Data in Brief 40 (2022) 107744

Contents lists available at ScienceDirect

Data in Brief

journal homepage: www.elsevier.com/locate/dib

Data Article

Microencapsulated Sertoli cells sustain myoblast proliferation without affecting the myogenic potential. *In vitro* data

Sara Chiappalupi[a,c,f,#], Laura Salvadori[b,c,f,#], Francesca Mancuso[a,#], Iva Arato[a], Mario Calvitti[a], Francesca Riuzzi[a,c,f], Riccardo Calafiore[a,d,*], Giovanni Luca[a,d,e,*], Guglielmo Sorci[a,c,d,e,f,*]

[a] *Department of Medicine and Surgery, University of Perugia, Perugia 06132, Italy*
[b] *Department of Translational Medicine, University of Piemonte Orientale, Novara 28100, Italy*
[c] *Interuniversity Institute of Myology (IIM), Perugia 06132, Italy*
[d] *Centro Biotecnologico Internazionale di Ricerca Traslazionale ad indirizzo Endocrino, Metabolico ed Embrio-Riproduttivo (CIRTEMER), Perugia 06132, Italy*
[e] *Centro Universitario di Ricerca sulla Genomica Funzionale (CURGeF), Perugia 06132, Italy*
[f] *Consorzio Interuniversitario Biotecnologie (CIB), Trieste 34127, Italy*

ARTICLE INFO

Article history:
Received 20 November 2021
Revised 16 December 2021
Accepted 20 December 2021
Available online 23 December 2021

Keywords:
Sertoli cell
Microencapsulation
Myoblast
Fusion index
Myogenic potential
Myoblast proliferation

ABSTRACT

Sertoli cells (SeC) isolated from porcine testes have shown direct effects on muscle precursor cells sustaining C2C12 myoblasts proliferation and inhibiting oxidative stress and apoptosis in the early phase of the differentiation process, and stimulating myoblast fusion into myotubes and the expression of markers of myogenic differentiation in the late phase. This suggested that the cocktail of factors secreted by SeC stimulates proliferation in myoblasts without weakening their myogenic potential resulting in the formation of the critical myoblast amount necessary to rebuild the re-

Abbreviations: SeC, Sertoli cells; MC-SeC, microencapsulated Sertoli cells; E-MC, empty microcapsules; DM, differentiation medium; AMH, anti-Müllerian hormone; INSL3, insulin-like3; ASMA, alpha-smooth muscle actin; PGP9.5, protein gene product 9.5; HG-DMEM, high-glucose Dulbecco's modified Eagle's medium; FBS, foetal bovine serum; HS, horse serum; P/S, penicillin/streptomycin; HBSS, Hanks' balanced salt solution; TRIS, tris(hydroxymethyl)aminomethane; ITS, insulin-transferrin-selenium; EB, ethidium bromide; FDA, fluorescein diacetate; ALG, sodium alginate; EDTA, ethylenediamine tetra-acetic acid; PFA, paraformaldehyde; BSA, bovine serum albumin; DAPI, 4′,6-diamidino-2-phenylindole.

* Corresponding authors at: Department of Medicine and Surgery, University of Perugia, Perugia 06132, Italy.
E-mail addresses: giovanni.luca@unipg.it (G. Luca), guglielmo.sorci@unipg.it (G. Sorci).
# Equally contributed
* Shared senior authorship

https://doi.org/10.1016/j.dib.2021.107744

quired muscle mass upon a damage. Here, we show that co-culturing C2C12 myoblasts with high doses of SeC microencapsulated in clinical grade alginate-based microcapsules (MC-SeC) for three days in differentiation medium (DM) translates into increased cell numbers and almost absence of myotube formation. However, after removal of MC-SeC, an intense fusion activity into myotubes was observed culminating in a fusion index similar to that of control after additional three days of culture in DM. These data definitely demonstrate that SeC-derived factors preserve the myogenic potential while sustaining cell proliferation in C2C12 myoblasts.

