AMNIOTIC FLUID-DERIVED MESENCHYMAL STEM CELLS

A SCIENTIFIC ABSTRACTS REVIEW

From June 2008 to September 2009

BIOCELL CENTER GROUP

COMPANY OVERVIEW

Biocell Center is a private company that offers the opportunity to cryopreserve amniotic fluid-derived stem cells as well as supports research collaborations for developing the amniotic stem cell medical applications.

Biocell Center was founded in Italy in 2006 by a group of distinguished medical doctors and businessmen, following the discovery that stem cells present in the amniotic fluid possess unique characteristics compared to the cells derived from other sources. Researchers from Biocell Center as well as many other groups worldwide have demonstrated that amniotic stem cells are able to differentiate into various cell types and can potentially lead to numerous clinical applications and medical treatments for a broad set of diseases

(http://www.biocellcenter.com/en/services_research/scientific_updates/)

Biocell Center scientists have developed a novel approach to preserve this important cellular material via the method that does not require any changes to the standard amniocentesis procedure performed during pregnancy. Biocell Center has since built several laboratories and amniotic stem cell cryobanks that focus on the standardization and optimization of the cryopreservation protocols for amniotic stem cells and offers the option to preserve this important biological material to the individual families and to the physicians and the medical centers.

Biocell Center has been successfully collaborating with many leading hospitals, universities, stem cell research laboratories and companies worldwide, including TOMA Laboratories (Italy), Istituto Neurologico “Besta” (Italy), ARTEMISA Roma, (Italy), Sintetica SA (Switzerland), National Council for Research (Italy), Politecnico di Milano (Italy), and Mass Eye and Ear Infirmary, Harvard Medical School (US.)

Biocell Center currently has offices and laboratories in Milan (Italy), Lugano (Switzerland), and Medford (MA, USA.)

ABSTRACTS TITLES

1. **Variations of Protein Levels in Human Amniotic Fluid Stem Cells CD117/2 Over Passages 5-25.** Chen WQ, Siegel N, Li L, Pollak A, Hengstschläger M, Lubec G. J Proteome Res. 2009 Sep 30.

2. **A microfluidic device for separation of amniotic fluid mesenchymal stem cells utilizing louver-array structures.** Wu HW, Lin XZ, Hwang SM, Lee GB. Biomed Microdevices. 2009 Sep 3.

3. **In vitro and in vivo study of human amniotic fluid-derived stem cell differentiation into myogenic lineage.** Gekas J, Walther G, Skuk D, Bujold E, Harvey I, Bertrand OF. Clin Exp Med. 2009 Sep 3.

4. **Escalated regeneration in sciatic nerve crush injury by the combined therapy of human amniotic fluid mesenchymal stem cells and fermented soybean extracts, Natto.** Pan HC, Yang DY, Ho SP, Sheu ML, Chen CJ, Hwang SM, Chang MH, Cheng FC. J Biomed Sci. 2009 Aug 23;16:75.

5. **Secretory Profiles and Wound Healing Effects of Human Amniotic Fluid-derived Mesenchymal Stem Cells.** Yoon BS, Moon JH, Jun EK, Kim J, Maeng I, Kim JS, Lee JH, Baik CS, Kim A, Cho KS, Lee JH, Lee HH, Whang KY, You S. Stem Cells Dev. 2009 Aug 17.

6. **Pluripotency can be rapidly and efficiently induced in human amniotic fluid-derived cells.** Li C, Zhou J, Shi G, Ma Y, Yang Y, Gu J, Yu H, Jin S, Wei Z, Chen F, Jin Y. Hum Mol Genet. 2009 Aug 13.

7. **Pluripotent stem cells isolated from human amniotic fluid and differentiation into pancreatic betacells.** Trovato L, De Fazio R, Annunziata M, Sdei S, Favaro E, Ponti R, Marozio L, Ghigo E, Benedetto C, Granata R. J Endocrinol Invest. 2009 Jul 2.

8. **Effects of different culture conditions on isolation and expansion of stem cells from second-trimester amniotic fluids** Liu H, Liu DQ, Guan LD, Yan ZF, Wang J,

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9. **Multipotent mesenchymal stem cells from amniotic fluid originate neural precursors with functional voltage-gated sodium channels.**Mareschi K, Rustichelli D, Comunanza V, De Fazio R, Cravero C, Morterra G, Martinoglio B, Medico E, Carbone E, Benedetto C, Fagioli F. Cytotherapy. 2009;11(5):534-47.

10. **Mesenchymal stromal cells multipotency and plasticity: induction toward the hepatic lineage.** Saulnier N, Lattanzi W, Puglisi MA, Pani G, Barba M, Piscaglia AC, Giachelia M, Alfieri S, Neri G, Gasbarrini G, Gasbarrini A. 10: Eur Rev Med Pharmacol Sci. 2009 Mar;13 Suppl 1:71-8.

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Variations of Protein Levels in Human Amniotic Fluid Stem Cells CD117/2 Over Passages 5-25.

Chen WQ, Siegel N, Li L, Pollak A, Hengstschläger M, Lubec G. J Proteome Res. 2009 Sep 30.

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Stability of cell lines is the prerequisite for all in vitro research, but literature on the stability of protein expression over passages is limited. Determination of specific stability markers, karyotyping, and morphology may not provide full information on this subject. It was the aim of the study to test protein level fluctuations in a human amniotic fluid stem cell line from passages 5, 7, 11, and 25. While karyotype, cell cycle, apoptosis rate, and 10 markers for characterization of the cell line remained unchanged (carried out at passages 5 and 25), cell volume was increased at passage 25. Significant protein fluctuations were observed for signaling, antioxidant, guidance cue, proteasomal, connective tissue, cytoskeleton proteins, chaperones, a chloride channel, and prothymosin at passages 5, 7, 11, and 25. Herein, the use of this gel-based proteomic screen, checking protein stability for the characterization of cell lines in addition to corresponding published markers, is proposed, in particular when experiments are run over several passages.

A microfluidic device for separation of amniotic fluid mesenchymal stem cells utilizing louver-array structures.

Wu HW, Lin XZ, Hwang SM, Lee GB. Biomed Microdevices. 2009 Sep 3.

Department of Engineering Science, National Cheng Kung University, Tainan, 701, Taiwan.

Human mesenchymal stem cells can differentiate into multiple lineages for cell therapy and, therefore, have attracted considerable research interest recently. This study presents a new microfluidic device for bead and cell separation utilizing a combination of T-junction focusing and tilted louver-like structures. For the first time, a microfluidic device is used for continuous separation of amniotic stem cells from amniotic fluids. An experimental separation efficiency as high as 82.8% for amniotic fluid mesenchymal stem cells is achieved. Furthermore, a two-step separation process is performed to improve the separation efficiency to 97.1%. These results are based on characterization experiments that show that this microfluidic chip is capable of separating beads with diameters of 5, 10, 20, and 40 mum by adjusting the volume-flow-rate ratio between the flows in the main and side channels of the T-junction focusing structure. An optimal volume-flow-rate ratio of 0.5 can lead to high separation efficiencies of 87.8% and 85.7% for 5-mum and 10-mum beads, respectively, in a one-step separation process. The development of this microfluidic chip may be promising for future research into stem cells and for cell therapy.

In vitro and in vivo study of human amniotic fluid-derived stem cell differentiation into myogenic lineage.

Gekas J, Walther G, Skuk D, Bujold E, Harvey I, Bertrand OF. Clin Exp Med. 2009 Sep 3.

Laboratoire de Génétique Humaine, Centre Hospitalier de l'Université Laval, Quebec City, QC, Canada.

Recent findings have shown that amniotic fluid (AF) could be a putative new source of multipotent stem cells (SC). We investigated whether these human SC could efficiently differentiate into myogenic lineage in vitro and integrate in vivo skeletal muscle in severe combined immunodeficiency (SCID) mice. C/kit immunomagnetic-sorted AF (AF c/kit+) SC were characterized by immunocytochemistry and Southern blotting for myogenic markers (desmin, MyoD). In vitro, AF c/kit+ SC phenotypic conversion into myogenic cells was assayed by myogenic-specific induction media. AF c/kit+ SC without ex vivo manipulation were transplanted into the tibialis anterior (TA) of (SCID) mice. Acquisition of a myogenic-like phenotype (desmin, MyoD) in AF c/kit+ SC was observed after culture in myogenic-specific induction media. In vivo, transplanted AF c/kit+ SC showed an engraftment in the skeletal muscle of SCID mice, but with unexpected tubular glandular tissue-like differentiation. Importantly, no immuno-rejection, inflammatory response or tumorigenicity of these cells was found. Within these experimental conditions, AF c/kit+ SC were able to differentiate into myogenic cells in vitro, but not in vivo after their transplantation into the skeletal muscle of SCID mice. Because AF c/kit+ SC survived and differentiated into tubular gland-like cells after their transplantation in the TA, an ex vivo engagement in myogenic pathway prior their transplantation could favor their differentiation into myogenic cells in vivo.

