonic stem cells, which can form most of the cells or tissues in an organism (Vants et al., 2002). However, it is beginning to emerge that adult multipotent stem cells show greater plasticity than was initially thought, and they are now being explored as a therapeutic alternative to embryonic stem cells.

Given its particular capacity for renewal, skeletal muscle was the first choice as a source of adult stem cells. Skeletal muscle has a large population of myogenic cells (satellite cells) (Kuehnle and Goodell, 2002), functionally defined as the cells responsible for differentiation towards myoblasts, which subsequently fuse to form myocytes, and a second population of cells known as muscle-derived stem cells (MDSC). This last group of cells could be the predecessors of the cells in the first population (Seale and Rudnicki, 2000) and have a broader capacity for differentiation, not confined to the myogenic cell lineage. The identification of the cells of these two populations is difficult since their expression of distinguishing markers depends on their activation state, which is in turn affected by the degree of differentiation. Asakura et al. (2001) report that primary satellite cells express the myogenic markers MyoD, Myf5, desmin and Pax-7, and can differentiate into myogenic, osteoblastic and adipose lineages. In subsequent studies by this author (Asakura et al., 2002), it was shown how these two cell populations, MDSC and satellite cells, are independent: MDSC were found to express the hematopoietic stem cell marker Sca-1 and CD45, thus had a hematopoietic capacity, and the satellite cells did not express these markers or form hematopoietic colonies. As a satellite cell marker, some authors defend the use of Pax 7 (Seale et al., 2000) and others that of Myf5 and CD34 (Beauchamp et al., 2000). Myosin has been used as a marker of myoblasts from skeletal muscle as possible candidates for treating ischemic heart disease (Mirabet et al., 2000).

Despite the present controversy over the different markers expressed by MDSC, these cells are being used in several tissue engineering applications, especially for producing constructs. Constructs are generally elaborated by seeding MDSC on a scaffold aimed at achieving stability and cell expansion and "recreating" a given tissue. So far, the time point selected for transplanting the MDSC onto the scaffold is based on no objective criteria in terms of stability at the transplant site and their ability to undergo suitable differentiation in the construct.

In this study, we identified and isolated muscle adult stem cells, and evaluated their proliferation capacity and changes in the expression of their markers during culture. Our ultimate goal was to obtain a population of MDSC, able to adhere well to the substrate before differentiating in their new environment, which could be suitable for regenerative therapy applications.

Materials and methods

Harvesting and culturing stem cells

Muscle cells were obtained under sterile conditions from a biopsy specimen comprised of 1g of the dorsal muscle of white New Zealand rabbits. For the biopsies, the animals were anesthetized with a mixture of ketamine chlorohydrate (70 mg/kg), diazepam (1.5 mg/kg), and chlorpromazine (1.5 mg/kg) administered intramuscularly. A fragment of dorsal muscle was then obtained through a small paravertebral incision.

The biopsy specimens were transported to the laboratory in minimal essential medium (MEM, Invitrogen, Barcelona). A portion of the muscle tissue was used as control, to identify the stem cell population in situ, and the remaining tissue was processed to isolate the cells. The muscle was washed in MEM, cut into small pieces and placed in an incubator at 37°C in a 0.1% suspension of type I collagenase (10 ml/g tissue) (Worthington, Iberlabo, Madrid) for 60 min. Following incubation, the suspension was centrifuged at 1050 rpm for 5 min. The resultant precipitate was resuspended in 10 ml of 1% trypsin-EDTA (Invitrogen, Barcelona) and placed in a water bath at 37°C with constant shaking for 40 min. The action of trypsin was stopped by adding 10 ml of bovine fetal serum (BFS) (Invitrogen, Barcelona). The final suspension was filtered through a 100 µm pore-size mesh and then centrifuged at 1050 rpm for 5 minutes. The supernatant was removed and the precipitate was resuspended in 10 ml of MEM, and centrifuged a further 3 times at 1050 rpm for 5 min. The pellet was resuspended in 10 ml of complete culture medium (Amniomax; Invitrogen, Barcelona) and transferred to a 75 cm^2 culture flask. The flask was incubated at 37°C in a 5% CO_2 atmosphere until 40-50% confluence. At this point the cells were treated with 1% con trypsin-EDTA and subcultured at a 1:3 ratio (Fig. 1). Fibroblasts isolated from dermal explants of the ear of the same experimental animal were used as the control cell population.

Morphological analyses

Cells for light microscopy were observed in culture using an inverted microscope (Olympus, Japan).

For the ultrastructural observations, cells were seeded onto sterile coverslips, fixed for 1 h in 3% glutaraldehyde, stored in Millonig buffer, pH 7.3, and postfixed in 2% osmium tetroxide. The cells were then dehydrated in a graded acetone series and embedded in Araldite to obtain thin cuts. Finally, the sections were contrasted with lead citrate and examined in a Zeiss 109 transmission electron microscope (TEM). Coverslips with cells for scanning electron microscopy (SEM) were immersed in 3% glutaraldehyde. Once transferred to Millonig buffer, pH 7.3 for 1 h they were dehydrated in a graded acetone series, reaching critical point in a Polaron E 3000 with CO_2. The cells were finally

metalized with gold-palladium before observation in a Zeiss 950 DSM scanning electron microscope.

