

Chapter 6

Generation and Use of Trophoblast Stem Cells and Uterine Myocytes to Study the Role of Connexins for Pregnancy and Labor

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Abstract

Transgenic mouse models have demonstrated critical roles for gap junctions in establishing a successful pregnancy. To study the cellular and molecular mechanisms, the use of cell culture systems is essential to discriminate between the effects of different connexin isoforms expressed in individual cells or tissues of the developing conceptus or in maternal reproductive tissues. The generation and analysis of gene-deficient trophoblast stem cell lines from mice clearly revealed the functions of connexins in regulating placental development. This chapter focuses on the use of connexin gene-deficient trophoblast stem cell cultures to reveal the individual role of gap junctions in regulating trophoblast differentiation and proliferation *in vitro* under controlled conditions. In addition, cultures of primary uterine myocytes, isolated from mice or rats, allow studying the effects of mechanical stretch or ovarian hormones on regulating connexin expression, and thus, to model the molecular mechanisms of uterine growth and development during pregnancy. Here, we describe the derivation of primary uterine myocyte cultures and their use in *in vitro* stretch experiments to study the mechanisms of myometrial remodeling essential to accommodate the growing fetus throughout gestation.

Key words Placenta, Trophoblast differentiation, Embryo, Uterus, Myometrium, Gestation, Stretch

1 Introduction

During pregnancy, the developing embryo establishes complex interactions with maternal uterine tissues. Physiological, genetic, or environmental impairment of the fetomaternal interaction can lead to pregnancy complications, such as preeclampsia, intrauterine growth restriction, or preterm labor, affecting the health of both the baby and the mother [1, 2]. Studying developmental and molecular mechanisms of the placenta or the pregnant uterus is challenging due to the presence of tissues of two genetically divergent organisms. Current experimental approaches include the use of different animal models to examine specific cellular, hormonal, or immunological aspects of fetal and/or maternal development.

Mutant mice have demonstrated that gap junctions play essential roles in establishing a successful pregnancy from regulating trophoblast differentiation during placental development and mediating uterine artery remodeling [3, 4], and to forming a functional myometrial syncytium, which enables forceful uterine contractions to expel the fetus during labor [5]. However, due to the presence of multiple connexin isoforms expressed by the placental trophoblasts (i.e., Cx31, C31.1, Cx26, and Cx43), endothelial cells (i.e., Cx40, Cx43, and Cx37), the decidua (i.e., Cx43), the fetal mesoderm (i.e., Cx43 and Cx45), and by uterine myocytes (i.e., Cx26, Cx43, and Cx45) [3], the specific effects of individual connexins are difficult to study. The derivation of tissue cell lines and long-term or short-term primary cell cultures that mimic the physiological processes in utero allow studying the molecular mechanisms leading to a successful gestation.

In the developing placenta, Cx31 and Cx31.1 are critical regulators of trophoblast differentiation. Both connexins are coexpressed in the proliferating postimplantation trophoblast lineage. The inactivation of either gene in mice results in partial loss (i.e., 60 and 30%, respectively) of conceptuses between embryonic days 10.5 and 13.5 [6, 7]. Surviving embryos are born viable, but growth restricted due to significantly reduced placental sizes [7, 8]. For both mutant mouse strains, the generation of Cx31 or Cx31.1 gene-deficient trophoblast stem cell lines and their *in vitro* analysis has provided fundamental insights into the role of these gap junction proteins in regulating trophoblast differentiation (Fig. 1) [9, 10].

Trophoblast stem cell (TSC) lines can be generated from a blastocyst's trophectoderm outgrowth or dissected extraembryonic ectoderm in the presence of fibroblast growth factor 4 (FGF4) and mouse embryonic fibroblast conditioned medium (MEF-CM). A later study showed that transforming growth factor beta/activin is the critical factor produced by MEFs [11]. By removing FGF4 and MEF-CM/activin from cell culture medium, TSC can differentiate into all placental trophoblast subpopulations [12, 13]. Earlier, we showed that during *in vitro* differentiation, wild-type TSC maintain a specific placental connexin expression pattern. In particular, undifferentiated TSC express both Cx31 and Cx31.1, and upon differentiation into syncytiotrophoblast, the expression of Cx26 is induced, while differentiation into trophoblast giant cells stimulates the expression of Cx43 [10, 14]. The generation of Cx31-deficient and Cx31.1-deficient TSC lines from blastocysts of the corresponding