Escalated regeneration in sciatic nerve crush injury by the combined therapy of human amniotic fluid mesenchymal stem cells and fermented soybean extracts, Natto.

Pan HC, Yang DY, Ho SP, Sheu ML, Chen CJ, Hwang SM, Chang MH, Cheng FC. J Biomed Sci. 2009 Aug 23;16:75.

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Attenuation of inflammatory cell deposits and associated cytokines prevented the apoptosis of transplanted stem cells in a sciatic nerve crush injury model. Suppression of inflammatory cytokines by fermented soybean extracts (Natto) was also beneficial to nerve regeneration. In this study, the effect of Natto on transplanted human amniotic fluid mesenchymal stem cells (AFS) was evaluated. Peripheral nerve injury was induced in SD rats by crushing a sciatic nerve using a vessel clamp. Animals were categorized into four groups: Group I: no treatment; Group II: fed with Natto (16 mg/day for 7 consecutive days); Group III: AFS embedded in fibrin glue; Group IV: Combination of group II and III therapy. Transplanted AFS and Schwann cell apoptosis, inflammatory cell deposits and associated cytokines, motor function, and nerve regeneration were evaluated 7 or 28 days after injury. The deterioration of neurological function was attenuated by AFS, Natto, or the combined therapy. The combined therapy caused the most significantly beneficial effects. Administration of Natto suppressed the inflammatory responses and correlated with decreased AFS and Schwann cell apoptosis. The decreased AFS apoptosis was in line with neurological improvement such as expression of early regeneration marker of neurofilament and late markers of S-100 and decreased vacuole formation. Administration of either AFS, or Natto, or combined therapy augmented the nerve regeneration. In conclusion, administration of Natto may rescue the AFS and Schwann cells from apoptosis by suppressing the macrophage deposits, associated inflammatory cytokines, and fibrin deposits.

Secretory Profiles and Wound Healing Effects of Human Amniotic Fluid-derived Mesenchymal Stem Cells.

Yoon BS, Moon JH, Jun EK, Kim J, Maeng I, Kim JS, Lee JH, Baik CS, Kim A, Cho KS, Lee JH, Lee HH, Whang KY, You S. Stem Cells Dev. 2009 Aug 17.

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Recent evidence shows that amniotic fluid contains multiple cell types derived from the developing fetus, and may represent a novel source of stem cells for cell therapy. In this study, we examined the paracrine factors released by human amniotic fluid-derived mesenchymal stem cells (AF-MSCs) and their ability to accelerate the wound healing process by stimulating proliferation and migration of dermal fibroblasts. AF-MSCs expressed the typical mesenchymal stem cell marker proteins CD13, CD29, and CD44 and differentiated into adipocytes, osteoblasts and chondrocytes when exposed to the appropriate differentiation media. In addition, AF-MSC-conditioned media (AF-MSC-CM) significantly enhanced proliferation of dermal fibroblasts. Antibody-based protein array and ELISA indicated that AF-MSC-CM contains various cytokines and chemokines that are known to be important in normal wound healing, including IL-8, IL-6, TGF-beta, TNFRI, VEGF, and EGF. Application of AF-MSC-CM significantly enhanced wound healing by dermal fibroblasts via the TGF-beta/SMAD2 pathway. Levels of p-SMAD2 were increased by AF-MSC-CM, and both the increase in p-SMAD2 and migration of dermal fibroblasts were blocked by inhibiting the TGF-beta/SMAD2 pathway. Moreover, in a mouse excisional wound model, AF-MSC-CM accelerated wound healing. These data provide the first evidence of the potential for AF-MSC-CM in the treatment of skin wounds.

Pluripotency can be rapidly and efficiently induced in human amniotic fluid-derived cells.

Li C, Zhou J, Shi G, Ma Y, Yang Y, Gu J, Yu H, Jin S, Wei Z, Chen F, Jin Y. Hum Mol Genet. 2009 Aug 13.

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Direct reprogramming of human somatic cells into pluripotency has broad implications in generating patient-specific induced pluripotent stem (iPS) cells for disease modeling and cellular replacement therapies. However, the low efficiency and safety issues associated with generation of human iPS cells have limited their usage in clinical settings. Cell types can significantly influence reprogramming efficiency and kinetics. To date, human iPS cells have been obtained only from a few cell types. Here, we report for the first time rapid and efficient generation of iPS cells from human amniotic fluid-derived cells (hAFDCs) via ectopic expression of four human factors: OCT4/SOX2/KLF4/C-MYC. Significantly, typical single iPS cell colonies can be picked up six days after viral infection with high efificiency. Eight iPS cell lines have been derived. They can be continuously propagated in vitro and express pluripotency markers such as AKP, OCT4, SOX2, SSEA4, TRA-1-60 and TRA-1-81, maintaining the normal karyotype. Transgenes are completely inactivated and the endogenous OCT4 promoter is adequately demethylated in the established iPS cell lines. Moreover, various cells and tissues from all three germ layers are found in embryoid bodies and teratomas respectively. In addition, microarray analysis demonstrates a high correlation coefficient between hAFDC-iPS cells and human embryonic stem cells, but a low correlation coefficient between hAFDCs and hAFDC-iPS cells. Taken together, these data identify an ideal human somatic cell resource for rapid and efficient generation of iPS cells, allowing us to establish human iPS cells using more advanced approaches and possibly to establish disease- or patient-specific iPS cells.

Pluripotent stem cells isolated from human amniotic fluid and differentiation into pancreatic betacells.

Trovato L, De Fazio R, Annunziata M, Sdei S, Favaro E, Ponti R, Marozio L, Ghigo E, Benedetto C, Granata R. J Endocrinol Invest. 2009 Jul 2.

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Human amniotic fluid (HAF) contains multipotent stem cells (AFSCs) which can differentiate into a variety of different cell types. Recently, we demonstrated that obestatin, a peptide encoded by the ghrelin gene, exerts antiapoptotic effects in pancreatic betacells and human islets and increases the expression of genes involved in betacell differentiation. We investigated whether: 1) AFSCs would differentiate into pancreatic betacells and 2) obestatin would increase betacell differentiation from AFSCs. FACS analysis and immunocytochemical staining showed the presence of mesenchymal and endothelial markers in AFSCs. Real-time PCR evidenced the expression of Oct-4, a marker of pluripotency, during early differentiation phase. However, the betacell differentiation marker duodenal homeobox factor-1 (PDX-1) could not be detected. Obestatin increased Oct-4 expression but had no effect on betacell differentiation. These results suggest that, at least under the experimental conditions used in this study, AFSCs do not differentiate into betacells and obestatin has no additional effect.

Effects of different culture conditions on isolation and expansion of stem cells from second-trimester amniotic fluids

Liu H, Liu DQ, Guan LD, Yan ZF, Wang J, He LJ, Lü Y, Nan X, Li YL, Pei XT. Zhonghua Fu Chan Ke Za Zhi. 2009 Apr;44(4):241-5.

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OBJECTIVE: To investigate the effects of different culture conditions on the isolation and expansion of stem cells from second-trimester amniotic fluids. METHODS: Amniotic fluids were obtained from 15 pregnant women undergone amniocenteses for medical indications between 16 - 24 gestation weeks by transabdominal amniocenteses from September 2007 to June 2008. Amniotic fluids (10 - 20 ml) samples were collected and each was cultured under different conditions or groups. (1) Low-glucose DMEM (LD) medium supplemented with 10% of fetal bovine serum (group of 10%FBS); (2) LD medium with 20% of FBS (group of 20%FBS); (3) LD medium with 15% of FBS and 4 ng/ml of basic fibroblast growth factor (group of bFGF); (4) LD medium with 10% of FBS as well as the culture plate coated with gelatin (group of gelatin). The effects of different conditions were evaluated by comparing the number of primary colonies, the cell morphology and the ability of expansion. The isolated stem cells were identified by flow cytometry, RT-PCR and differentiation ability to adipocyte. RESULTS: (1) The success rates of primary culture of the group of 10%FBS, 20%FBS, bFGF and gelatin were 60%, 73%, 73% and 60% respectively ($P > 0.05$). The numbers of colonies were 0.9 +/- 0.5, 2.6 +/- 1.5, 2.9 +/- 1.5, 1.1 +/- 0.8 ($P < 0.01$ when group of 10%FBS and gelatin compared with group of 20%FBS and bFGF); among the primary colonies, fibroblast-like colonies accounted for 46%, 49%, 64%, 44% respectively ($P > 0.05$). (2) The second passage cells obtained from all of these four groups could differentiate into adipocyte after induction. (3) In the group of bFGF, stem cells were isolated from 5 samples and expanded to nearly 10(7) cells after 5 passages ($P < 0.01$ compared with other groups). (4) Karyotype were normal in all samples. (5) Stem cells from bFGF group showed positive expression of SSEA-4, Oct-4 and Nanog gene detected by flow cytometry and RT-PCR. CONCLUSION: Stem cells can be isolated from second-trimester amniotic fluids; moderate serum concentration and supplementation of bFGF can improve the efficiency of isolation and expansion of amniotic fluid of stem cells.