Cell proliferation

To determine the proliferative capacity of the adult stem cells, $5x10^4$ cells were seeded on glass coverslips. Proliferation rates were estimated by adding ^{3}H-thymidine (1 µCi/ml) to the culture medium for 24 h and evaluating changes after 14 days of culture. Fibroblasts from the same animal were used as controls. At each follow-up time, the cultures were fixed in 10% formaldehyde and subjected to autoradiography. For this, the coverslips were immersed in NTB-2 emulsion (Eastman Kodak, Rochester, NY, USA) diluted 1:1 in water at 40°C and stored at 4°C in the dark for 2 weeks. The coverslips were developed in D-19 (Kodak) for 5 min with shaking, washed briefly in 0.5% acetic acid and fixed using Rapid Fix (Kodak) for 5 min. They were then washed for 1 h under running water and left to dry at room temperature. The coverslips were stained with Carazzi's hematoxylin and mounted with plasdone.

Identifying the cell population

Stem cells were identified in situ and in culture using antibodies against m-cadherin, CD34 and Myf-5 (Santa Cruz, California, USA). We also used the following antibodies to identify markers of muscle differentiation: anti-vimentin (Sigma, St. Louis, MO, USA), anti-α-actin (Sigma, St. Louis, MO, USA), anti-desmin (Sigma, St. Louis, MO, USA) and anti-myosin (Sigma, St. Louis, MO, USA).

Labeling was performed on 5 µm-thick muscle biopsy sections previously fixed in 2% paraformaldehyde, Bouin's fixative or methanol, or on cell cultures fixed at 4°C for 5 min in 2% paraformaldehyde or methanol, depending on the antibody used. As secondary antibodies, we used an anti-mouse rabbit IgG (Sigma, St. Louis, MO, USA), an anti-goat mouse IgG (Sigma, St. Louis, MO, USA), both biotin-conjugated and diluted 1/300 in PBS, pH 7.6, and an anti-mouse rabbit IgG conjugated with fluorescein isothiocyonate (FITC) (Zymed Laboratories, San Francisco, USA) diluted 1/20 in PBS, pH 7.6. For the standard immunohistochemical procedures, avidin-biotin labeled with alkaline phosphatase was used to detect the antigen-antibody reactions. Cell nuclei were counterstained with hematoxylin. Labeling was detected under a light microscope (Zeiss, Jena, Germany). Images were recorded on Ektachrome 160T film (Eastman Kodak, Rochester, NY, USA).

In the immunofluorescence analysis, when the secondary antibody was not directly conjugated with a fluorochrome, the antibody-antigen reaction was detected using an amplification system TSA TM Plus Fluorescence System (Perkin Elmer Life Sciences, Boston, MA, USA) with cyanine 3 (Cy3). Cell nuclei were counterstained with 4',6-diamino-2-phenylindole-dihydrochloride (DAPI). Specimens were examined using a Zeiss Axiophot microscope fitted with a fluorescence lamp. Ten visual fields of each cell population were examined at random (40X) to obtain labeled cell and non-labeled cell counts, giving expression percentages for the different antibodies. All counts were performed by two different observers. Images were recorded on Fujichrome 400 film (Fuji Photo Film Co., Ltd., Tokyo).

Western blots

Two hundred thousand cells were seeded in 25-cm^2 flasks with the appropriate culture medium. Once the cells were confluent, they were lysed and their proteins precipitated with trichloroacetic acid solution (TCA). The proteins were then separated by electrophoresis on a sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) under reducing conditions and in the presence of 4% 2-beta-mercaptoethanol. Equal aliquots (20µl) diluted in sample buffer 2x were boiled for 5 minutes before loading. After 2 hours of electrophoresis at 100v, the separated proteins were transferred to a nitrocellulose membrane (Bio Rad Laboratories, Hercules, CA, USA) at 210 mA for two hours at room temperature. Membranes were blocked overnight at 4°C with 5% dry milk in phosphate-buffered saline (PBS1x) with 0.05% Tween 20. The following primary antibodies were applied to the membranes for 2 h at room temperature: anti-vimentin (1:1000), anti-α-actin (1:1000), anti-desmin (1:500), anti-myosin (1:1000) and anti-TGFß1 (R&D Systems Inc., Minneapolis, MN, USA) (1:5000). The secondary antibodies anti-mouse and anti-goat IgG-horseradish peroxidase (Sigma, St. Louis, MO, USA) (both 1:10000) were incubated with

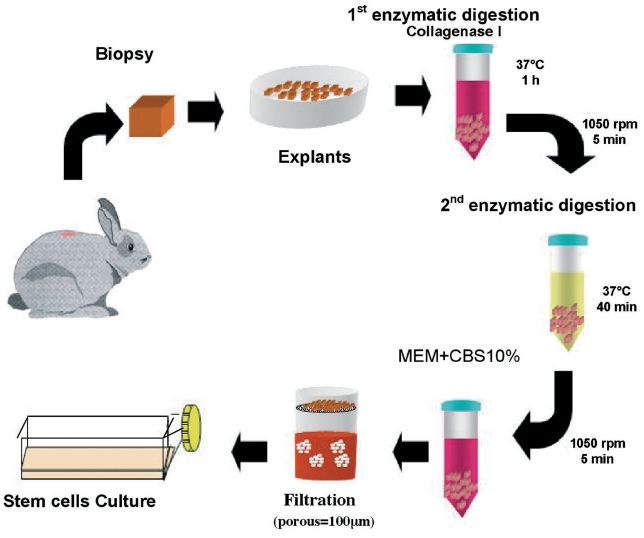


Fig. 1. Diagram showing the stem cell extraction procedure.

the membranes for 1.5 h at room temperature. The blots were developed using the SuperSignal Westpico Chemioluminiscent kit (Pierce, Rockford, IL, USA). Positive bands were visualized on X-ray film.