Specifications Table

Subject	Biological Sciences
Specific subject area	Cell Biology
Type of data	Image Graph Figure
How data were acquired	Peristaltic pump (Gilson) Inverted phase contrast microscope (Olympus IX51) Bight-field microscope (Olympus BX51) Epifluorescence microscope (Olympus BX41)
Data format	Raw
Parameters for data collection	C2C12 myoblasts were cultured in the absence or presence of high doses of microencapsulated porcine SeC (equivalent amount, 8.0×10^5 SeC/ml).
Description of data collection	Evaluation of myogenic indexes, total nuclei/field, and numbers of nuclei/myotubes were performed by three independent operators blinded to treatments.
Data source location	Institution: University of Perugia City/Town/Region: Perugia Country: Italy
Data accessibility	https://data.mendeley.com/datasets/2gvbndvtnp/3
Related research article	L. Salvadori, S. Chiappalupi, I. Arato, F. Mancuso, M. Calvitti, M.C. Marchetti, F. Riuzzi, R. Calafiore, G. Luca, G. Sorci. *Sertoli cells improve myogenic differentiation, reduce fibrogenic markers, and induce utrophin expression in human DMD myoblasts.* Biomolecules 2021 Oct 12; 11(10):1504. doi: 10.3390/biom11101504.

Value of the Data

- Our data demonstrate that microencapsulated Sertoli cells (MC-SeC) release factors [1–4] that exert direct effects on muscle precursor cells, stimulating myoblast proliferation (Salvadori, 2021) without affecting the myogenic potential, even at high doses.
- In addition to reported potential applications [2,6,7], our data suggest that SeC might be beneficial (i) in muscle regeneration, when myoblasts have to proliferate to reach the critical density needed to replace damaged muscle mass [8]; (ii) in muscle atrophy conditions, sustaining myoblast proliferation and differentiation [9]; and (iii) in muscular dystrophy, where continuous cycles of muscle degeneration/regeneration occur leading to depletion of muscle precursor cells [10].
- The effects induced on myoblasts by MC-SeC are the final result of a cocktail of factors the exact composition of which remains to be determined [1–4]. Starting from our data, acellular

SeC derivatives (e.g., SeC-conditioned media or SeC-derived extracellular vesicles) should be evaluated for their effects in muscle regeneration, muscle atrophy, and muscular dystrophies.
- Together with experimental results obtained in human DMD myoblasts with different mutations [5], these data support the potential application of MC-SeC as a universal treatment to DMD patients.
- In a translational perspective, the concentration of SeC to be applied in a certain muscle pathology or atrophying condition has to be carefully evaluated in order to allow myoblasts to differentiate after the early proliferation phase.

1. Data Description

Sertoli cells (SeC) isolated and purified from testes of Large White piglets (Fig. 1, Step 1) were characterized for the expression of anti-Müllerian hormone (AMH), insulin-like3 (INSL3), alpha-smooth muscle actin (ASMA), and protein gene product 9.5 (PGP9.5) as markers of prepubertal SeC, Leydig cells, peritubular cells, and gonocyte and spermatogonial cells, respectively (Fig. 1, Step 2). SeC preparations with very high purity were obtained, as demonstrated by the high percentages of AMH-positive cells (96.7±2.8%) and the negligible contamination by INSL3-positive (0.9±0.5%), ASMA-positive (2.0±0.3%), and PGP9.5-positive (0.4±0.1%) cells (Fig. 1, Step 3). Purified SeC were counted and encapsulated into clinical grade alginate microcapsules (MC-SeC) through a dedicated air-jet apparatus (Fig. 1, Step 4A). SeC viability remained high (95.7±1.3% viable cells) in freshly-prepared MC-SeC as evaluated after ethidium bromide/fluorescein diacetate staining (Fig. 1, Step 4C). Empty microcapsules (E-MC) were prepared following the same procedure in the absence of SeC (Fig. 1, Step 4B) and were used as control.