Multipotent mesenchymal stem cells from amniotic fluid originate neural precursors with functional voltage-gated sodium channels.

Mareschi K, Rustichelli D, Comunanza V, De Fazio R, Cravero C, Morterra G, Martinoglio B, Medico E, Carbone E, Benedetto C, Fagioli F. Cytotherapy. 2009;11(5):534-47.

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BACKGROUND AIMS: Amniotic fluid (AF) contains stem cells with high proliferative and differentiative potential that might be an attractive source of multipotent stem cells. We investigated whether human AF contains mesenchymal stem cells (MSC) and evaluated their phenotypic characteristics and differentiation potential in vitro. METHODS: AF was harvested during routine pre-natal amniocentesis at 14-16 weeks of pregnancy. AF sample pellets were plated in alpha-minimum essential medium (MEM) with 10% fetal bovine serum (FBS). We evaluated cellular growth, immunophenotype, stemness markers and differentiative potential during in vitro expansion. Neural progenitor maintenance medium (NPMM), a medium normally used for the growth and maintenance of neural stem cells, containing hFGF, hEGF and NSF-1, was used for neural induction. RESULTS: Twenty-seven AF samples were collected and primary cells, obtained from samples containing more than 6 mL AF, had MSC characteristics. AF MSC showed high proliferative potential, were positive for CD90, CD105, CD29, CD44, CD73 and CD166, showed Oct-4 and Nanog molecular and protein expression, and differentiated into osteoblasts, adypocytes and chondrocytes. The NPMM-cultured cells expressed neural markers and increased Na(+) channel density and channel inactivation rate, making the tetrodotoxin (TTX)-sensitive channels more kinetically similar to native neuronal voltage-gated Na(+) channels. CONCLUSIONS: These data suggest that AF is an important multipotent stem cell source with a high proliferative potential able to originate potential precursors of functional neurons.

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Mesenchymal stromal cells multipotency and plasticity: induction toward the hepatic lineage.

Saulnier N, Lattanzi W, Puglisi MA, Pani G, Barba M, Piscaglia AC, Giachelia M, Alfieri S, Neri G, Gasbarrini G, Gasbarrini A. 10: Eur Rev Med Pharmacol Sci. 2009 Mar;13 Suppl 1:71-8.

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BACKGROUND: Human mesenchymal stromal cells (MSCs) can be isolated from a variety of adult and perinatal tissues and exert multipotency and self renewal properties which make them suitable for cell-based therapy. Their potential plasticity extended to non-mesodermal-derived tissues has been indicated, although it is still a debated issue. In this study we have isolated MSCs from both adult and fetal tissues. Their growth, immunophenotype and multi-lineage differentiation potentials have been analyzed, focusing, in particular, on the hepatic differentiation. METHODS: Cells were isolated from bone marrow (BMSC), adipose tissue (ATSC) and second trimester amniotic fluid (AFSC), upon a written informed consent obtained from donor patients. Cells were expanded and growth kinetics was assessed by means of proliferation assay. Their immunophenotype was analyzed using cytometry and multi-lineage differentiation potential was evaluated by means of in vitro differentiation assays. Finally, the expression of tissue-specific markers was also assessed by mean of semi-quantitative PCR. RESULTS: Bipolar spindle-shaped cells were successfully isolated from all these tissues. Interestingly, ATSCs and AFSCs showed a higher proliferation potential than BMSCs. Mesodermal differentiation capacity was verified in all MSC populations, even if AFSCs were not able to undergo adipogenesis in our culture conditions. Furthermore, we showed that MSC cultured in appropriate conditions were able to induce hepatic-associated genes, such as ALB and TDO2. CONCLUSION: Taken together the data here reported suggest that MSCs from both adult and fetal tissues are capable of tissue-specific commitment along mesodermal and non-mesodermal lineages. In particular we have demonstrated that a specific hepatogenic commitment can be efficiently induced, proposing these cells as suitable tool for cell-based applications aimed at liver regeneration.

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Sternal repair with bone grafts engineered from amniotic mesenchymal stem cells.

Steigman SA, Ahmed A, Shanti RM, Tuan RS, Valim C, Fauza DO. J Pediatr Surg. 2009 Jun;44(6):1120-6; discussion 1126.

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PURPOSE: We aimed at determining whether osseous grafts engineered from amniotic mesenchymal stem cells (aMSCs) could be used in postnatal sternal repair. METHODS: Leporine aMSCs were isolated, identified, transfected with green fluorescent protein (GFP), expanded, and seeded onto biodegradable electrospun nanofibrous scaffolds (n = 6). Constructs were dynamically maintained in an osteogenic medium and equally divided into 2 groups with respect to time in vitro as follows: 14.6 or 33.9 weeks. They were then used to repair full-thickness sternal defects spanning 2 to 3 intercostal spaces in allogeneic kits (n = 6). Grafts were submitted to multiple analyses 2 months thereafter. RESULTS: Chest roentgenograms showed defect closure in all animals, confirmed at necropsy. Graft density as assessed by microcomputed tomographic scans increased significantly in vivo, yet there were no differences in mineralization by extracellular calcium measurements preimplantation and postimplantation. There was a borderline increase in alkaline phosphatase activity in vivo, suggesting ongoing graft remodeling. Histologically, implants contained GFP-positive cells and few mononuclear infiltrates. There were no differences between the 2 construct groups in any comparison. CONCLUSIONS: Engineered osseous grafts derived from amniotic mesenchymal stem cells may become a viable alternative for sternal repair. The amniotic fluid can be a practical cell source for engineered chest wall reconstruction.

12

Endothelial Differentiation of Amniotic Fluid-derived Stem Cells: Synergism of biochemical and shear force stimuli.

Zhang P, Baxter J, Vinod K, Tulenko TN, Dimuzio P. 12: Stem Cells Dev. 2009 Jun 9.

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Human amniotic fluid-derived stem (AFS) cells possess several advantages over embryonic and adult stem cells, as evidenced by expression of both types of stem cell markers and ability to differentiate into cells of all three germ layers. Herein, we examine endothelial differentiation of AFS in response to growth factors, shear force and hypoxia. We isolated human AFS from amniotic fluid samples (1-4cc/specimen) obtained from patients undergoing amniocentesis at 15-18 weeks of gestation (n=10). Isolates maintained in non-differentiating medium expressed the stem cell markers CD13, CD29, CD44, CD90, CD105, OCT4 and SSEA-4 through passage eight. After three weeks of culture in Endothelial Growth Media (EGM2), the stem cells exhibited an endothelial-like morphology, formed cord-like structures when plated on Matrigel, and up took acetylated-LDL/lectin. Additionally, mRNA and protein levels of CD31 and von Willebrand factor (vWF) significantly increased in response to culture in EGM2, with further up-regulation when stimulated by physiological levels (12 dyne/cm2) of shear force. Culture in hypoxic conditions (5% O2) resulted in significant expression of Vascular Endothelial Growth Factor (VEGF) and Placental Growth Factor (PGF) mRNA. This study suggests that AFS, isolated from minute amounts of amniotic fluid, acquire endothelial cell characteristics when stimulated by growth factors and shear force, and produce angiogenic factors (VEGF, PGF and HGF) in response to hypoxia. Thus, amniotic fluid represents a rich source of mesenchymal stem cells potentially suitable for use in cardiovascular regenerative medicine.

Lost in Translation: What is Limiting Cardiomyoplasty and Can Tissue Engineering Help?

Simpson D, Dudley SC Jr. Curr Stem Cell Res Ther. 2009 Sep 1.

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Heart failure accounts for more deaths in the United States than any other detrimental human pathology. Recently, repairing the heart after seemingly irreversible injury leading to heart failure appears to have come within reach. Cellular cardiomyoplasty, transplanting viable cell alternatives into the diseased myocardium, has emerged as a promising possible solution. Translating this approach from the laboratory to the clinic, however, has been met with several challenges, leaving many questions unanswered. This review assesses the state of investigation of several progenitor cell sources, including induced pluripotent stem cells, embryonic stem cells, bone marrow stem cells, adipose-derived adult stem cells, amniotic fluid stem cells, skeletal muscle progenitors, induced pluripotent stem cells and cardiac progenitors. Several current roadblocks to maximum success are discussed. These include understanding the need for cardiomyocyte differentiation, appreciating the role of paracrine factors, and addressing the low engraftment rates using current techniques. Tissue engineering strategies to address these obstacles and to help maximize cellular cardiomyoplasty success are reviewed.

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Induced pluripotent stem cells offer new approach to therapy in thalassemia and sickle cell anemia and option in prenatal diagnosis in genetic diseases.

Ye L, Chang JC, Lin C, Sun X, Yu J, Kan YW. Proc Natl Acad Sci U S A. 2009 Jun 16;106(24):9826-30.