Experimental design

Muscle biopsies were obtained from 20 animals. Before isolating the stem cells, a portion of muscle tissue was processed to identify the cell population required.

Follow-up evaluations of the cell cultures were performed 5 and 14 days after harvesting the cells.

Data derived from the stem cell and control fibroblast populations were compared by ANOVA and the Mann Whitney U-test.

Results

Morphological analyses

The cells in culture showed a pleomorphic appearance including different shapes and sizes (Fig. 2a), although in the culture flask their general appearance was fibroblast-like. On electron microscopy, most of the cells showed a secretory phenotype with an intensely swollen reticulum, dense bodies in the

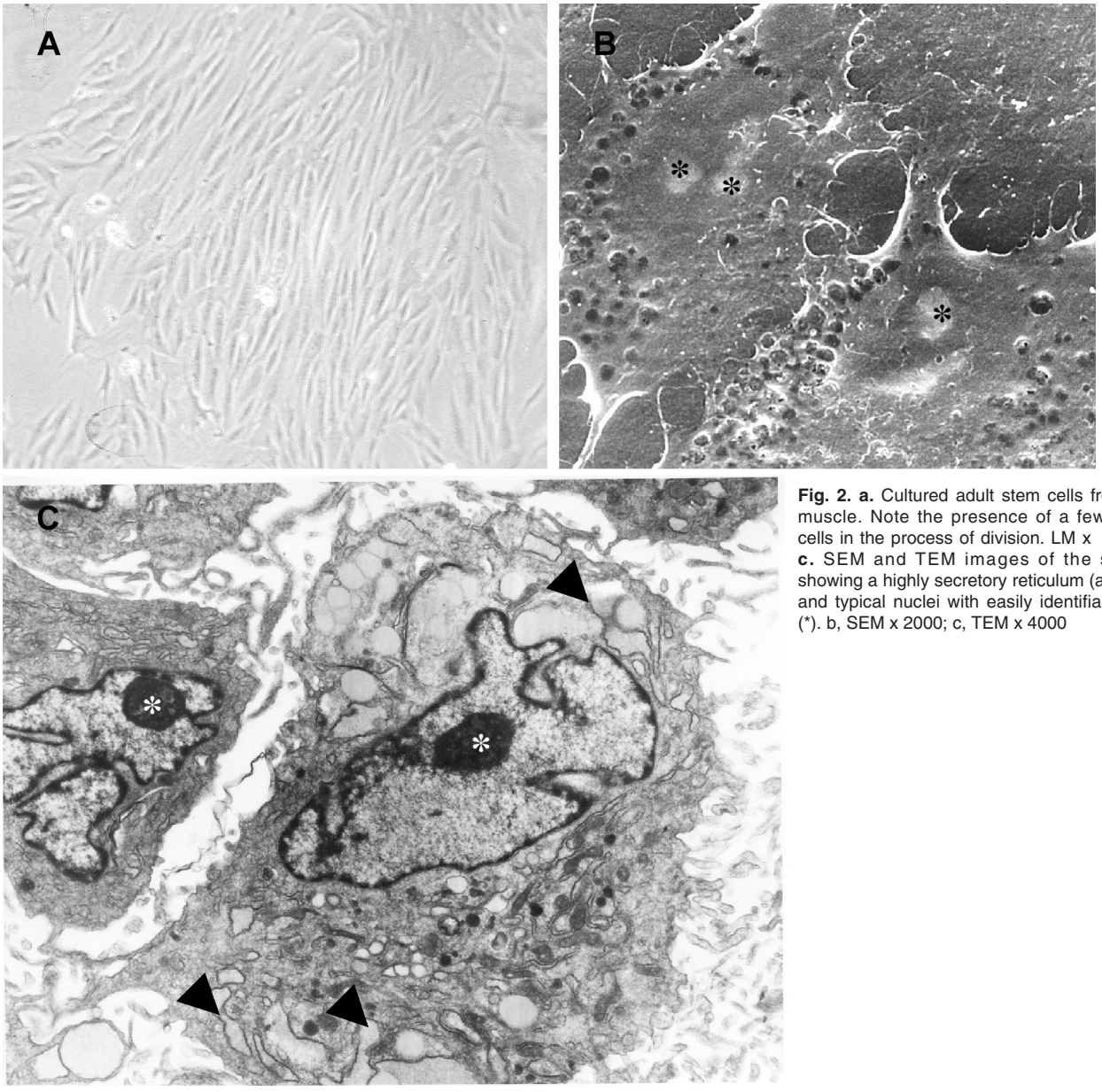


Fig. 2. a. Cultured adult stem cells from skeletal muscle. Note the presence of a few refringent cells in the process of division. LM x 100; **b and c.** SEM and TEM images of the stem cells showing a highly secretory reticulum (arrowheads) and typical nuclei with easily identifiable nucleoli (*). b, SEM x 2000; c, TEM x 4000

cytoplasm, and characteristic nuclei with easily discernable nucleoli (Fig. 2b,c).