Fig. 1 (continued) Cx31 preserves the diploid, proliferating trophoblasts, whereas Cx31.1 promotes differentiation. **(b–e)** *Quantitative real-time polymerase chain reaction analysis of each 3 Cx31.1^{+/-} (HZ) and Cx31.1^{-/-} (KO) TSC lines differentiated in vitro for 6 days. Cx31.1 deficiency results in a block of terminal differentiation from the Mash2-expressing intermediate trophoblast population into placental glycogen cells, Pcdh12, spongiotrophoblast, Tpbpa, and giant cells, Pl-2 as indicated by repressed marker gene expression compared to Cx31.1^{+/-} (HZ) controls*

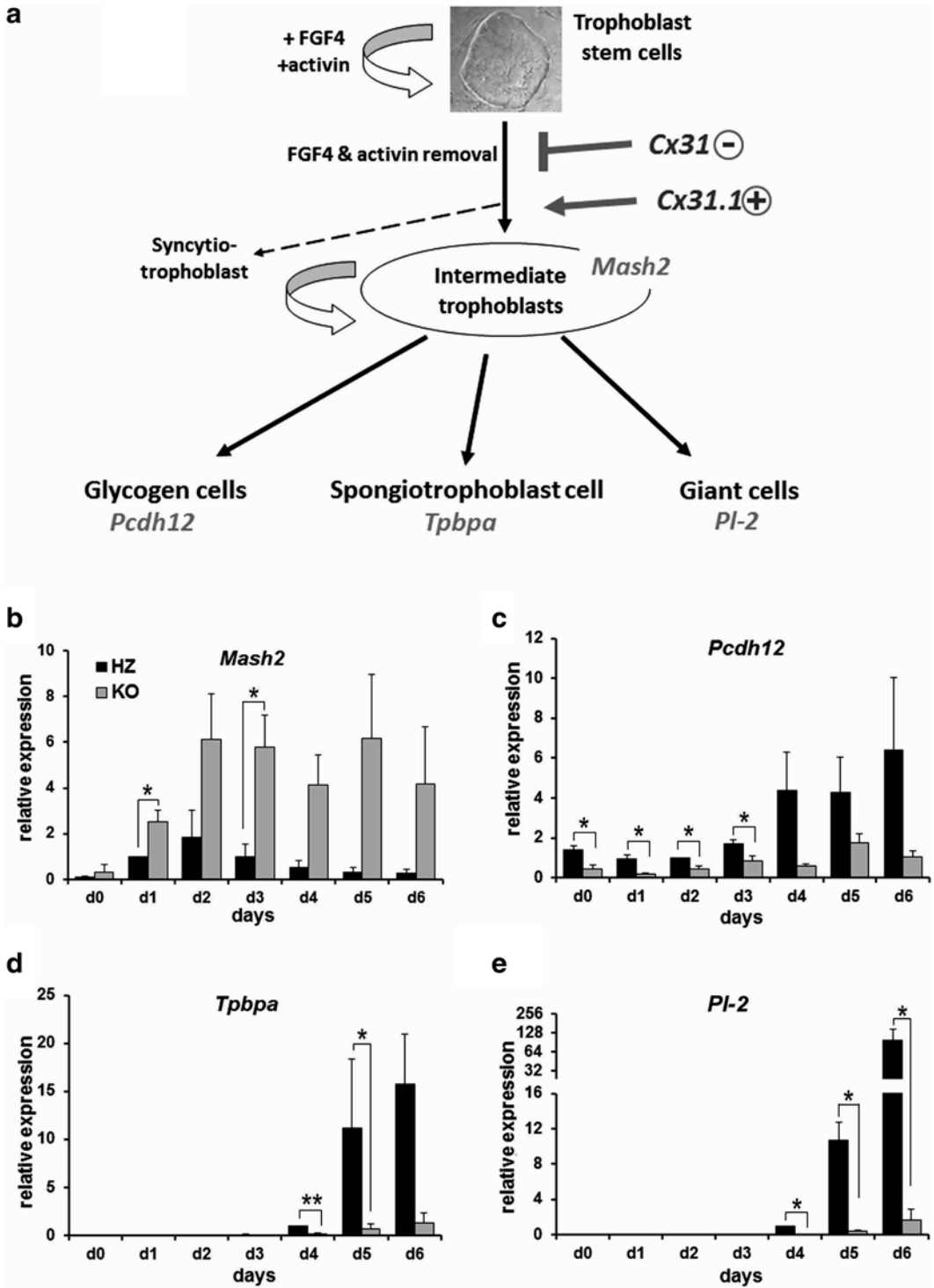


Fig. 1 (a) Scheme demonstrating the opposite roles of *Cx31* and *Cx31.1* in regulation TSC differentiation. Upon removal of FGF4 and activin, TSC differentiate into intermediate trophoblasts characterized by *Mash2* expression and further into the placental trophoblast subpopulation, namely, glycogen cells (protocadherin12, *Pcdh12*), spongiotrophoblast (trophoblast binding protein A, *Tpbpa*), and trophoblast giant cells (placental lactogen 2, *PI-2*).