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The innovation of reprogramming somatic cells to induced pluripotent stem cells provides a possible new approach to treat beta-thalassemia and other genetic diseases such as sickle cell anemia. Induced pluripotent stem (iPS) cells can be made from these patients' somatic cells and the mutation in the beta-globin gene corrected by gene targeting, and the cells differentiated into hematopoietic cells to be returned to the patient. In this study, we reprogrammed the skin fibroblasts of a patient with homozygous beta(0) thalassemia into iPS cells, and showed that the iPS cells could be differentiated into hematopoietic cells that synthesized hemoglobin. Prenatal diagnosis and selective abortion have been effective in decreasing the number of beta-thalassemia births in some countries that have instituted carrier screening and genetic counseling. To make use of the cells from the amniotic fluid or chorionic villus sampling that are used for prenatal diagnosis, we also showed that these cells could be reprogrammed into iPS cells. This raises the possibility of providing a new option following prenatal diagnosis of a fetus affected by a severe illness. Currently, the parents would choose either to terminate the pregnancy or continue it and take care of the sick child after birth. The cells for prenatal diagnosis can be converted into iPS cells for treatment in the perinatal periods. Early treatment has the advantage of requiring much fewer cells than adult treatment, and can also prevent organ damage in those diseases in which damage can begin in utero or at an early age.

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Amniotic stem cells for cellular cardiomyoplasty: promises and premises.

Walther G, Gekas J, Bertrand OF. 15: Catheter Cardiovasc Interv. 2009 Jun 1;73(7):917-24.

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Cellular cardiomyoplasty is undergoing intensive investigation as a new form of therapy for severely damaged hearts. Among several cell types, mesenchymal stem cells (MSCs) have been proposed as a potential cell source. MSC can be found in adult tissues or in fetal tissues like the umbilical chord blood, amniotic membrane, or amniotic fluid (AF). AF-MSCs have properties intermediate between embryonic and adult MSC, which make them particularly attractive for cellular regeneration. It has been shown that MSC could differentiate in cardiomyocytes-like cells in vitro. In some animal models, it has also been shown that transplanted MSC could engraft and show some cardiomyocytes-like characteristics. Since MSC do not express HLA-DR and present in vitro and in vivo immunosuppressive properties, they can be envisioned to be used in allogenic cellular cardiomyoplasty. Based on these promises, MSC from adult donors are currently used in small safety and feasibility trials. No clinical trial using AF-MSC has been performed yet. Still, the exact role of true cell repopulation and in situ cardiomyocytes differentiation versus pure paracrine effect after cell transplantation is currently much debated. Cellular cardiomyoplasty is a fascinating new area of investigation in regenerative medicine. Although considerable knowledge has been gained over the last decade on the use of MSC as a potential stem cell (SC) source, many issues remain unsolved. Because of several limitations in animal models, clinical studies in highly selected patients balancing the risks and benefits are required. In that regard, MSCs obtained from the fetal AF are a potential new source of SCs that need to be further investigated for cellular cardiomyoplasty. (c) 2009 Wiley-Liss, Inc.

16

Novel sources of fetal stem cells: where do they fit on the developmental continuum?

Pappa KI, Anagnou NP. Regen Med. 2009 May;4(3):423-33.

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The recent isolation of fetal stem cells from several sources either at the early stages of development or during the later trimesters of gestation, sharing similar growth kinetics and expressing pluripotency markers, provides strong support to the notion that these cells may be biologically closer to embryonic stem cells, actually representing intermediates between embryonic stem cells and adult mesenchymal stem cells, regarding proliferation rates and plasticity features, and thus able to confer an advantage over postnatal mesenchymal stem cells derived from conventional adult sources such as bone marrow. This conclusion has been strengthened by the different pattern of growth potential between the two stage-specific types of sources, as assessed by transcriptomic and proteomic analysis. A series of recent studies regarding the numerous novel features of fetal stem cells has reignited our interest in the field of stem-cell biology and in the possibilities for the eventual repair of damaged organs and the generation of in vitro tissues on biomimetic scaffolds for transplantation. These studies, employing elegant approaches and novel technologies, have provided new insights regarding the nature and the potential of fetal stem cells derived from placenta, amniotic fluid, amnion or umbilical cord. In this update, we highlight the major progression that has occurred in fetal stem-cell biology and discuss the most important areas for future investigation in the field of regenerative medicine.

Expression of mTOR pathway proteins in human amniotic fluid stem cells.

Siegel N, Valli A, Fuchs C, Rosner M, Hengstschläger M. Int J Mol Med. 2009 Jun;23(6):779-84.

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The discovery of human amniotic fluid stem cells initiated a new and promising stem cell research field. These cells harbor a high proliferative capacity and the potential to differentiate into cells of all three embryonic germ layers. The facts that they do not form tumors in vivo and do not raise the ethical concerns associated with human embryonic stem cells support their role as an optimal tool to study the underlying molecular mechanisms of cell differentiation processes and of their deregulation in human genetic diseases. Deregulation of the protein kinase mammalian target of rapamycin (mTOR) pathway is a hallmark of a wide variety of human genetic diseases. Here we report the establishment of an amniotic fluid stem cell line. We analysed the endogenous expression of the mTOR pathway proteins tuberin, mTOR, raptor, rictor, sin1, mLST8, Akt and p70S6K in human amniotic fluid stem cells. In addition, we studied the endogenous activity of the kinase p70S6K, one of the major targets of the mTOR complex 1 kinase, by analysing the p70S6K T389 phosphorylation status. The activity of the Akt kinase, the major mTOR complex 2 target, was studied by analysing its phosphorylation at S473. In addition, the mTOR inhibitor rapamycin was found to affect the phosphorylation status of p70S6K in amniotic fluid stem cells. Taken together, we provide evidence that the mTOR pathway is fully active in human amniotic fluid stem cells. These data demonstrate that amniotic fluid stem cell lines can be used as new tools to study the molecular and cell biological consequences of natural occurring alterations of the mTOR pathway being responsible for a wide variety of different human genetic diseases.

Rare finding of 2n/4n mixoploidy in mother and fetus with severe immune hydrops.

Sharma A, Paliwal P, Dadhwal V, Sharma Y, Deka D. Cytogenet Genome Res. 2009;124(1):90-3.

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The findings of diploid-tetraploid mosaicism in cultured amniotic fluid cells is considered a culture artifact. On rare occasions its presence indicates a chromosomally abnormal fetus with multiple congenital defects. We present here a patient who in her previous pregnancies had delivered one hydrops and two macerated fetuses. The reported pregnancy also resulted in a fetus with severe hydrops. Cytogenetic analysis of cultured amniotic fluid cells revealed diploid-tetraploid mosaicism in the fetus also confirmed in fetal cord blood and skin fibroblasts. Chromosomal analysis of the parents revealed mixoploidy in the mother. These findings are extremely important for prenatal diagnosis and prompt us to not uniformly dismiss tetraploidy as artifactual but to confirm it especially in cases with ultrasound abnormalities.

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The culture and differentiation of amniotic stem cells using a microfluidic system.

Wu HW, Lin XZ, Hwang SM, Lee GB. Biomed Microdevices. 2009 Aug;11(4):869-81.

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Human mesenchymal stem cells (MSCs) have the potential to differentiate into multiple tissue lineages for cell therapy and, therefore, have attracted considerable interest recently. In this study, a new microfluidic system is presented which can culture and differentiate MSCs in situ. It is composed of several components, including stem cell culture areas, micropumps, microgates, seeding reservoirs, waste reservoirs and fluid microchannels; all fabricated by using micro-electro-mechanical-systems (MEMS) technology. The developed automated system allows for the long-term culture and differentiation of MSCs. Three methods, including Oil Red O staining for adipogenic cells, alkaline phosphatase staining and immunofluorescence staining are used to assess the differentiation of MSCs. Experimental results clearly demonstrate that the MSCs can be cultured for proliferation and different types of differentiation are possible in this microfluidic system, which can maintain a suitable and stable pH value over long time periods. This prototype microfluidic system has great potential as a powerful tool for future MSC studies.

Amniotic Fluid Stem Cells Produce Robust Mineral Deposits on Biodegradable Scaffolds.

Peister A, Deutsch ER, Kolambkar Y, Hutmacher DW, Guldberg R. Tissue Eng Part A. 2009 Apr 5.

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Bone regeneration through cell-based therapies is limited by insufficient availability of osteogenic cells. This study investigated the potential of amniotic fluid-derived stem cells (AFS cells) to synthesize mineralized extracellular matrix within porous medical-grade poly-epsilon-caprolactone (mPCL) scaffolds. The AFS cells were initially differentiated in 2D culture to determine appropriate osteogenic culture conditions and verify physiologic mineral production by the AFS cells. The AFS cells were then cultured on 3D mPCL scaffolds (6 mm diameter x 9 mm height) and analyzed for their ability to differentiate to osteoblastic cells in this environment. The amount and distribution of mineralized matrix production was quantified throughout the mPCL scaffold using nondestructive microCT analysis and confirmed through biochemical assays. Sterile microCT scanning provided longitudinal analysis of long-term cultured mPCL constructs to determine the rate and distribution of mineral matrix within the scaffolds. The AFS cells deposited mineralized matrix throughout the mPCL scaffolds and remained viable after 15 weeks of 3D culture. The effect of pre-differentiation of the AFS cells on the subsequent bone formation in vivo was determined in a rat subcutaneous model. Cells that were pre-differentiated for 28 days in vitro produced a 7-fold increase in mineralized matrix when implanted subcutaneously in vivo. This study demonstrated the potential of AFS cells to produce 3D mineralized bioengineered constructs in vitro and in vivo and suggests that AFS cells may be an effective cell source for functional repair of large bone defects.