Cell proliferation

The rate of tritiated thymidine incorporation was appreciably higher for the population of undifferentiated cells (Fig. 3) than for control fibroblasts.

Immunohistochemical analysis

Control muscle tissue before cell isolation

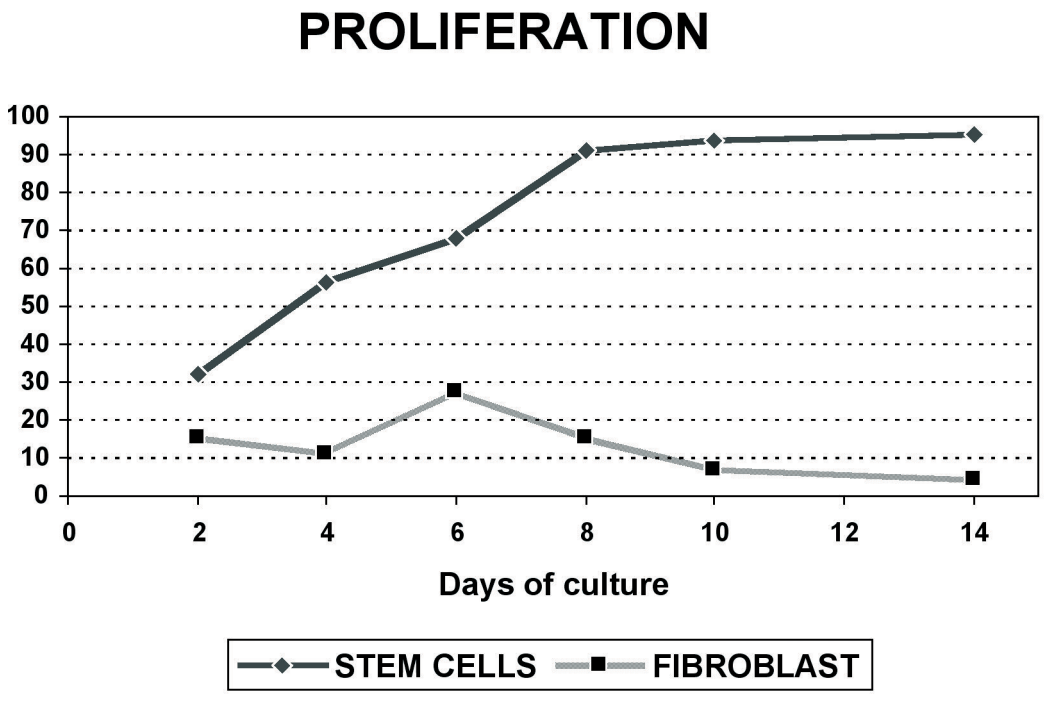
Skeletal muscle cells were surrounded by cells showing the immunophenotype m-cadherin, CD34, Myf-5 (Fig. 4a), α-actin, vimentin. This cell profile corresponds to that of vascular cells, fibroblasts or mesenchymal cells and it was from this cell pool that we isolated cells for subsequent culture.

Cells cultured for 5 days

During the first few days of culture, most cells were negative for all the markers with the exception of m-cadherin, CD34 and Myf-5 (Fig. 4b). However, we did observe the incipient expression of vimentin, such that 10.3±1.8% (Table 1) of the cell population was positive for the vimentin antibody.

Cells cultured for 14 days

By the second week of culture, changes started to emerge in the phenotypic expression of the culture. All cells continued to express m-cadherin (Fig. 4c) and a



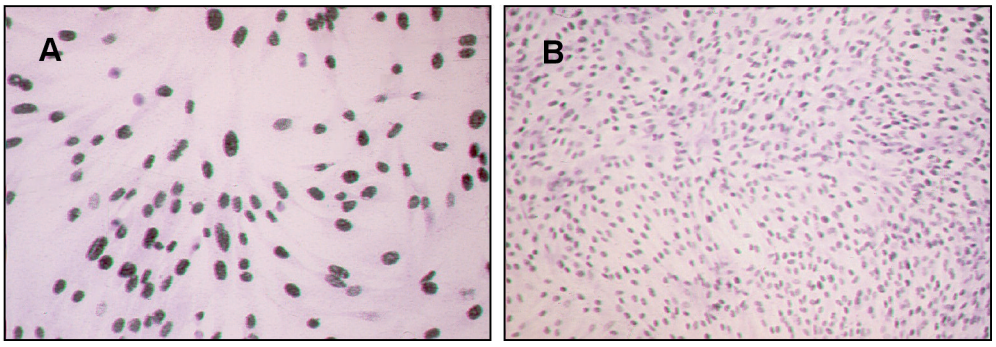


Fig. 3. Proliferation of stem cells versus fibroblasts. Stem cells in culture showing incorporation of ^{3}H-tymidine in the nucleus by autoradiography at 5 **(a)** and 14 days **(b)**.

Table 1. Proportions of the stem cell population immunohistochemically expressing markers of muscle differentiation (mean±SD) (*p<0.05)

ANTIBODY	Sc 5 DAYS	Sc 14 DAYS	Fb CONTROL
Vimentin	10.3±1.8%	94.2±5.3%	99.3±2.1%
Desmin	-	33.8±5.1%	1.6±0.8%*
Myosin	-	16.2±1.6%	0.9±0.6%
α-Actin	-	8.3±2.1%	62.2±3.1%*

SC: stem cell population; Fb: fibroblast population.