knock-out (KO) mice revealed an antipodal role for both connexin isoforms. Cx31-deficient TSC differentiate faster along the trophoblast lineage compared to wild-type control cells [10], whereas Cx31.1-deficient TSC differentiate slower into placental trophoblast subpopulations compared to controls [9], as indicated by the analysis of specific marker genes and proliferation capabilities (Fig. 1). This clearly indicates that Cx31 is critical to maintaining TSCs in the undifferentiated proliferative state during placental development and the coexpression of Cx31.1 has an opposite function of promoting trophoblast lineage differentiation into placental subpopulations [9, 10]. Therefore, TSC cultures provide a unique and valuable tool for investigating the role of gap junction proteins in lineage differentiation during placental development.

During pregnancy, the uterus undergoes dramatic changes to accommodate the growing fetus. The uterine smooth muscle (i.e., myometrium) is noticeably increasing in size. This growth is regulated by mechanical stretch of the uterine wall by the growing fetus(es) and by pregnancy-related changes of the ovarian hormones estrogen and progesterone [15]. In addition, uterine remodeling is critically regulated by the invading trophoblast populations, paracrine and endocrine signals, and by the infiltration of immune cells into the uterine tissues [16, 17]. The expression of gap junction proteins (i.e., Cx43, Cx26, and Cx45) is regulated throughout gestation to ensure tissue integrity during the development of cellular hypertrophy and later for coordinated forceful uterine contractions during labor to expel the fetus/es [18]. Tissue-specific deletion of Cx43 from uterine myocytes in mice leads to irregular uterine contractions and delayed delivery of pups, revealing the importance of gap junctional communication for successful pregnancies [19].

The establishment of primary rodent myocyte cultures allows to analyze *in vitro* the effect of mechanical stretch and/or hormonal changes on gap junctional communication between uterine myocytes independent of vascular cells, resident or infiltrating leukocytes or fetal tissues [19, 20].

This chapter describes the development of different cell culture systems derived from fetal (i.e., trophoblasts) and maternal (i.e., myocytes) rodent tissues to study the complex physiological mechanisms underlying pregnancy.

2 Materials

2.1 Culture and Differentiation of Mouse Trophoblast Stem Cells

1. TS medium: Roswell Park Memorial Institute (RPMI) medium 1640, 20% fetal bovine serum (FBS) (Thermo-Fisher Scientific, USA) (*see Note 1*), 2 mM glutamine, 1 mM sodium pyruvate, 100 mM β -mercaptoethanol, and 100 U/mL penicillin/streptomycin.

2. Mouse embryonic fibroblast (MEF) conditioned TS medium (TS/CM) [14] (*see Note 2*).
3. TSC lines cultured in 70% MEF conditioned medium (TS/CM) and 30% TS medium, supplemented with 25 ng/mL FGF4 (R&D Systems, USA) (*see Note 3*) and 1 U/mL heparin (Sigma-Aldrich, Canada).
4. TrypLE dissociation reagent (Life Technologies, Canada) (*see Note 4*).
5. Incubator at 37 °C, 5% CO₂, 5% CO₂ and 90% humidity, and biosafety cabinet.
6. Cell culture plastic dishes and 40 µm cell strainer.
7. Dulbecco's phosphate buffered saline (DPBS) at pH 7.4 (Sigma-Aldrich, Canada).

2.2 Colorimetric TSC Proliferation Assay

1. Staining solution: 1.6 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, Canada) dissolved in DPBS.
2. 24-Well culture plates.
3. Dimethylsulfoxide (DMSO).
4. Plate reader photometer.

2.3 Derivation and Culture of Primary Rodent Uterine Myocytes

1. Estrogen solution: 0.25 mg/mL 17β-estradiol (Sigma-Aldrich, Canada) dissolved in 90% corn oil with 10% ethanol.
2. Buffer A: sterile Hank's Basic Salt Solution (HBSS) containing calcium and magnesium, 25 mM 2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), 100 U/mL penicillin/streptomycin, and 2.5 µg/mL amphotericin B. Adjust to pH 7.4.
3. Buffer B: buffer A without calcium and magnesium.
4. Buffer C: buffer B supplemented with 1 mg/mL collagenase type II (Sigma-Aldrich, Canada), 0.15 mg/mL DNase I (Roche, Canada), 0.1 mg/mL soybean trypsin inhibitor, 10% FBS (Cansera, Canada), and 1 mg/mL bovine serum albumin (BSA).
5. Cell culture medium: phenol red-free Dulbecco's Modified Eagle's Medium (DMEM) (Life Technologies, Canada) supplemented with 10% FBS, 25 mM HEPES, 100 U/mL penicillin/streptomycin, and 2.5 µg/mL amphotericin B.
6. Serum-free medium: phenol red-free DMEM supplemented with insulin-, transferrin-, selenium-, sodium pyruvate solution (ITS-A) (Life Technologies, Canada), 25 mM HEPES, 100 U/mL penicillin/streptomycin, and 2.5 µg/mL amphotericin B.