Amniotic membrane and amniotic fluid-derived cells: potential tools for regenerative medicine?

Parolini O, Soncini M, Evangelista M, Schmidt D. Regen Med. 2009 Mar;4(2):275-91.

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Human amniotic membranes and amniotic fluid have attracted increasing attention in recent years as a possible reserve of stem cells that may be useful for clinical application in regenerative medicine. Many studies have been conducted to date in terms of the differentiation potential of these cells, with several reports demonstrating that cells from both the amniotic fluid and membrane display high plasticity. In addition, cells from the amniotic membrane have also been shown to display immunomodulatory characteristics both in vivo and in vitro, which could make them useful in an allotransplantation setting. Here, we provide an overview comparing the latest findings regarding the stem characteristics of cells from both the amniotic membrane and amniotic fluid, as well as on the potential utility of these cells for future clinical application in regenerative medicine.

Stem cells derived from amniotic fluid: new potentials in regenerative medicine.

Cananzi M, Atala A, De Coppi P. Reprod Biomed Online. 2009;18 Suppl 1:17-27.

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Human amniotic fluid cells have been used as a diagnostic tool for the prenatal diagnosis of fetal genetic anomalies for more than 50 years. Evidence provided in the last 5 years, however, suggests that they can also harbour a therapeutic potential for human diseases, as different populations of fetal-derived stem cells have been isolated from amniotic fluid. Mesenchymal stem cells were the first to be described, which possess the higher proliferation and differentiation plasticity of adult mesenchymal stem cells and are able to differentiate towards mesodermal lineages. Amniotic fluid stem cells have more recently been isolated. They represent a novel class of pluripotent stem cells with intermediate characteristics between embryonic and adult stem cells, as they are able to differentiate into lineages representative of all three germ layers but do not form tumours when injected in vivo. These characteristics, together with the absence of ethical issues concerning their employment, suggest that stem cells present in the amniotic fluid might be promising candidates for tissue engineering and stem cell therapy of several human disorders.

Porcine mesenchymal stem cells--current technological status and future perspective.

Rho GJ, Kumar BM, Balasubramanian SS. Front Biosci. 2009 Jan 1;14:3942-61.

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Similarities of porcine mesenchymal stem/progenitor cells (MSCs) with human counterpart allow them to be considered as a valuable model system for in vitro studies and preclinical assessments. Effective isolation and expansion of porcine MSCs from different origins, namely bone marrow, umbilical cord Wharton's jelly, amniotic fluid, umbilical cord blood and peripheral blood has been reported. The differentiation of porcine MSCs into mesenchymal lineages under in vitro conditions is consistent and growing evidence has also suggested their transdifferentiation abilities. Results of preclinical studies unveil a time dependent retention, engraftment, migration, ex vivo and in vivo differentiation characteristics and possibility for genetic modification of MSCs. Findings on immunogenicity and the immunomodulatory capacity of porcine MSCs are encouraging and valuable to understand the host compatibility following transplantation. Furthermore, suitability of porcine MSCs as donors in nuclear transfer offers a greater potential to medicine and biopharming. Here, we highlight recent findings in the areas of porcine MSC sources, differentiation ability, transplantation applications and their potential as nuclear donors for somatic cell nuclear transfer.

Development of cloned embryos from porcine neural stem cells and amniotic fluid-derived stem cells transfected with enhanced green fluorescence protein gene.

Zheng YM, Zhao HY, Zhao XE, Quan FS, Hua S, He XY, Liu J, He XN, Lin H. Reproduction. 2009 May;137(5):793-801.

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We assessed the developmental ability of embryos cloned from porcine neural stem (NS) cells, amniotic fluid-derived stem (AFS) cells, fetal fibroblast cells, adult fibroblast, and mammary gland epithelial cells. The five cell lines were transfected with enhanced green fluorescence protein gene respectively using lipofection. NS and AFS cells were induced to differentiate in vitro. Stem cells and their differentiated cells were harvested for analysis of the markers using RT-PCR. The five cell lines were used for nuclear transfer. The two-cell stage-cloned embryos derived from each cell line were transferred into the oviducts of surrogate mothers. The results showed that both NS and AFS cells expressed POU5F1, THY1 and SOX2, and they were both induced to differentiate into astrocyte (GFAP+), oligodendrocyte (GalC+), neuron (NF+, ENO2+, and MAP2+), adipocyte (LPL+ and PPARG-D+), osteoblast (osteonectin+ and osteocalcin+), myocyte (MYF6+ and MYOD+), and endothelium (PECAM1+, CD34+, CDH5+, and NOS3+) respectively. Seven cloned fetuses (28 days and 32 days) derived from stem cells were obtained. The in vitro developmental ability (morula-blastocyst rate was 28.26-30.07%) and in vivo developmental ability (pregnancy rate were 1.67-2.17%) of the embryos cloned from stem cells were higher ($P<0.05$) than that of the embryos cloned from somatic cells (morula-blastocyst rate was 16.27-19.28% and pregnancy rate was 0.00%), which suggests that the undifferentiated state of the donor cells increases cloning efficiency.

Human and murine amniotic fluid c-Kit+Lin- cells display hematopoietic activity.

Ditadi A, de Coppi P, Picone O, Gautreau L, Smati R, Six E, Bonhomme D, Ezine S, Frydman R, Cavazzana-Calvo M, André-Schmutz I. Blood. 2009 Sep 10;114(11):2362.

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We have isolated c-Kit(+)Lin(-) cells from both human and murine amniotic fluid (AF) and investigated their hematopoietic potential. In vitro, the c-Kit(+)Lin(-) population in both species displayed a multilineage hematopoietic potential, as demonstrated by the generation of erythroid, myeloid, and lymphoid cells. In vivo, cells belonging to all 3 hematopoietic lineages were found after primary and secondary transplantation of murine c-Kit(+)Lin(-) cells into immunocompromised hosts, thus demonstrating the ability of these cells to self-renew. Gene expression analysis of c-Kit(+) cells isolated from murine AF confirmed these results. The presence of cells with similar characteristics in the surrounding amnion indicates the possible origin of AF c-Kit(+)Lin(-) cells. This is the first report showing that cells isolated from the AF do have hematopoietic potential; our results support the idea that AF may be a new source of stem cells for therapeutic applications.

Isolation of osteogenic progenitors from human amniotic fluid using a single step culture protocol.

Antonucci I, Iezzi I, Morizio E, Mastrangelo F, Pantalone A, Mattioli-Belmonte M, Gigante A, Salini V, Calabrese G, Tetè S, Palka G, Stuppia L. BMC Biotechnol. 2009 Feb 16;9:9.

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BACKGROUND: Stem cells isolated from amniotic fluid are known to be able to differentiate into different cells types, being thus considered as a potential tool for cellular therapy of different human diseases. In the present study, we report a novel single step protocol for the osteoblastic differentiation of human amniotic fluid cells. RESULTS: The described protocol is able to provide osteoblastic cells producing nodules of calcium mineralization within 18 days from withdrawal of amniotic fluid samples. These cells display a complete expression of osteogenic markers (COL1, ONC, OPN, OCN, OPG, BSP, Runx2) within 30 days from withdrawal. In order to test the ability of these cells to proliferate on surfaces commonly used in oral osteointegrated implantology, we carried out cultures onto different test disks, namely smooth copper, machined titanium and Sandblasted and Acid Etching titanium (SLA titanium). Electron microscopy analysis evidenced the best cell growth on this latter surface. CONCLUSION: The described protocol provides an efficient and time-saving tool for the production of osteogenic cells from amniotic fluid that in the future could be used in oral osteointegrated implantology.

The biological characteristics of human third trimester amniotic fluid stem cells.

You Q, Tong X, Guan Y, Zhang D, Huang M, Zhang Y, Zheng J. J Int Med Res. 2009 Jan-Feb;37(1):105-12.

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Third trimester amniotic fluid (AF)-derived human mesenchymal stem cells (MSCs) can be greatly expanded in vitro and induced to differentiate into multiple mesenchymal cell types. This study aimed to investigate the biological characteristics of MSCs from third trimester AF as a new source of therapeutic stem cells. Forty third trimester AF samples were obtained from healthy women who underwent elective caesarean section for breech presentation. A simple culture protocol for MSCs was used. A cell growth curve was drawn, and cell surface antigens and cytokines were analysed by immunofluorescent staining, reverse transcription-polymerase chain reaction and flow cytometry. MSCs from third-trimester AF were successfully isolated, cultured and enriched. MSCs expanded extensively without feeders, they were not tumourigenic and were induced to differentiate into osteocytes. Surface antigens were analysed and found to express the pluripotency marker Oct-4. Considering the great feasibility of biomedical engineering using MSCs, third trimester AF may provide a rich source for investigation of human MSCs.