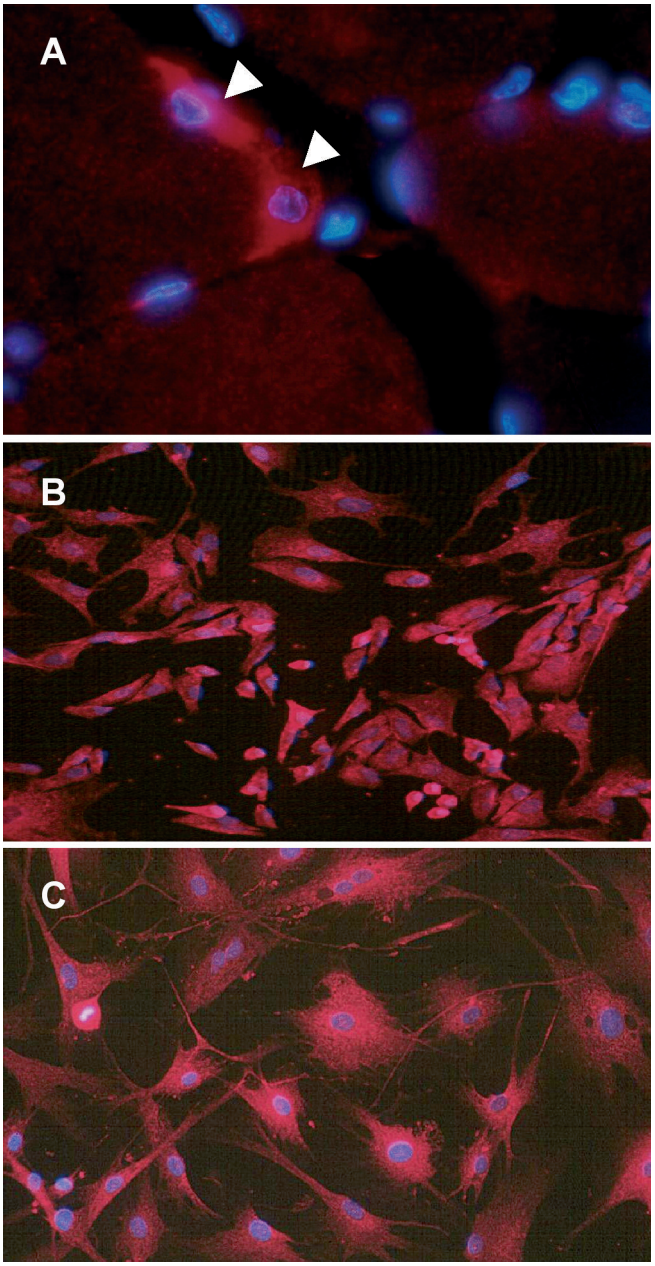


Fig. 4. a. Expression of m-cadherin in stem cells from the dorsal muscle of the rabbit and at 5 and 14 days of culture **(b and c)**.

large proportion showed vimentin expression (94.2±5.3%). The expression of most of the remaining markers dropped, with values of 33.8±5.1% recorded for desmin and 16.2±1.6% for myosin. α-actin occurred in isolated cell colonies; the proportion of cells immunostained with its antibody was 8.3±2.1% (Fig. 5).

Control fibroblasts

Almost the entire population was found to express vimentin (99.3±2.1%). The numbers of cells expressing myosin and desmin were reduced compared to the stem cells, with values under 2% recorded for both proteins (Table 1). The proportion of fibroblasts labeled with the desmin antibody differed significantly with respect to the stem cell population ($p<0.05$). In contrast, α-actin was detected in a significantly ($p<0.05$) higher number of fibroblasts than stem cells, reaching proportions above 60% (62.2±3.1%) (Fig. 5).

Western blotting

The different markers of muscle cell differentiation and the TGFß1 were also determined in control fibroblasts and stem cells by immunoblotting. Both cell populations were found to express all the markers examined (Fig. 6). The characteristic vimentin band (of approximately 58 KDa) was similarly observed in fibroblasts and stem cells. A further two bands appeared below the vimentin band, which could reflect protein degradation during the extraction process. In contrast, immunoblots using the anti-α-actin antibody (molecular weight 42 KDa) revealed a significant difference ($p<0.05$) between the fibroblast and stem cell populations. Myosin expression (204-200 KDa) was similar, yet desmin (50-55 KDa) expression differed ($p<0.05$) between the two cell populations. Three bands corresponding to TGFß1 were observed for the two cell populations. The most intense band appeared at around 120KDa, while the others appeared as dimers of molecular weights above 79KDa. TGFß1 expression was significantly higher ($p<0.05$) in the fibroblasts than the stem cells for each of the three bands (Fig. 7). Overall, the results of the Western blot experiments were consistent with our immunohistochemistry data.