2.4 In Vitro Stretch of Primary Uterine Myocyte Cultures

1. Flexcell FX-3000 Tension System (Flexcell Inc., USA).
2. Collagen I-coated Bio-Flex 6-well plates with rubber membranes (Flexcell Inc., USA).

3. ProNectin-coated 6-well culture plates with rubber membranes (Flexcell Inc., USA).
4. Collagen I-coated HT Bio-Flex 24-well plates with rubber membranes (Flexcell Inc., USA).
5. ProNectin-coated HT Bio-Flex 24-well plates with rubber membranes (Flexcell Inc., USA).
6. Covalently bound matrix surfaces: amino, collagen (i.e., type I or IV), elastin and pronectin (Flexcell Inc., USA).

3 Methods

3.1 Comparative Analysis of Connexin-Deficient TSC Differentiation

1. Use gene-deficient TSC lines and wild-type control lines, which were derived in parallel (*see Note 5*) using the same batch of TS/CM (*see Note 3*). Change cell culture media every other day.
2. At 80% confluency, remove cell culture medium, add 1 mL TrypLE, and incubate for 3 min at room temperature. Dissociate TSC to single cells by pipetting up and down with a 1 mL pipette tip. Add 1 mL of TS medium and pass the suspension through a 40 μ m cell strainer to remove clumps and aggregates of differentiated cells.
3. Spin the suspension at 800 $\times g$ for 3 min, remove supernatant, and resuspend cells in 1 mL of TS/CM. Count cells and seed in 12-well plates at a density of 10,000 cells *per* well in 1 mL of TS/CM. Incubate plates for 48 h before inducing differentiation (*see Note 7*).
4. To induce TSC differentiation, remove the cell culture medium and wash monolayers with DPBS to remove any traces of TSC/CM. Add 1 mL of TS medium and culture plates for up to 10 days (*see Note 6*).
5. Take samples at days 0, 2, 4, 6, 8, and 10. Isolate RNA and perform standard quantitative real-time polymerase chain reaction analysis for specific trophoblast differentiation markers (Fig. 1).

3.2 MTT-Proliferation Assay for TSC

1. Plate TSC in 24-well plastic plates at a density of 10,000 cells *per* well in 0.5 mL of TSC/CM. Use four technical replicates for each measuring point. Culture plates for 48 h in the incubator.
2. At 48 h postplating, establish the reference point for the proliferation curve. Remove cell culture medium and add 1 mL of prewarmed 10% staining solution in TS medium to the wells at a final MTT concentration 160 μ g/mL (*see Note 7*). Incubate plates for 2 h in the incubator.

3. Carefully remove medium and add 0.5 mL of DMSO to each well. Shake the plates for 5 min at low speed on a shaker to lyse cells and release the blue dye.
4. Place the plates into a plate reader or transfer solutions into cuvettes and measure in a photometer at 570 nm wave length with DMSO as a background (i.e., blank).
5. Measure four technical replicates for each time point (i.e., day) of the proliferation curve.
6. Normalize data to day 0, graph and analyze using appropriate computer software (Fig. 2).

3.3 Derivation and Culture of Primary Rodent Uterine Myocytes

Primary rodent uterine myocytes are prepared as follows [21, 22]:

1. Virgin, female, Sprague-Dawley rats weighing 150–200 g are subcutaneously injected with estrogen (i.e., 50 μg in 200 μL).
2. 24 h after injection, excise the whole uterus under sterile conditions and place in a 10 cm cell culture dish with 25 mL of buffer A.
3. Place the sterile cell culture dish with uterine tissue in a biosafety cabinet with laminar flow, clean the uterine horns from fat and connective tissue, cut into 1 mm wide rings, and place them in a 50 mL flask with 25 mL of buffer B.
4. Wash the tissue pieces three times with buffer B at room temperature.
5. Perform an enzymatic digestion of the tissue by incubation in buffer C (i.e., 10 mL/g of tissue) for 30 min at 37 °C on an orbital shaker (i.e., 100 rounds *per* min).
6. Following incubation, gently agitate the mixture by repeated trituration with a glass and plastic pipette to mechanically disrupt uterine tissue (*see* **Note 8**).
7. Add an equal volume of ice-cold buffer B with 10% FBS to stop enzymatic digestion, pass the suspension through a 70 μm cell strainer to remove clumps of cells, and store on ice.
8. Put the remaining undigested tissue into a new flask, add 10 mL of fresh buffer C to the tissue, and repeat the incubation and aspiration process five times.
9. Collect the dissociated cells from **steps 2–6** by centrifugation at 200 $\times g$ for 15 min and resuspend the cell pellet in DMEM supplemented with 10% FBS (*see* **Note 9**).