Bidirectional communication between neural and cardiac cells in human amniotic fluid.

Stefanidis K, Loutradis D, Anastasiadou V, Dinopoulou V, Eleni E, Arampatzi E, Lekka K, Mesogitis S, Antsaklis A. Fetal Diagn Ther. 2009;25(1):62-6.

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OBJECTIVE: To evaluate whether amniotic fluid cells contain cardiomyocyte- and neuron-like cells. STUDY DESIGN: In this experimental study, cells from human amniotic fluid samples were analyzed for mRNA expression of microtubule-associated protein 2 (MAP-2), vimentin and oxytocin (OT) receptor via RT-PCR. Immunocytochemistry was also performed with MAP-2, OT receptor, vimentin and troponin I antibodies. RESULTS: In all samples, MAP-2, vimentin and mRNA expression were detectable. OT receptor was also detectable. The cells showed strong immunoreactivity for molecular markers of neurogenic cells including MAP-2 and vimentin. The cells also showed strong immunoreactivity for molecular markers of cardiac muscle such as troponin I and for OT receptors. This report also shows that atosiban (an OT antagonist) added to culture medium of amniotic fluid cells did not induce neurogenic and cardiomyogenic differentiation. CONCLUSIONS: The observed concurrent development of cardiomyocyte- and neuron-like cells suggests that amniotic fluid contains progenitor cells and there is bidirectional communication between both cell types. (c) 2009 S. Karger AG, Basel.

Human amniotic fluid mesenchymal stem cells in combination with hyperbaric oxygen augment peripheral nerve regeneration.

Pan HC, Chin CS, Yang DY, Ho SP, Chen CJ, Hwang SM, Chang MH, Cheng FC. Neurochem Res. 2009 Jul;34(7):1304-16.

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PURPOSE: Attenuation of pro-inflammatory cytokines and associated inflammatory cell deposits rescues human amniotic fluid mesenchymal stem cells (AFS) from apoptosis. Hyperbaric oxygen (HBO) suppressed stimulus-induced pro-inflammatory cytokine production in blood-derived monocyte-macrophages. Herein, we evaluate the beneficial effect of hyperbaric oxygen on transplanted AFS in a sciatic nerve injury model. METHODS: Peripheral nerve injury was produced in Sprague-Dawley rats by crushing the left sciatic nerve using a vessel clamp. The AFS were embedded in fibrin glue and delivered to the injured site. Hyperbaric oxygen (100% oxygen, 2 ATA, 60 min/day) was administered 12 h after operation for seven consecutive days. Transplanted cell apoptosis, oxidative stress, inflammatory cell deposits and associated chemokines, pro-inflammatory cytokines, motor function, and nerve regeneration were evaluated 7 and 28 days after injury. RESULTS: Crush injury induced an inflammatory response, disrupted nerve integrity, and impaired nerve function in the sciatic nerve. However, crush injury-provoked inflammatory cytokines, deposits of inflammatory cytokines, and associated macrophage migration chemokines were attenuated in groups receiving hyperbaric oxygen but not in the AFS-only group. No significant increase in oxidative stress was observed after administration of HBO. In transplanted AFS, marked apoptosis was detected and this event was reduced by HBO treatment. Increased nerve myelination and improved motor function were observed in AFS-transplant, HBO-administrated, and AFS/HBO-combined treatment groups. Significantly, the AFS/HBO combined treatment showed the most beneficial effect. CONCLUSION: AFS in combination with HBO augment peripheral nerve regeneration, which may involve the suppression of apoptotic death in implanted AFS and the attenuation of an inflammatory response detrimental to peripheral nerve regeneration.

Stem cell sources to treat diabetes.

Furth ME, Atala A. J Cell Biochem. 2009 Mar 1;106(4):507-11.

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We review progress towards the goal of utilizing stem cells as a source of engineered pancreatic beta-cells for therapy of diabetes. Protocols for the in vitro differentiation of embryonic stem (ES) cells based on normal developmental cues have generated beta-like cells that produce high levels of insulin, albeit at low efficiency and without full responsiveness to extracellular levels of glucose. Induced pluripotent stem (iPS) cells also can yield insulin-producing cells following similar approaches. An important recent report shows that when transplanted into mice, human ES-derived cells with a phenotype corresponding to pancreatic endoderm matured to yield cells capable of maintaining near-normal regulation of blood sugar [Kroon et al., 2008]. Major hurdles that must be overcome to enable the broad clinical translation of these advances include teratoma formation by ES and iPS cells, and the need for immunosuppressive drugs. Classes of stem cells that can be expanded extensively in culture but do not form teratomas, such as amniotic fluid-derived stem cells and hepatic stem cells, offer possible alternatives for the production of beta-like cells, but further evidence is required to document this potential. Generation of autologous iPS cells should prevent transplant rejection, but may prove prohibitively expensive. Banking strategies to identify small numbers of stem cell lines homozygous for major histocompatibility loci have been proposed to enable beneficial genetic matching that would decrease the need for immunosuppression.

Hyperthermia-treated mesenchymal stem cells exert antitumor effects on human carcinoma cell line.

Cho JA, Park H, Kim HK, Lim EH, Seo SW, Choi JS, Lee KW. Cancer. 2009 Jan 15;115(2):311-23.

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BACKGROUND: Mesenchymal stem cells (MSCs) possess the potential for differentiation into multilineages. MSCs have been reported to play a role as precursors for tumor stroma in providing a favorable environment for tumor progression. Hyperthermia destroys cancer cells by raising the temperature of tumor-loaded tissue to 40 degrees C to 43 degrees C and causes indirect sensitizing effects when combined with chemo- and/or radiotherapy. However, how hyperthermia affects the tumor-supportive stroma is unknown. Here, the authors investigated the effects of hyperthermia-treated MSCs, from different sources, on the human ovarian cancer cell line SK-OV-3. METHODS: MSCs from adipose tissue and amniotic fluid were untreated or heat-treated (HS-MSCs). The culture supernatant of each treatment group was collected and transferred to the SK-OV-3 cells. RESULTS: The morphological analysis and cell proliferation assay showed a reduced viability of the tumor cells in the conditioned medium with the HS-MSCs. Further investigations revealed that the conditioned medium of the HS-MSCs induced a higher nuclear condensation and a greater number of sub-G1 cells among the tumor cells. Analysis of the mRNA expression demonstrated that the conditioned medium of the HS-MSCs induced up-regulation or down-regulation of several tumor-associated molecules. Finally, the cytokine array of each conditioned medium showed that angiogenin, insulin-like growth factor binding protein 4, neurotrophin 3, and chemokine (C-C motif) ligand 18 are involved as main factors. CONCLUSIONS: This study showed that the conditioned medium of the HS-MSCs exerted a suppressive effect on tumor progression and malignancy, suggesting that hyperthermia enables tumor stromal cells to provide a sensitizing environment for tumor cells to undergo cell death.

Fetal mesenchymal stem cells: isolation, properties and potential use in perinatology and regenerative medicine.

Gucciardo L, Lories R, Ochsenbein-Kölble N, Done' E, Zwijsen A, Deprest J. BJOG. 2009 Jan;116(2):166-72.

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The fetus is a source of nonembryonic stem cells (SC), with potential applications in perinatal medicine. Cells derived from the placenta, membranes, amniotic fluid or fetal tissues are higher in number, expansion potential and differentiation abilities compared with SC from adult tissues. Although some obstacles keep SC biology at distance from clinical application, the feasibility of using (homologous) SC for tissue engineering for the fetus with a congenital birth defect has been demonstrated. Also, other pathologies may benefit from SC technology.

Baculovirus-transduced mouse amniotic fluid-derived stem cells maintain differentiation potential.

Liu ZS, Xu YF, Feng SW, Li Y, Yao XL, Lu XL, Zhang C. Ann Hematol. 2009 Jun;88(6):565-72.

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Amniotic fluid-derived stem cells have attracted considerable attention in the field of regenerative medicine. Approach of genetic modification probably enhances their regenerative potential. In this work, we wanted to determine whether baculovirus as a new gene vector could efficiently and safely transduce mouse amniotic fluid-derived stem cells (mAFSs). Cells were isolated from mouse amniotic fluid and cultured in vitro. These cells were analyzed by examining phenotypes and differentiation potential. They were further transduced with baculovirus. Baculovirus-transduced mAFSs were induced to differentiate into adipogenic, osteogenic, myogenic, and neurogenic lineages. Mouse amniotic fluid-derived stem cells were successfully isolated and cultured in vitro. They were positive for CD29 and Sca-1, but negative for CD34, CD45, or CD11b. Furthermore, they could differentiate into adipocytes, osteocytes, myocytes, and neurocytes in vitro. Baculovirus could efficiently transduced mAFSs. More importantly, baculovirus-transduced mAFSs retained differentiation potential. Thus, baculovirus vector effective and safe transduction is an attractive promise for genetic modification of mAFSs. Baculovirus genetically modified mAFSs will probably be more suitable as vehicles for regenerative medicine.