Discussion

Muscular tissue contains several cell types including satellite cells, muscle associated fibroblasts, and pericytes, smooth muscle cells and endothelial cells related to blood vessels (Zammit and Beauchamp, 2001). All these cell types contribute to the so-called muscle-derived stem cell (MDSC) population of adult tissues with regenerative capacity. The initial controversy in the literature refers to the type of muscle stem cells that can be isolated from skeletal muscle. According to some authors, satellite cells have stem capacity (Cheng and Goldhamer, 2003), while others believe these cells arise

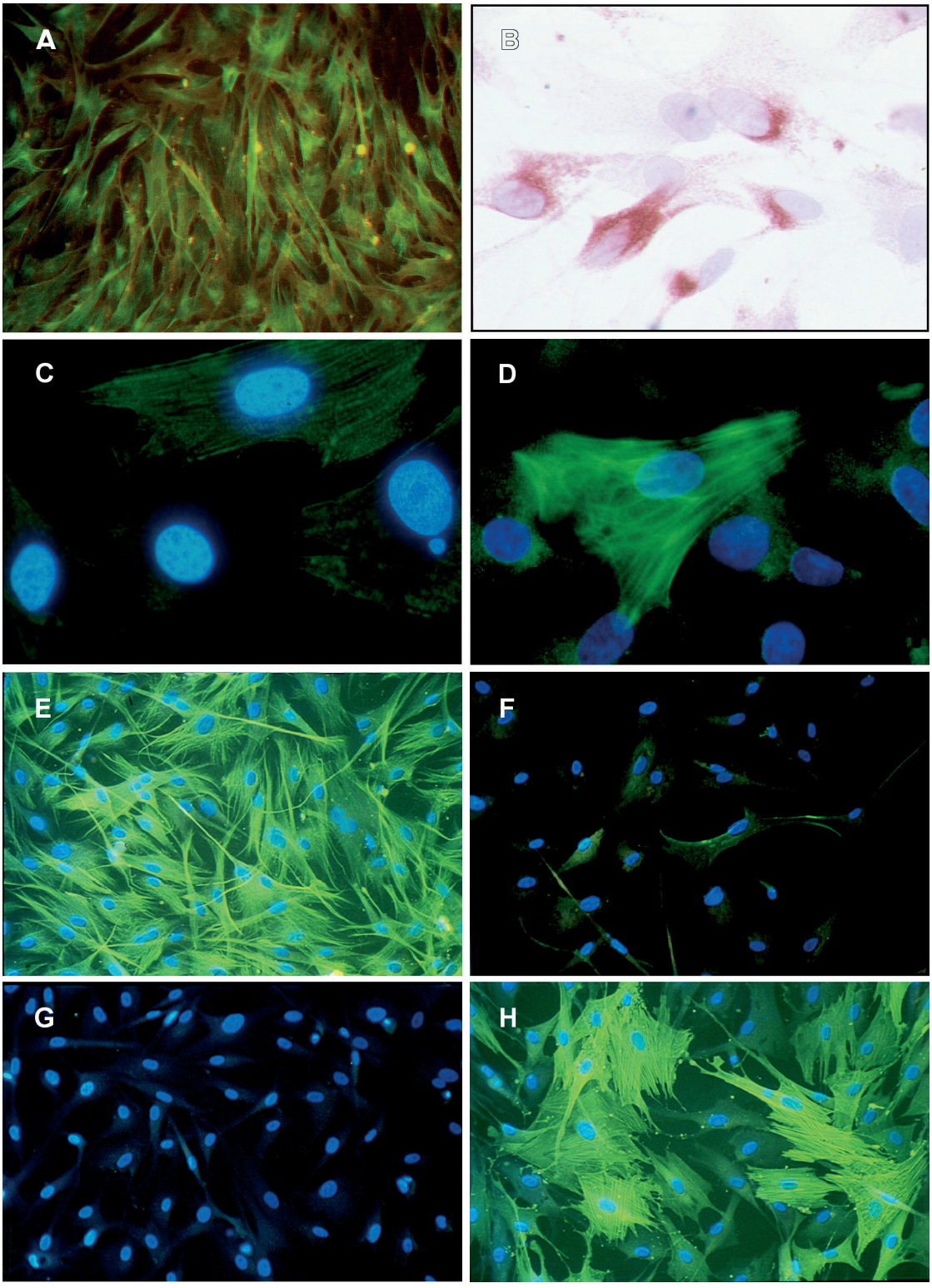


Fig. 5. Expression of the markers of muscle differentiation in the adhering cell population after 14 days of culture: **a)** vimentin (x 200); **b)** desmin (x 630); **c)** myosin (x 1000); **d)** α-actin (x 1000). Expression of the same markers in the control fibroblast population: **e)** vimentin (x 200); **f)** desmin (x 200); **g)** myosin (x 200); **h)** α-actin (x 200).