3.4 Application of Stretch to Rat Smooth Muscle Cells

1. Plate freshly isolated myometrial cells with an initial density of 3×10^6 cells *per* well in 3 mL of cell culture medium into 6-well Flexcell plates coated with type I collagen or other extracellular matrix proteins. Leave for 3 days to attach and proliferate in a humidified 5% CO_2 incubator at 37 °C to 75% confluence.

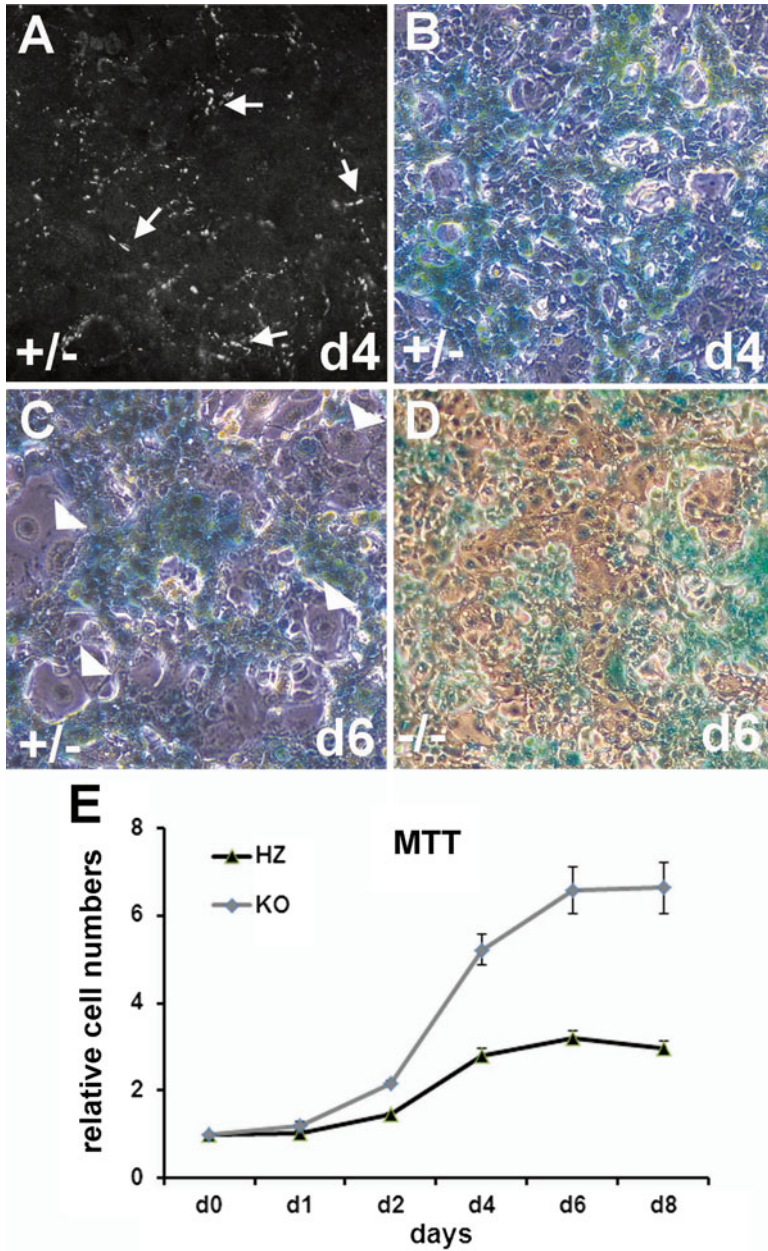


Fig. 2 (a) Immunolabeling for Cx31.1 (arrows) and (b) corresponding phase-contrast image of Cx31.1^{+/-} TSC at day 4 of differentiation. (c) At day 6, giant cells (arrow heads) are prominent in Cx31.1^{+/-} TSC cultures, whereas the delayed differentiation of Cx31.1^{+/-} TSC (d) leads to absence of giant cells forming cultures at day 6. Blue LacZ stain indicates the Cx31.1 expression cell populations. (e) MTT proliferation assay shows increased proliferation rates of Cx31.1^{-/-} during differentiation compared to Cx31.1^{+/-} controls (magnification 10×)

2. Incubate the cells for 24 h in serum-free medium to render quiescence.
3. Expose the cell culture plates to static stretch ranging between 0 and 25% elongation for predetermined time points (i.e., 2,