C2C12 myoblasts were cultured in low-serum DMEM (differentiation medium, DM) in the absence or presence of high doses of MC-SeC (equivalent amount, 8.0×10^5 SeC/ml) with the use of cell culture inserts (Fig. 2A). After 72 h, increased cell numbers and almost absence of myotube formation were seen in myoblasts co-cultured with MC-SeC in comparison with myoblasts cultivated in the presence of E-MC (Fig. 2B). Indeed, C2C12 cells co-cultured with MC-SeC showed a fusion index of only 7.4±4.8% in comparison with the 68.8±3.4% fusion index of E-MC-treated myoblasts (Fig. 2C), and an average nuclei/myotube ratio of 5.3 versus 21.1 nuclei/myotube observed in E-MC-treated myoblasts (Fig. 2D), suggesting that high concentrations of SeC-derived factors sustain an intense proliferative activity inhibiting the myogenic differentiation.

After 72 h of co-culture, MC-SeC and E-MC were removed or not removed and myoblasts/myotubes were cultivated for additional 72 h without renewing the culture medium. Myoblasts cultured in the presence of E-MC for the entire duration of the experiment showed a fusion index of 59.1% and a nuclei/myotube ratio of 12.9 (Fig. 2E–G). Instead, myoblasts cultured constantly in the presence of MC-SeC showed very low values of fusion index (11.9%) and nuclei/myotube ratio (7.7) (Fig. 2E–G), supporting an inhibitory role of SeC in the myogenic process.

Myoblasts/myotubes cultivated after the removal of E-MC showed similar values of fusion indexes (62.8%) and nuclei/myotube ratios (13.3) (Fig. 2H–J) to those of myoblasts/myotubes in which E-MC were maintained in the culture inserts (Fig. 2E–G), as expected. In contrast, when myoblasts cultivated with MC-SeC were cultured for additional 72 h in the absence of MC-SeC, an extensive myotube formation could be seen (Fig. 2H) reaching fusion indexes (61.1%) (Fig. 2I) similar to those of myoblasts cultured in the presence of E-MC for the first 72 h (Fig. 2I) or for the entire duration of the experiment (Fig. 2F). Interestingly, the evaluation of nuclei/myotube ratios showed even higher values for myoblasts previously treated with MC-SeC (15.5 nuclei/myotubes) (Fig. 2J) in comparison with myoblasts previously cultured (13.3 nuclei/myotubes) (Fig. 2J) or constantly cultured (12.9 nuclei/myotubes) (Fig. 2G) with E-MC.

Altogether, these results were strongly indicative of preservation of the myogenic potential by myoblasts treated with (high-dose) SeC.