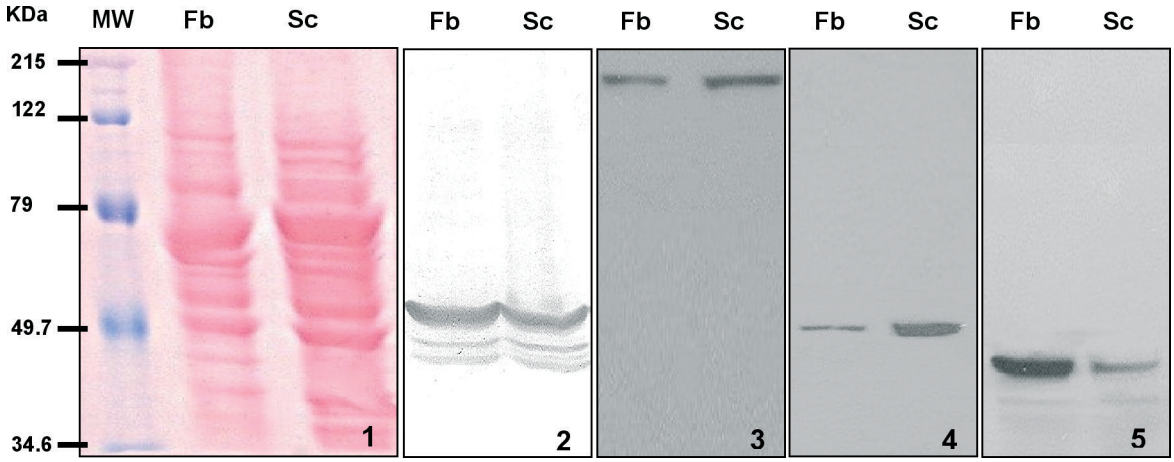


Fig. 6. Immunoblots of the muscle differentiation markers examined in the different cell populations. **(1)** Ponceau red-stained proteins, left lane, molecular mass markers (MW), fibroblasts (Fb) and stem cells (Sc). The antibodies anti-vimentin **(2)**, anti-myosin **(3)**, anti-desmin **(4)** and anti-α-actin **(5)** were used for the Western blots.

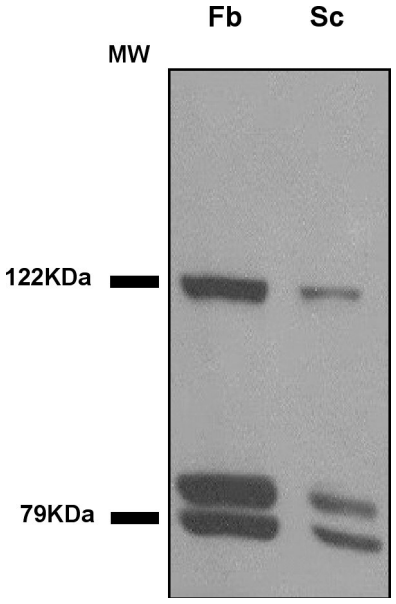


Fig. 7. Western blot analysis using anti-TGFß1 in the fibroblast (Fb) and stem cell populations (Sc). Left lane, molecular mass markers (MW).

from other more undifferentiated cells (Stewart et al., 2003). The muscle cell population examined in the present study was heterogenous, since only the muscle fibers were excluded. In this mixed cell population, we confirmed the immunohistochemical presence of cells expressing satellite cell markers (m-cadherin) along with vascular cells expressing markers such as α-actin.

The in vitro characterization of MDSC was difficult since to date there is no validated procedure defining the most appropriate markers for their identification. The method most accepted today is based on the analysis of their in vitro replication, along with the expression of certain markers. These cells are highly sensitive to the environment, and have a great capacity for differentiation. It is therefore difficult to find a single specific marker that also persists in time. This is the reason for the existing controversy over the expression of the different markers and whether the cells are progenitors or successors (Morgan and Partridge, 2003).

Given this situation, attention has turned to the processes that take place during embryonic development (Tajbakhsh, 2003), and authors such as Kablar et al. (2003), by generating knockout mice lacking the genes involved in muscle differentiation, have reported that these cells do not differentiate, rather they persist as multipotent mesenchymal cells. Future studies using these embryonic cell lines will no doubt shed some light on the cells remaining as stem cells in muscle tissue.

We used markers of both undifferentiated cells and of differentiated cells, at three stages of differentiation. The first outstanding feature of our cultured cell population was the high proliferation rate, indicating a much greater capacity than the control fibroblast population. Along with their capacity for asymmetric division, this confirms one of the characteristics defining a tissue-residing stem cell population (Janes et al., 2002; Tajbakhsh, 2003).

A further significant finding was that at the initial stages of culture, cells were only positive for the m-cadherin antibody and did not appear to express markers indicating differentiation via a single, or several different, pathways. It was not until two weeks of culture that the emerging cell population started to show the characteristics we were looking for. Thus, in agreement with the findings of other authors in undifferentiated populations (Jackson et al., 1999; Seale et al., 2000), the cells showed intense phenotypic vimentin expression. However, this feature is shared by differentiated cells in culture, and in our case, in the fibroblast population used as control. The importance of the development of adhesion properties at two weeks in our population may lead us to define this as a prerequisite for optimizing the

time of transplant, since good adhesion to the scaffold is essential for creating constructs.

Nonetheless, differentiation was not always similar in both populations and over time, markers such as desmin and myosin started to be expressed in the stem cell population, and labeling for α-actin was restricted to small isolated colonies. The expression patterns of these markers differed from the profiles of control fibroblasts even when both cell populations were under the influence of the same culture medium. The dermal fibroblasts acquired the characteristics of myofibroblasts, as revealed by the immunohistochemical expression of α-actin in over half the population examined. We suggest that the development of intracellular stress fibers containing cytoplasmic actin isoforms (Hinz and Gabbiani, 2003) would be initially induced by the presence of cytokines and TGFß1 in the culture medium and subsequently through an autocrine mechanism, accounting for the considerable TGFß1 synthesis indicated by the Western blots. Such a mechanism would promote the differentiation of fibroblasts into proto-myofibroblasts (Hinz and Gabbiani, 2003).