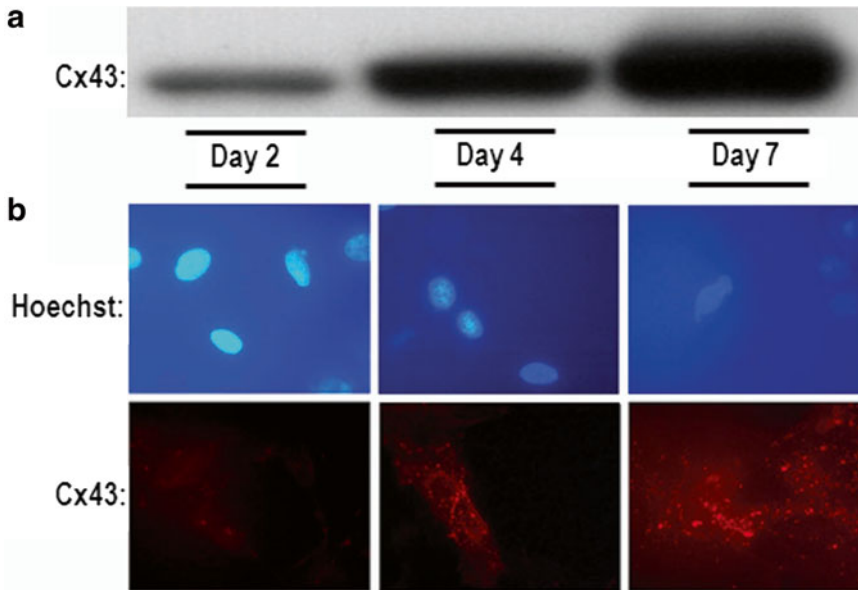


Fig. 3 Basal Cx43 protein levels in myometrial smooth muscle cells following 2, 4, and 7 days in culture. (a) Representative immunoblot analysis showing the dramatic increase in Cx43 protein levels with increasing days in culture. (b) Indirect immunofluorescence of Cx43 in myometrial smooth muscle cells showing intense punctate staining for Cx43 on day 7 of culture. Cells were simultaneously stained with Hoechst to mark cell nuclei (magnification 1000 \times)

6, or 24 h) by applying a vacuum generated by a pump and controlled by a computer-driven system (*see Note 10*).

4. Extract proteins or RNA, or fix with 4% paraformaldehyde (*see Note 11*).
5. Analyze gap junction expression (Fig. 3) and viability of cells (Fig. 4).

4 Notes

1. The source of FBS and percentage of conditioned TS medium have major effects on the undifferentiated growth of TSC. FBS should be tested to promote undifferentiated growth of TSC over several passages. Depending on the performance of FBS, the concentration of CM might be reduced [14].
2. We routinely use MEFs derived from the same mouse strain as the TSC lines. The autologous MEFs always lead to successful generation and culture of TSC lines.
3. It is crucial for comparison of differentiation and proliferation of mutant and wild-type TSC to use the same batch of TS medium as well as 70% TS/CM. We strongly recommend preparing large volumes of TS/CM and freezing at -20°C . The