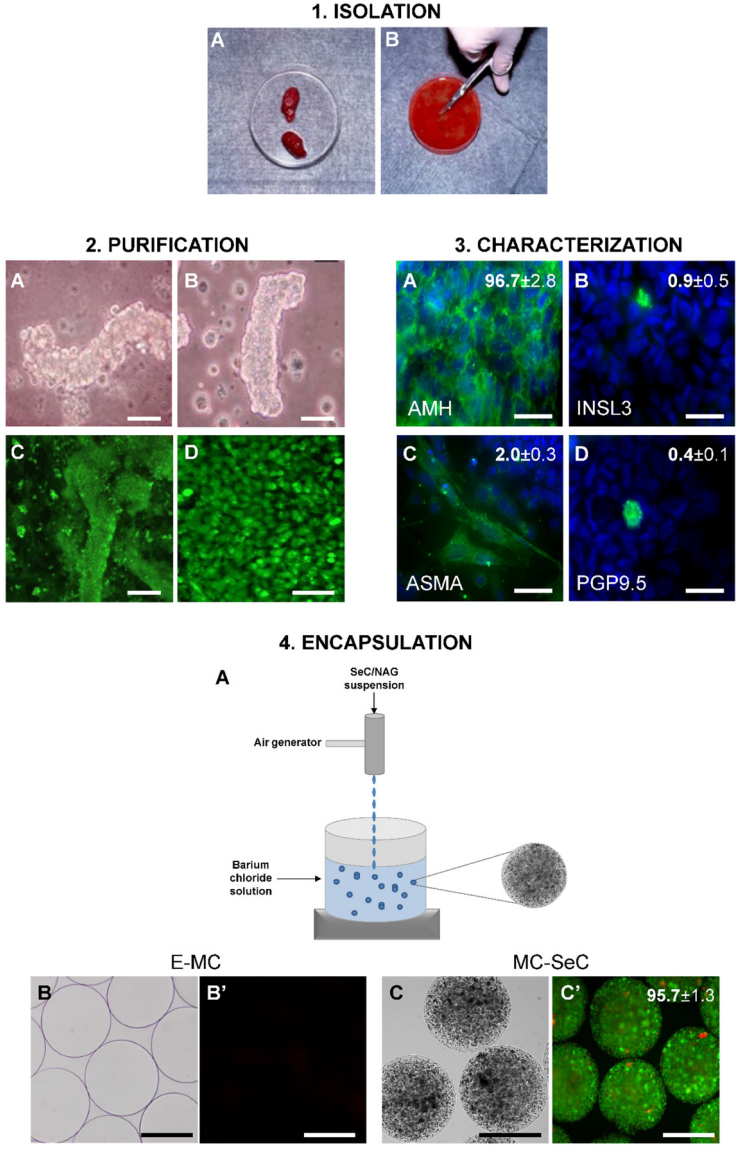

Fig. 1. Experimental steps of the procedure used to obtain MC-SeC. (1) Isolation step. Chopping phase in which the testes decorticated by the tunica albuginea (A) are finely minced (B). (2) Purification step. Phase contrast photomicrographs showing SeC culture after enzymatic digestion with trypsin and DNase I (A), followed by treatment with collagenase P (B). (C,D) Fluorescence photomicrographs of SeC after double staining with ethidium bromide (EB; *orange*) and fluorescein diacetate (FDA; *green*) to assess cell viability before (C) and after (D) plating. Scale bars, 100 µm (A-C) and 50 µm (D). (3) Characterization step. Isolated SeC were characterized for the expression of anti-Müllerian hormone (AMH) (A), insulin-like 3 (INSL3) (B), alpha-smooth muscle actin (ASMA) (C) or protein gene product 9.5 (PGP9.5) (D) by immunofluorescence staining (*green*). Nuclei were counterstained with DAPI (*blue*). Reported are representative images with the percentages (means ±SEM) of positive cells in three different purifications. Scale bars, 20 µm. (4) Encapsulation step. (A) Schematics of the air-jet system used for SeC microencapsulation, consisting in a peristaltic pump, an air-jet generator of microdroplets, and a flow meter. ALG, sodium alginate. Reported are representative phase contrast images and fluorescence images after double staining with EB/FDA of empty microcapsules (E-MC) (B and B', respectively) and MC-SeC (C and C', respectively). The percentage of viable cells (means ± SEM) of three different encapsulation procedures is indicated (C'). Scale bars, 200 µm.