On the contrary, in the MDSC population, expression levels of intracellular proteins such as desmin and myosin increased, suggesting their progression towards muscle cell lineages. Despite there being considerable variation in the expression of differentiation markers among muscle cells, in this study of cultured muscle stem cells, the patterns observed over time resemble those shown by arterial smooth muscle cells in fetal stages (Kocher et al., 1985) in that there were similar proportions of vimentin- and desmin-positive cells. Thus, it would seem that after 14 days of culture, our population was mainly comprised of cells with a capacity for adhesion, proliferation and migration. In addition, the population showed signs of differentiation, suggesting that these cells could be suitable for therapies of a regenerative nature.

Acknowledgements. This work was supported by grants from the Comunidad Autonoma de Madrid (CAM. Nº Expediente: 08.4/0022.1/2003) and from the Fondo Investigación Sanitaria (FIS PI031611). We thank Vanesa Montes Anguita for excellent technical assistance.

References

Asakura A., Komaki M. and Rudnicki M.A. (2001). Muscle satellite cells are multipotential stem cells that exhibit myogenic, osteogenic and adipogenic differentiation. Differentiation 68, 245-253.

Asakura A., Seale P., Girgis-Gabardo A. and Rudnicki M.A. (2002). Myogenic specification of side population cells in skeletal muscle. J. Cell Biol. 159, 123-134.

Beauchamp J.R., Heslop, L., Yu, D.S.W., Tajbakhshs S., Kelly R.G., Wernig A., Buckingham M.E., Partridge T.A and Zammit P.S. (2000). Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. J. Cell Biol. 151, 1221-1233.

Bellón J.M., Buján J., Garcia-Honduvilla N., Hernando A. and Navlet J. (1993). Endothelial cells seeding on PTFE vascular prostheses coated with a fibroblastic matriz. Ann. Vasc. Surg. 7, 549-555.

Chen J.C.J. and Goldhamer D.J. (2003). Skeletal muscle stem cells. Rep. Biol. Endocrinol. I, 101.

Fuchs E. and Segre J.A. (2000). Stem cells: A new lease on life. Cell 100, 143-155.

Hernando A., Garcia-Honduvilla N., Bellón J.M., Buján J. and Navlet J. (1994). Coatings for vascular prostheses: Mesothelial cells express specific markers for muscle and have biological activity similar to that of endothelial cells. Eur. J. Vasc. Surg. 8, 531-536.

Hinz B. and Gabbiani G. (2003). Mechanisms of force generation and transmission by myofibroblasts. Curr. Opin. Biotechnol. 14, 538-546.

Jackson K.A., Mi T. and Goodell M.A. (1999). Hematopoietic potential of stem cells isolated from murine skeletal muscle. Proc. Natl. Acad. Sci. USA 96, 14482-14486.

Janes S.M., Lowell S. and Hutter C. (2002). Epidermal stem cells. J. Pathol. 197, 479-491.

Kablar B., Krastel K., Tajbakhsh S. and Rudnicky M.A. (2003). Myf 5 and MyoD activation define independent myogenic compartments during embryonic development. Dev. Biol. 258, 307-318

Kocher O, Skalli O, Cerutti D., Gabbiani F. and Gabbiani G. (1985). Cytoskeletal features of rat aortic cells during development. An electron microscopic, immunohistochemical, and biochemical study. Circ. Res. 56, 829-838.

Kuehnle I. and Goodell M.A. (2002). The therapeutic potential of stem cells from adults. Br. Med. J. 325, 372-376.

Mirabet V., Lequerica J.L., Montero J.A., Hurtado C. and Carbonell F. (2000). Cultivo in vitro de células satélite de músculo esquético. ¿Una alternativa terapeútica para el tratamiento de la cardiopatía isquémica?. Investigación Cardiovascular 3, 91-103.

Morgan J.E. and Partridge T.A. (2003). Muscle satellite cells. Int. J. Biochem. Cell Biol. 35, 1151-1156.

Rao M.S. and Mattson M.P. (2001). Stem cells and aging: expanding the possibilities. Mechanism. Aging Dev. 122, 713-734.

Seale P. and Rudnicki M.A. (2000). A new look at the origin, function and stem-cell status of muscle satellite cells. Dev. Biol. 218, 115-124.

Seale P., Sabourin L., Girgis-Gabardo A., Mansouri A., Gruss P. and Rudnicki M.A. (2000). Pax7 is required for the specification of myogenic satellite cells. Cell 102, 777-786.

Stewart J.D., Masi T.L., Cumming AE., Molnar G.M., Wentworth B.M, Sampath K., McPherson J.M. and Yaeger P.C. (2003). Characterization of proliferating human skeletal muscle-derived cells in vitro: differential modulation of myoblast markers by TGF-ß2. J. Cell Physiol. 196, 70-78.

Stock U.A. and Vacanti J.P. (2001). Tissue enginnering: current state and prospects. Annu. Rev. Med. 52, 443-451.

Tajbakhsh S. (2003). Stem cells to tissue: molecular, cellular and anatomical heterogeneity in skeletal muscle. Curr. Opin. Genet. Dev. 13, 413-422.

Vacanti J.P. and Langer R. (1999). Tissue enginnering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. Lancet 354, 32-34.

Vants A., Tolley N.S., Polak J.M. and Buttery L.D. (2002). Stem cells sources and applications. Clin. Otolaryngol. 27, 227-232.

Zammit P. and Beauchamp J. (2001). The skeletal muscle satellite cell: stem cell or son of stem cell? Differentiation 68, 193-204.

Accepted April 15, 2005