Human amniotic fluid stem cells do not differentiate into dopamine neurons in vitro or after transplantation in vivo.

Donaldson AE, Cai J, Yang M, Iacovitti L. Stem Cells Dev. 2009 Sep;18(7):1003-12.

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Although embryonic stem (ES) cells can generate dopamine (DA) neurons that are potentially useful as a cell replacement therapy in Parkinson's disease (PD), associated ethical and practical concerns remain major stumbling blocks to their eventual use in humans. In this study, we examined human amniotic fluid stem (hAFS) cells derived from routine amniocenteses for their potential to give rise to DA neurons in vitro and following transplantation into the 6-hydroxydopamine-lesioned rat brain. We show that undifferentiated hAFS cells constitutively expressed mRNAs and proteins typical of stem cells but also cell derivatives of all three germ layers, including neural progenitors/neurons (nestin, beta-tubulin III, neurofilament). Additionally, these cells expressed mRNAs of an immature DA phenotype (Lmx1a, Pitx-3, Nurr1, Aldh1a1) but not the corresponding proteins. Importantly, treatment with DA differentiation factors using a variety of protocols did not further promote the development of fully differentiated DA neurons from hAFS cells. Thus, Lmx1a, Aldh1a1, AADC, TH, and DAT proteins were not detected in hAFS cells in culture or after transplantation into the PD rat brain. Moreover, by 3 weeks after implantation, there were no surviving AFS cells in the graft, likely as a result of an acute immunorejection response, as evidenced by the abundant presence of CD11+ macrophage/microglia and reactive GFAP+ astrocytes in the host brain. Taken together, these results suggest that further studies will be needed to improve differentiation procedures in culture and to prolong cell survival in vivo if hAFS cells are to be useful as replacement cells in PD.

Prospective full-term-derived pluripotent amniotic fluid stem (AFS) cells.

Ferdaos N, Nathan S, Nordin N. Med J Malaysia. 2008 Jul;63 Suppl A:75-6.

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Amniotic fluid (AF) serves as an excellent alternative source of pluripotent stem cells, as they are not bound with ethical issues and the stem cells are more primitive than adult stem (AS) cells. Hence, they have higher potential. Here we aim to isolate and characterize pluripotent stem cells from mid-term and full-term pregnant rat amniotic fluid. The results demonstrate the evidence of heterogeneous population of cells in the amniotic fluid and some of the cells morphology shows similarity with ES cells.

Differential immunomodulatory effects of fetal versus maternal multipotent stromal cells.

Roelen DL, van der Mast BJ, in't Anker PS, Kleijburg C, Eikmans M, van Beelen E, de Groot-Swings GM, Fibbe WE, Kanhai HH, Scherjon SA, Claas FH. Hum Immunol. 2009 Jan;70(1):16-23.

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Protective mechanisms are likely to be present at the fetomaternal interface because fetus-specific alloreactive T cells present in the decidua do not harm the fetus. We tested the immunosuppressive capacity of maternal and fetal multipotent stromal cells (MSC). Single cell suspensions were made from second-trimester amnion, amniotic fluid, and decidua. Culture-expanded cells were identified as MSC based on phenotype and multilineage potential. Coculture of MSC in a primary mixed lymphocyte culture of unrelated responder-stimulator combinations resulted in a dose-dependent inhibition of proliferation. Fetal MSC demonstrated a significantly higher inhibition compared with maternal MSC. This stronger inhibition by fetal MSC was even more prominent in a secondary mixed lymphocyte reaction (MLR) with primed alloreactive T cells. Analysis of cytokine production revealed that fetal MSC produced significantly more interleukin (IL)-10 and vascular endothelial growth factor than maternal MSC. Cell-cell contact is needed for part of the inhibitory effects of MSC. In addition, soluble factors play a role because blocking experiments with anti-IL-10 revealed that the inhibition of the MLR response by fetal MSC is mainly mediated by IL-10. For maternal MSC, other soluble factors seem to be involved. Fetal MSC derived from the fetomaternal interface have a stronger inhibitory effect on naive and antigen-experienced T cells compared with maternal MSC, which is probably related to their higher IL-10 production.

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Stem cells in urology.

Aboushwareb T, Atala A. Nat Clin Pract Urol. 2008 Nov;5(11):621-31.

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The shortage of donors for organ transplantation has stimulated research on stem cells as a potential resource for cell-based therapy in all human tissues. Stem cells have been used for regenerative medicine applications in many organ systems, including the genitourinary system. The potential applications for stem cell therapy have, however, been restricted by the ethical issues associated with embryonic stem cell research. Instead, scientists have explored other cell sources, including progenitor and stem cells derived from adult tissues and stem cells derived from the amniotic fluid and placenta. In addition, novel techniques for generating stem cells in the laboratory are being developed. These techniques include somatic cell nuclear transfer, in which the nucleus of an adult somatic cell is placed into an oocyte, and reprogramming of adult cells to induce stem-cell-like behavior. Such techniques are now being used in tissue engineering applications, and some of the most successful experiments have been in the field of urology. Techniques to regenerate bladder tissue have reached the clinic, and exciting progress is being made in other areas, such as regeneration of the kidney and urethra. Cell therapy as a treatment for incontinence and infertility might soon become a reality. Physicians should be optimistic that regenerative medicine and tissue engineering will one day provide mainstream treatment options for urologic disorders.

Neuronal characteristics of amniotic fluid derived cells after adenoviral transformation.

Arnhold S, Post C, Glüer S, Hoopmann M, Wenisch S, Volpers C, Addicks K. Cell Biol Int. 2008 Dec;32(12):1559-66.

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Efficient transformation of primary human amniocytes by E1 gene functions of human adenovirus serotype 5 (Ad5) yield in stable cell lines, which exhibit morphological features of epithelial like cells. A thorough investigation using immunocytochemistry confirmed the expression of epithelial cell markers. The analysis also revealed the expression of neuronal and glial marker proteins, such as nestin, vimentin, A2B5 and GFAP. Using RT-PCR, transcripts of the neurotrophic factors nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), glial cell line derived neurotrophic factor (GDNF), and neurotrophin 3 (NT-3) could be detected. Neurotrophic factors could also be detected in the cell culture supernatants of transformed amniocytes. In line with previous experimental data on a human Ad5 E1-transformed embryonal kidney cell line (HEK-293), the results suggest a co-expression of epithelial and neuronal marker proteins in E1-transformed human amniotic fluid derived cells and thus a preferential transformation into neuronal-like cells.

Potential role of culture mediums for successful isolation and neuronal differentiation of amniotic fluid stem cells.

Orciani M, Emanuelli M, Martino C, Pugnaloni A, Tranquilli AL, Di Primio R. Int J Immunopathol Pharmacol. 2008 Jul-Sep;21(3):595-602.

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In recent years, the use of stem cells has generated increasing interest in regenerative medicine and cancer therapies. The most potent stem cells derive from the inner cell mass during embryonic development and their use yields serious ethical and methodological problems. Recently, a number of reports suggests that another suitable source of multipotent stem cells may be the amniotic fluid. Amniotic fluid mesenchymal stem cells (AFMSCs) are capable of extensive self-renewal, able to differentiate in specialized cells representative of all three germ layers, do not show ethical restriction, and display minimal risks of teratomas and a very low immunogenity. For all these reasons, amniotic fluid appears as a promising alternative source for stem cell therapy. Their recent discovery implies a lack of knowledge of their specific features as well as the existence of a protocol universally recognized as the most suitable for their isolation, growth and long-term conservation. In this study, we isolated stem cells from six amniotic fluids; these cells were cultured with three different culture mediums (Mesenchymal Stem Cell Medium (MSCGM), PC-1 and RPMI-1640), characterized by cytofluorimetric analysis, and then either frozen or induced to neuronal differentiation. Even if the immunophenotype seemed not to be influenced by culture medium (all six samples cultured in the above-mentioned mediums expressed surface antigens commonly found on stem cells), cells showed different abilities to differentiate into neuron-like cells and to re-start the culture after short/long-term storage. Cells isolated and cultured in MSCGM showed the highest proliferation rate, and formed neuron-like cells when sub-plated with neuronal differentiation medium. Cells from PC-1, on the contrary, displayed an increased ability to re-start culture after short/long term storage. Finally, cells from RPMI-1640, even if expressing stem cells markers, were not able to differentiate in neuron-like cells. Further studies are still needed in order to assess the effective role of culture medium for a successful isolation, growth, differentiation and storage of AFMSCs, but our data underline the importance of finding a universally accepted protocol for the use of these cells.

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Different cardiovascular potential of adult- and fetal-type mesenchymal stem cells in a rat model of heart cryoinjury.