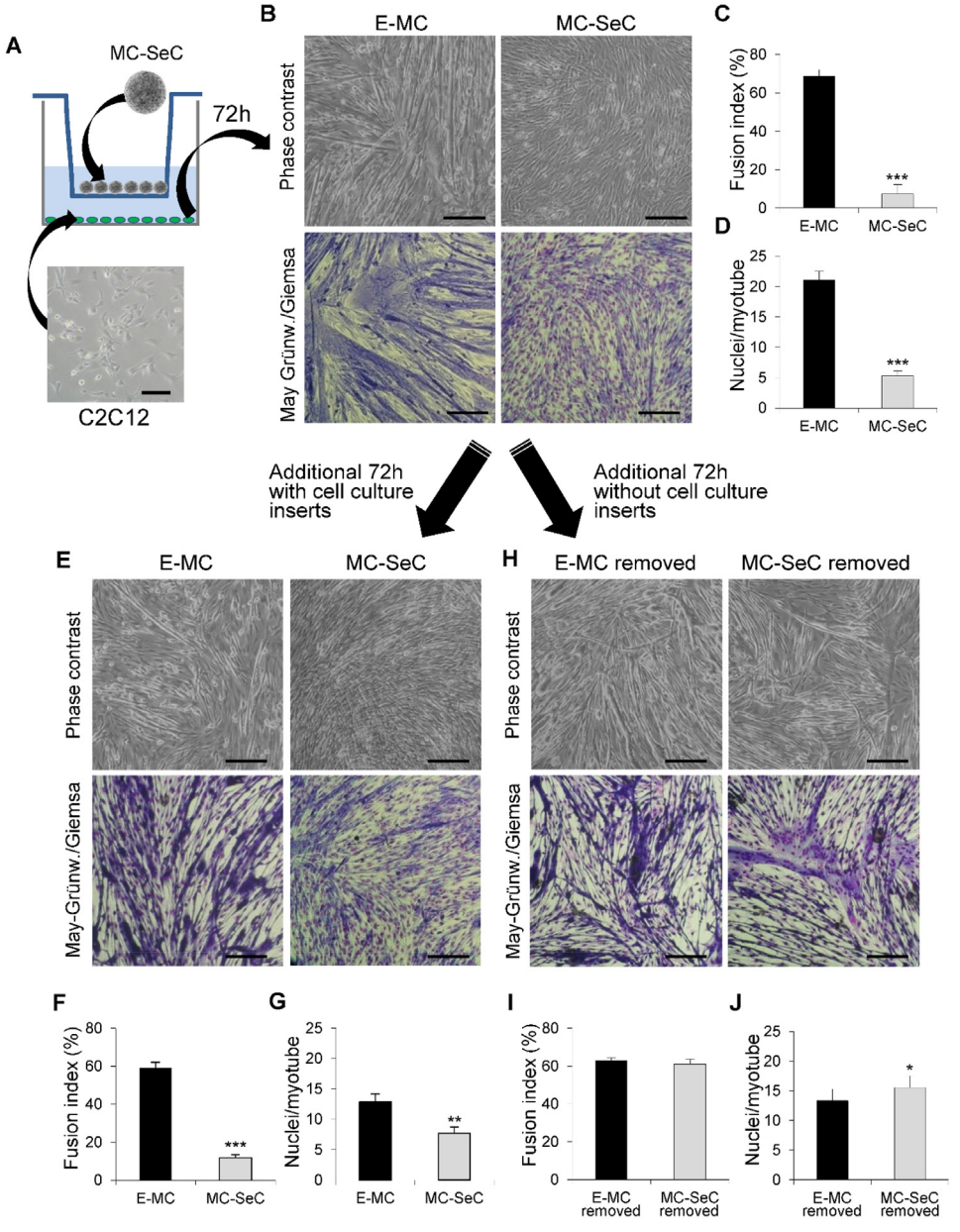

Fig. 2. (A–D) C2C12 myoblasts cultivated in GM in 6-multiwells plates were switched to differentiation medium (DM) for 72 h in the presence of empty microcapsules (E-MC) or microencapsulated SeC (MC-SeC; equivalent amount, 8.0×10^5 SeC/ml) by the use of cell culture inserts. (E–G) Myoblasts were cultured for additional 72 h in the same conditions without removing the culture inserts and without replacing the culture medium. (H–J) In parallel, the inserts with E-MC or MC-SeC were removed and myoblasts were cultivated for additional 72 h without replacing the medium. (A) Reported is a schematic illustration of the culture conditions. (B,E,H) Representative images of myoblast cultures as viewed with an inverted phase contrast microscope or after May-Grünwald/Giemsa staining. The fusion indexes (C,F,I) and the average numbers of nuclei/myotube (D,G,J) are reported. Results are means (±SD) of three independent experiments. *, ** and ***, significantly different from control (E-MC) ($P<0.05$, $P<0.01$ and $P<0.001$, respectively). Scale bars, 200 µm.

2. Experimental Design, Materials and Methods

2.1. C2C12 culture

Murine C2C12 myoblasts obtained from the American Type Culture Collection (Manassas, VA, USA) were cultured in growth medium (GM) composed of high-glucose Dulbecco's modified Eagle's medium (HG-DMEM) supplemented with 20% foetal bovine serum (FBS; Invitrogen, USA), and 100 U/ml penicillin and 100 mg/ml streptomycin (P/S). Cells were maintained in a humidified atmosphere containing 5% CO_2 at 37°C. Cells were seeded 2.9×10^4 cells/cm^2, cultured for 24 h in GM, and then shifted to differentiation medium (DM) made of HG-DMEM supplemented with 2% horse serum (HS, Invitrogen) and P/S. Co-culture of C2C12 myoblasts with MC-SeC or E-MC was obtained by the use of 0.4 µm cell culture inserts (BD Falcon).