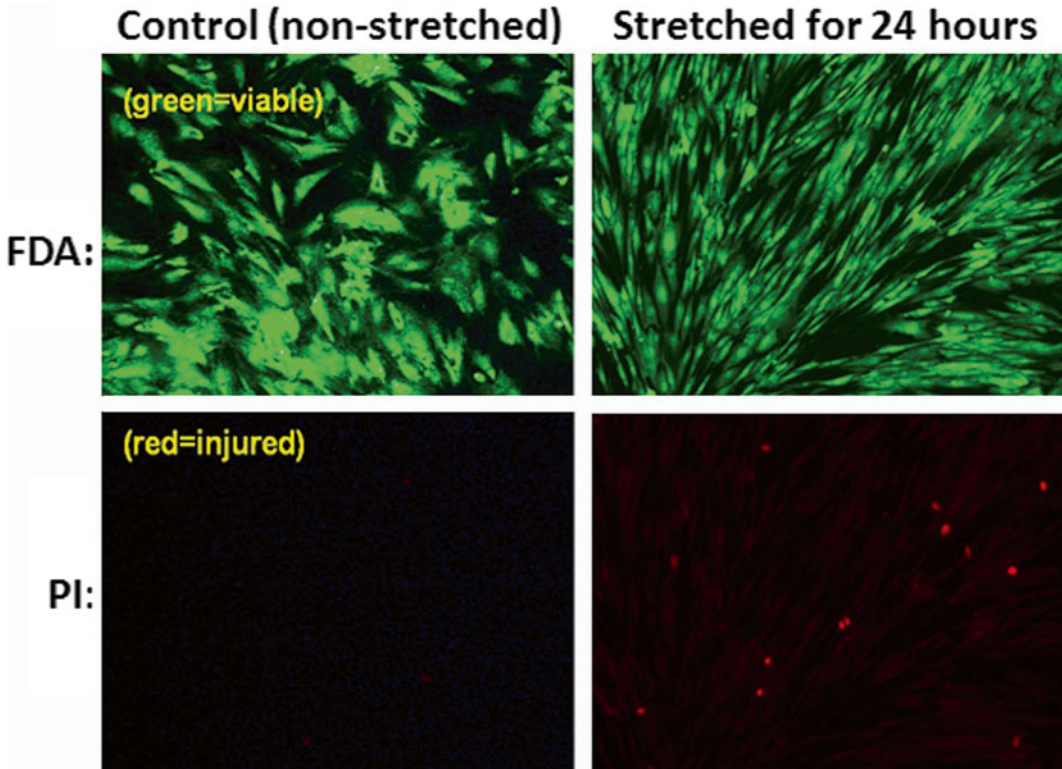


Fig. 4 Static mechanical stretch applied by Flexcell FX-3000 does not induce cell injury. Cell viability assay by fluorescein diacetate (FDA)-propidium iodide (PI) staining of primary rat myometrial smooth muscle cells. FDA (green) and PI (red) staining of nonstretched cells (control) and primary uterine myocytes following 24 h of static stretch (25% elongation) (magnification 200 \times)

quality of FGF4 has major effects on undifferentiated growth of TSC. Vendors should provide quality testing of FGF4 activity on data sheets, which is usually a sign of quality.

4. TrypLE is a recombinant cell dissociation enzyme, which is more effective on TSC compared to porcine trypsin-based solutions. In particular, when using TrypLE, dissociation of TSC to a single cell suspension is much faster and more reproducible.
5. For the differentiation assay, using connexin-deficient TSC is important to compare mutant and control TSC derived from the same mouse strain. Ideally, TSC should be derived in 1 experiment using the same batch of feeders and TS media. This approach will help to identify true differences in the marker gene profile during cell differentiation. TSC derived from different mouse strains (i.e. SV129 and C57BL/J) show significant difference in expression levels of marker genes, though the temporal expression profile is still comparable. We routinely mate heterozygous females with homozygous males for

blastocyst isolation to simultaneously generate several heterozygous and homozygous connexin-deficient TSC lines. When TSC from different rounds of derivation are used, we recommend first to normalize the data of mutant TSC to corresponding controls before combining all data into a study.

6. We do not recommend plating TSC in TS medium directly to induce differentiation. Seeding TSC in TSC/CM and allowing them to adhere and form initial colonies will significantly increase cell attachment and reduce spontaneous differentiation. Thus, for better performance and less variability of differentiation results as well as proliferation assays, we strongly recommend 48 h of incubation time.
7. MTT at high concentrations (i.e., 0.5 mg/mL) normally used for cancer cell lines is stressful to TSC and leads to detachment of cells over time. We found that an MTT concentration of 60 μ g/mL and using a 2 h incubation time led to reproducible experimental results of TSC proliferation assays without losing cells. The MTT assay is most useful when studying proliferation during TSC differentiation, as differentiated trophoblast cells are hard to detach enzymatically and therefore cannot be reliably counted as dissociated cells.
8. We recommend at the end of the incubation cycle (i.e., 30 min) that the mixture is gently agitated by repeated titration (i.e., 3–4 min) with a 25 mL large-hole glass pipette to aid enzymatic dispersion of uterine tissue. Due to the decreasing size of tissues during the procedure, large-hole and small-hole plastic transfer pipettes can be used for the final two incubation steps, respectively.
9. The first incubation solution is discarded, since it contains debris and damaged cells. To selectively enrich for uterine myocytes, we recommend subjecting the freshly isolated cell suspension to a differential attachment technique. For this purpose, preplate dissociated cells on polystyrene culture dishes for 30–45 min at 37 °C, during which period the quickly adhering nonmyocytes, mostly fibroblasts, will readily attach to the bottom of the cell culture dish. The supernatant containing slowly adhering uterine smooth muscle cells should be collected and plated on the Flexcell plates. Both cell count and cell viability could be assessed by trypan blue exclusion using a hemocytometer.
10. The Flexcell strain unit has been characterized in detail [23]. It consists of a vacuum unit regulated by a solenoid valve and a computer program. When a precise vacuum level is applied to the system, the cell culture plate bottoms are deformed in downward direction to a known percentage elongation, which is translated to the cultured cells. When the vacuum is released,

the plate bottoms return to their original conformation. The magnitude, duration, and frequency of the applied force can be varied in this system. The force on the attached cells is predominantly uniaxial. However, the deformation of the flexible membrane is not uniform, but rather generates a gradient stretch, with the greatest deformation occurring at the periphery. Therefore, the results of the stretch experiment represent an average of cells exposed to different degrees of stretch. For 25 % elongation, the average elongation is approximately 10 % over the entire cell culture plate surface.

11. Stretched and control (i.e., nonstretched) Flexcell plates should be established simultaneously with the same pool of cells in each experiment to match for temperature, CO₂ content, or pH of the cell culture medium.

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References

1. Roberts DJ, Post MD (2008) The placenta in pre-eclampsia and intrauterine growth restriction. *J Clin Pathol* 61:1254–1260
2. Romero R, Dey SK, Fisher SJ (2014) Preterm labor: one syndrome, many causes. *Science* 345:760–765
3. Kibschull M, Gellhaus A, Carette D et al (2015) Physiological roles of connexins and pannexins in reproductive organs. *Cell Mol Life Sci* 72:2879–2898
4. Winterhager E, Kidder GM (2015) Gap junction connexins in female reproductive organs: implications for women's reproductive health. *Hum Reprod Update* 21:340–352
5. Shynlova O, Lee YH, Srikhajon K et al (2013) Physiologic uterine inflammation and labor onset: integration of endocrine and mechanical signals. *Reprod Sci* 20: 154–167
6. Plum A, Winterhager E, Pesch J et al (2001) Connexin31-deficiency in mice causes transient placental dysmorphogenesis but does not impair hearing and skin differentiation. *Dev Biol* 231:334–347
7. Zheng-Fischhofer Q, Kibschull M, Schnichels M et al (2007) Characterization of connexin31.1-deficient mice reveals impaired placental development. *Dev Biol* 312:258–271
8. Kibschull M, Magin TM, Traub O et al (2005) Cx31 and Cx43 double-deficient mice reveal independent functions in murine placental and skin development. *Dev Dyn* 233:853–863
9. Kibschull M, Colaco K, Matysiak-Zablocki E et al (2014) Connexin31.1 (Gjb5) deficiency blocks trophoblast stem cell differentiation and delays placental development. *Stem Cells Dev* 23:2649–2660
10. Kibschull M, Nassiry M, Dunk C et al (2004) Connexin31-deficient trophoblast stem cells: a model to analyze the role of gap junction communication in mouse placental development. *Dev Biol* 273:63–75
11. Erlebacher A, Price KA, Glimcher LH (2004) Maintenance of mouse trophoblast stem cell proliferation by TGF-beta/activin. *Dev Biol* 275:158–169
12. Quinn J, Kunath T, Rossant J (2006) Mouse trophoblast stem cells. *Methods Mol Med* 121:125–148
13. Tanaka S, Kunath T, Hadjantonakis AK et al (1998) Promotion of trophoblast stem cell proliferation by FGF4. *Science* 282:2072–2075

14. Kibschull M, Winterhager E (2006) Connexins and trophoblast cell lineage development. *Methods Mol Med* 121:149–158
15. Shynlova O, Tsui P, Jaffer S et al (2009) Integration of endocrine and mechanical signals in the regulation of myometrial functions during pregnancy and labour. *Eur J Obstet Gynecol Reprod Biol* 144:S2–S10
16. Dunk CE, Gellhaus A, Drewlo S et al (2012) The molecular role of connexin 43 in human trophoblast cell fusion. *Biol Reprod* 86:115
17. Shynlova O, Nedd-Roderique T, Li Y et al (2013) Infiltration of myeloid cells into decidua is a critical early event in the labour cascade and post-partum uterine remodelling. *J Cell Mol Med* 17:311–324
18. Orsino A, Taylor CV, Lye SJ (1996) Connexin-26 and connexin-43 are differentially expressed and regulated in the rat myometrium throughout late pregnancy and with the onset of labor. *Endocrinology* 137:1545–1553
19. Doring B, Shynlova O, Tsui P et al (2006) Ablation of connexin43 in uterine smooth muscle cells of the mouse causes delayed parturition. *J Cell Sci* 119:1715–1722
20. Shynlova O, Nedd-Roderique T, Li Y et al (2013) Myometrial immune cells contribute to term parturition, preterm labour and post-partum involution in mice. *J Cell Mol Med* 17:90–102
21. Shynlova OP, Oldenhof AD, Liu M et al (2002) Regulation of c-fos expression by static stretch in rat myometrial smooth muscle cells. *Am J Obstet Gynecol* 186:1358–1365
22. Mollard P, Mironneau J, Amedee T et al (1986) Electrophysiological characterization of single pregnant rat myometrial cells in short-term primary culture. *Am J Physiol* 250:C47–C54
23. Gilbert JA, Weinhold PS, Banes AJ et al (1994) Strain profiles for circular cell culture plates containing flexible surfaces employed to mechanically deform cells in vitro. *J Biomech* 27:1169–1177