2.2. Sertoli cell isolation and characterization

SeC were isolated from Large White neonatal pigs, according to established methods modified in our laboratory [11–13]. After removal of the fibrous capsules the testes were finely chopped to obtain a homogeneous dense tissue that underwent sequential enzymatic digestion with Hanks' balanced salt solution (HBSS; Sigma-Aldrich, USA) containing 2 mg/ml trypsin and 50 ug/ml DNase I (both from Sigma-Aldrich, USA) for 15 min at 37°C. The digestion continued until physical separation of the seminiferous tubules was achieved. After washing twice with HBSS and centrifuging at 118 g for 3 min, the tissue suspension was incubated with 2 mg/ml collagenase P (Roche Diagnostics, Italy) solution in HBSS for 30 min at 37°C. The pellet was washed twice in HBSS and centrifuged at 118 g for 3 min. The pellet was passed through a 500-µm stainless steel mesh and resuspended in 1 M glycine, 1 mM EDTA buffer, pH 7.2 to eliminate any residual Leydig and peritubular cells [14]. The resulting SeC were cultured in HAM'S F12 (Euroclone, Italy) supplemented with 0.166 nM retinoic acid (Sigma-Aldrich, USA) and 1% Insulin-Transferrin-Selenium (ITS) + Premix (Corning, USA; Cat. 354352) in 95% air/5% CO2 at 37°C. After 3 days of culture, SeC were incubated with 10 mM TRIS [tris(hydroxymethyl)aminomethane] buffer, pH 7.4 (Sigma-Aldrich, USA) to eliminate any residual germ cells [15]. To characterize the isolated cells, immunofluorescence staining for markers of prepubertal SeC [anti-Müllerian hormone (AMH)], Leydig cells [insulin-like 3 (INSL3)], peritubular cells [alpha-smooth muscle actin (ASMA)], and gonocyte and spermatogonial cells [protein gene product 9.5 (PGP9.5)] were performed according to previously established methods [11,13] with minor changes (see *Immunofluorescence*).

2.3. Sertoli cell microencapsulation

After isolation, and upon 3 days of culture, SeC underwent envelopment in alginate microcapsules according to established method [11]. Confluent monolayers of SeC were collected with 0.05% trypsin-ethylene-diamine tetra-acetic acid (EDTA; Gibco, USA) (2 min), washed, counted by hemocytometer, and assayed for viability by ethidium bromide (EB; Sigma-Aldrich) and fluorescein diacetate (FDA; Sigma-Aldrich) staining. SeC were suspended in 1.6% aqueous solution of highly purified, clinical grade sodium alginate (ALG) (Stern Italia, Italy) [16]. The ALG-SeC suspension was continuously aspirated by a peristaltic pump at a flow rate of 12-14 ml/min and then extruded through a microdroplet generator (air flow rate: 4 l/min) under sterile conditions. The microdroplets were collected on a barium chloride (BaCl2, 1.2%, w/v) bath, which immediately turned them into gel microbeads. The obtained MC-SeC were washed twice in saline and suspended in HAM'S F12 in 95% air/5% CO2 at 37°C.

2.4. Immunofluorescence

SeC monolayers grown on glass chamber slides (Thermo Fisher, USA) were fixed in 4% paraformaldehyde (PFA) for 30 min and permeabilized with 0.2% Triton X-100 in phosphate-buffered saline (PBS) for 10 min at room temperature (RT). Cells were blocked with 0.5% bovine serum albumin (BSA, Sigma-Aldrich) in PBS for 1 h prior to exposure to the primary antibody overnight at 4°C. The following antibodies were used: polyclonal goat anti-AMH (cat. C-20; Santa Cruz Biotechnology, USA) diluted 1:100; polyclonal rabbit anti-INSL-3 (cat. NBP1-18706; Novus Biologicals, USA) diluted 1:200; polyclonal rabbit anti-ASMA (cat. ab5694; Abcam, USA) diluted 1:200; monoclonal rabbit anti-PGP9.5 (cat. ab108986; Abcam, USA) diluted 1:200. The cells were washed three times in PBS for 5 min before exposure to the secondary antibody. The following antibodies were used diluted 1:500: Alexa 488-conjugated donkey anti-goat antibody (Molecular Probes); Alexa 488-conjugated donkey anti-rabbit antibody (Molecular Probes). The cells were treated with RNAse (10 mg/ml; Sigma-Aldrich) and counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich) for 1 min at R.T. Negative controls were obtained by omitting the primary antibody. Stained cells were mounted on slides with ProLong Gold antifade reagent (Molecular Probes), and the percentages of cells positive for AMH, INSL3, ASMA or PGP9.5 were determined using an epifluorescence microscope (Olympus BX-41) equipped with a digital camera (F-viewer, Olympus). Images were processed with Cell F imaging software (Olympus), and ten different preparations containing at least 500 cells were counted by independent investigators blind to the treatments.

2.5. May-Grünwald/Giemsa staining and fusion index evaluation

C2C12 cells were fixed in cold absolute methanol for 8 min and stained with May-Grünwald (Sigma-Aldrich, USA) for 3 min. Then, the cells were added with Sorensen's phosphate buffer (0.067 M, pH 6.8) for 4 min, May-Grünwald was removed and replaced with filtered Giemsa solution diluted in Sorensen's phosphate buffer 1:10 for 12 min. Giemsa solution was refreshed every 2 min for six times. Giemsa was removed and the cells were dried and observed with an Olympus BX51 microscope. Fusion indexes, total nuclei and nuclei/myotube ratios were determined after May-Grünwald/Giemsa staining at 10X magnification in five randomly selected fields/well of three different experiments using Image J software by independent investigators blind to the treatments. The fusion indexes were calculated as the percentages of nuclei inside myotubes (i.e., syncytia containing at least three nuclei) versus the total number of nuclei.

Ethics Statement

For primary culture of porcine prepubertal SeC, three Large White neonatal pigs (15 to 20 days old) were used as SeC donors. Animals were treated in agreement with the guidelines adopted by the Italian Approved Animal Welfare Assurance (A-3143-01) and the European Communities Council Directive of November 1986 (86/609/EEC). The experimental protocols were approved by the University of Perugia.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

CRediT Author Statement

Sara Chiappalupi: Conceptualization, Investigation, Visualization, Writing – original draft; **Laura Salvadori:** Conceptualization, Investigation, Visualization, Writing – original draft; **Francesca Mancuso:** Conceptualization, Resources, Investigation, Validation, Visualization; **Iva Arato:** Resources, Validation; **Mario Calvitti:** Resources; **Francesca Riuzzi:** Writing – review & editing; **Riccardo Calafiore:** Writing – review & editing, Funding acquisition; **Giovanni Luca:** Supervision, Writing – review & editing, Funding acquisition; **Guglielmo Sorci:** Supervision, Writing – review & editing, Funding acquisition.

Acknowledgments

This work was supported by Parent Project aps (Italy) to G.S., and Altucell Inc. (3 Astor Court, Dix Hills, NY, USA) to G.L. and R.C. S.C. was recipient of a fellowship granted by Parent Project aps (Italy). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

[1] M. Skinner, Sertoli cell secreted regulatory factors, in: M. Skinner, M. Griswold (Eds.), Sertoli Cell Biology, Academic Press, 2005, pp. 107–120.
[2] P. Mital, G. Kaur, J.M. Dufour, Immunoprotective sertoli cells: making allogeneic and xenogeneic transplantation feasible, Reproduction 139 (2010) 495–504, doi:10.1530/REP-09-0384.
[3] G. Kaur, C.R. Long, J.M. Dufour, Genetically engineered immune privileged Sertoli cells: a new road to cell based gene therapy, Spermatogenesis 2 (2012) 23–31, doi:10.4161/spmg.19119.
[4] S. Chiappalupi, L. Salvadori, G. Luca, F. Riuzzi, R. Calafiore, R. Donato, G. Sorci, Employment of microencapsulated sertoli cells as a new tool to treat duchenne muscular dystrophy, J. Funct. Morphol. Kinesiol. 2 (2017) 47, doi:10.3390/jfmk2040047.
[5] L. Salvadori, S. Chiappalupi, I. Arato, F. Mancuso, M. Calvitti, M.C. Marchetti, F. Riuzzi, R. Calafiore, G. Luca, G. Sorci, Sertoli cells improve myogenic differentiation, reduce fibrogenic markers, and induce utrophin expression in human DMD myoblasts, Biomolecules 11 (10) (2021) 1504, doi:10.3390/biom11101504.
[6] G. Kaur, L.A. Thompson, J.M. Dufour, Therapeutic potential of immune privileged Sertoli cells, Anim. Reprod. 12 (2015) 105–117.
[7] S. Chiappalupi, L. Salvadori, G. Luca, F. Riuzzi, R. Calafiore, R. Donato, G. Sorci, Do porcine Sertoli cells represent an opportunity for Duchenne muscular dystrophy? Cell Prolif. 52 (2019) e12599, doi:10.1111/cpr.12599.
[8] S.B. Chargé, M.A. Rudnicki, Cellular and molecular regulation of muscle regeneration, Physiol. Rev. 84 (2004) 209–238, doi:10.1152/physrev.00019.2003.
[9] B.M. Scicchitano, G. Dobrowolny, G. Sica, A. Musarò, Molecular insights into muscle homeostasis, atrophy and wasting, Curr. Genomics 19 (2018) 356–369, doi:10.2174/1389202919666180101153911.
[10] S. Brunelli, P. Rovere-Querini, The immune system and the repair of skeletal muscle, Pharmacol. Res. 58 (2008) 117–121, doi:10.1016/j.phrs.2008.06.008.
[11] G. Luca, F. Mancuso, M. Calvitti, I. Arato, G. Falabella, A. Bufalari, V. De Monte, E. Tresoldi, C. Nastruzzi, G. Basta, F. Fallarino, C. Lilli, C. Bellucci, T. Baroni, M.C. Aglietti, S. Giovagnoli, D.F. Cameron, M. Bodo, R. Calafiore, Long-term stability, functional competence, and safety of microencapsulated specific pathogen-free neonatal porcine Sertoli cells: a potential product for cell transplant therapy, Xenotransplantation 22 (2015) 273–283, doi:10.1111/xen.12175.
[12] S. Chiappalupi, G. Luca, F. Mancuso, L. Madaro, F. Fallarino, C. Nicoletti, M. Calvitti, I. Arato, G. Falabella, L. Salvadori, A. Di Meo, A. Bufalari, S. Giovagnoli, R. Calafiore, R. Donato, G. Sorci, Effects of intraperitoneal injection of microencapsulated Sertoli cells on chronic and presymptomatic dystrophic mice, Data Brief 5 (2015) 1015–1021, doi:10.1016/j.dib.2015.11.016.
[13] S. Chiappalupi, G. Luca, F. Mancuso, L. Madaro, F. Fallarino, C. Nicoletti, M. Calvitti, I. Arato, G. Falabella, L. Salvadori, A. Di Meo, A. Bufalari, S. Giovagnoli, R. Calafiore, R. Donato, G. Sorci, Intraperitoneal injection of microencapsulated Sertoli cells restores muscle morphology and performance in dystrophic mice, Biomaterials 75 (2016) 313–326, doi:10.1016/j.biomaterials.2015.10.029.
[14] J.P. Mather, D.D. Philips, Primary culture of testicular somatic cells, in: Barnes, D.W., Sirbasku, D.A., and Sato, G.H. (Eds.), Methods for Serum Free Culture of Cells of the Endocrine System, New York Liss, New York, pp. 24-45.
[15] M. Galdieri, E. Ziparo, F. Palombi, M.A. Russo, M.J. Stefanini, Pure Sertoli cell cultures: a new model for the study of somatic-germ cell interactions, J. Androl. 2 (1981) 249–254, doi:10.1002/j.1939-4640.1981.tb00625.x.
[16] S. Giovagnoli, G. Luca, P. Blasi, F. Mancuso, A. Schoubben, I. Arato, M. Calvitti, G. Falabella, G. Basta, M. Bodo, R. Calafiore, M. Ricci, Alginates in pharmaceutics and biomedicine: is the future so bright? Curr. Pharm. Des. 21 (2015) 4917–4935, doi:10.2174/1381612821666150820105639.