Iop L, Chiavegato A, Callegari A, Bollini S, Piccoli M, Pozzobon M, Rossi CA, Calamelli S, Chiavegato D, Gerosa G, De Coppi P, Sartore S. Cell Transplant. 2008;17(6):679-94.

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Efficacy of adult (bone marrow, BM) versus fetal (amniotic fluid, AF) mesenchymal stem cells (MSCs) to replenish damaged rat heart tissues with new cardiovascular cells has not yet been established. We investigated on the differentiation potential of these two rat MSC populations in vitro and in a model of acute necrotizing injury (ANI) induced by cryoinjury. Isolated BM-MSCs and AF-MSCs were characterized by flow cytometry and cytocentrifugation and their potential for osteogenic, adipogenic, and cardiovascular differentiation assayed in vitro using specific induction media. The left anterior ventricular wall of syngeneic Fisher 344 (n = 48) and athymic nude (rNu) rats (n = 6) was subjected to a limited, nontransmural epicardial ANI in the approximately one third of wall thickness without significant hemodynamic effects. The time window for in situ stem cell transplantation was established at day 7 postinjury. Fluorochrome (CMTMR)-labeled BM-MSCs (2 x 10(6)) or AF-MSCs (2 x 10(6)) were injected in syngeneic animals (n = 26) around the myocardial lesion via echocardiographic guidance. Reliability of CMTMR cell tracking in this context was ascertained by transplanting genetically labeled BM-MSCs or AF-MSCs, expressing the green fluorescent protein (GFP), in rNu rats with ANI. Comparison between the two methods of cell tracking 30 days after cell transplantation gave slightly different values (1420,58 +/- 129,65 cells/mm2 for CMTMR labeling and 1613.18 +/- 643.84 cells/mm2 for genetic labeling; p = NS). One day after transplantation about one half CMTMR-labeled AF-MSCs engrafted to the injured heart (778.61 +/- 156.28 cells/mm2) in comparison with BM-MSCs (1434.50 +/- 173.80 cells/mm2, p < 0.01). Conversely, 30 days after cell transplantation survived MSCs were similar: 1275.26 +/- 74.51/mm2 (AF-MSCs) versus 1420.58 +/- 129.65/mm2 for BM-MSCs (p = NS). Apparent survival gain of AF-MSCs between the two time periods was motivated by the cell proliferation rate calculated at day 30, which was lower for BM-MSCs (6.79 +/- 0.48) than AF-MSCs (10.83 +/- 3.50; p < 0.01), in the face of a similar apoptotic index (4.68 +/- 0.20 for BM-MSCs and 4.16 +/- 0.58 for AF-MSCs; p = NS). These cells were also studied for their expression of markers specific for endothelial cells (ECs), smooth muscle cells (SMCs), and cardiomyocytes (CMs) using von Willebrand factor (vWf), smooth muscle (SM) alpha-actin, and cardiac troponin T, respectively. Grafted BM-MSCs or AF-MSCs were found as single cell/small cell clusters or incorporated in the wall of microvessels. A larger number of ECs (227.27 +/- 18.91 vs. 150.36 +/- 24.08 cells/mm2, p < 0.01) and CMs (417.91 +/- 100.95 vs. 237.43 +/- 79.99 cells/mm2, p < 0.01) originated from AF-MSCs than from BM-MSCs. Almost no SMCs were seen with AF-MSCs, in comparison to BM-MSCs (98.03 +/- 40.84 cells/mm2), in concordance with lacking of arterioles, which, instead, were well expressed with BM-MSCs (71.30 +/- 55.66 blood vessels/mm2). The number of structurally organized capillaries was slightly different with the two MSCs (122.49 +/- 17.37/mm2 for AF-MSCs vs. 148.69 +/- 54.41/mm2 for BM-MSCs; p = NS). Collectively, these results suggest that, in the presence of the same postinjury microenvironment, the two MSC populations from different sources are able to activate distinct differentiation programs that potentially can bring about a myocardial-capillary or myocardial-capillary-arteriole reconstitution.

Isolation of mesenchymal stem cells from amniotic fluid and placenta.

Steigman SA, Fauza DO. Curr Protoc Stem Cell Biol. 2007 Jun;Chapter 1:Unit 1E.2.

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Diverse progenitor cell populations, including mesenchymal, hematopoietic, trophoblastic, and possibly more primitive stem cells can be isolated from the amniotic fluid and the placenta. At least some of the amniotic and placental cells share a common origin, namely the inner cell mass of the morula. Indeed, most types of progenitor cells that can be isolated from these two sources share many characteristics. This unit will focus solely on the mesenchymal stem cells, the most abundant progenitor cell population found therein and, unlike some of the other stem cell types, present all through gestation. Protocols for isolation, expansion, freezing, and thawing of these cells are presented. Preference is given to the simplest methods available for any given procedure.

Characterization and hepatogenic differentiation of mesenchymal stem cells from human amniotic fluid and human bone marrow: a comparative study.

Zheng YB, Gao ZL, Xie C, Zhu HP, Peng L, Chen JH, Chong YT. Cell Biol Int. 2008 Nov;32(11):1439-48.

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Since stem cells can differentiate into hepatocyte, stem cell-based therapy becomes a potential alternative treatment for terminal liver diseases. However, an appropriate source of human mesenchymal stem cells (hMSCs) for hepatocytes has not yet been clearly elucidated. The aim of the present study was to investigate the in vitro biological characterization and hepatic differentiation potential of human amniotic fluid-derived mesenchymal stem cells (AF-hMSCs) and human bone marrow-derived mesenchymal stem cells (BM-hMSCs). Our results show that AF-hMSCs possess higher proliferation and self-renewal capacity than BM-hMSCs. Cytogenetic studies indicate that AF-hMSCs are as genetically stabile as BM-hMSCs. Following incubation with specific hepatogenic agents, AF-hMSCs showed a higher hepatic differentiation potential than BM-hMSCs. Expression of several liver-specific markers was significantly greater in AF-hMSCs than in BM-hMSCs, as shown by real time RT-PCR and immunofluorescence (IF). In conclusion, AF-hMSCs possess superior potential for hepatic differentiation, making them more suitable for diverse terminal liver diseases.

New paths to pluripotent stem cells.

Tweedell KS. Curr Stem Cell Res Ther. 2008 Sep;3(3):151-62.

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Stem cells obtained from early mammalian embryos and the subsequent establishment of self replicating embryonic stem cell lines (ES) provided a legacy resource of pluripotent cells capable of differentiating into specific cell lineages of the adult organism. Still the most versatile source of pluripotent cells, their application to potential human therapeutic use has been encumbered by various technical and ethical objections. New sources of embryonic pluripotent stem cells have been sought, the isolation of ES cell lines from a single blastomere that avoids destruction of the human embryo, the use of arrested embryos no longer capable of completing development or using post-implantation embryos as stem cell providers. The successful cloning and reprogramming of adult animal cell nuclei by somatic cell nuclear transplantation (SCNT) or nuclear transfer (NT) provides stem cells tailored to the donor organism, though a step away for human use. Variations in this procedure are altered SCNT, that would block human use for reproduction and the use of parthenotes to induce pluripotent stem cell lines. All of these NT methods depend upon a very limited supply of healthy oocyte host cells. Enucleated fertilized eggs have been substituted for oocytes and the production of stem cell somatic cell hybrids by cell fusion have potential use for nuclear transfer ES cells not directly dependent on oocytes. Recovery of cells from human amniotic fluid has yielded stem cells that share some pluripotent characteristics but are multipotent stem cells. Adult somatic cells have been reprogrammed recently by retroviral transduction using four transcription factors to induce pluripotent stem cells (iPS) with great promise. Each of these procedures has limitations at present for extensive use in human regenerative medicine.

Isolation of human mesenchymal stem cells from third-trimester amniotic fluid.

You Q, Cai L, Zheng J, Tong X, Zhang D, Zhang Y. Int J Gynaecol Obstet. 2008 Nov;103(2):149-52.

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OBJECTIVE: To determine whether mesenchymal stem cells (MSCs) can be isolated in third-trimester amniotic fluid (AF) and their differentiation induced. METHOD: Sufficient numbers of MSCs were isolated from the AF of 15 healthy women undergoing cesarean delivery in the third trimester to be cultured and induced to differentiate into osteocytes. RESULTS: Reverse-transcriptase polymerase chain reaction showed the MSCs to express the pluripotency marker gene OCT4, and flow cytometry showed these cells to be positive for CD29, CD73, CD90, and CD105 and negative for CD31, CD45, and CD61. The MSCs were also determined to be nontumorigenic. CONCLUSION: Multipotent MSCs can be obtained from AF in the third trimester, which may be less dangerous than the second trimester to women and their fetuses.

Cryopreserved amniotic fluid-derived cells: a lifelong autologous fetal stem cell source for heart valve tissue engineering.

Schmidt D, Achermann J, Odermatt B, Genoni M, Zund G, Hoerstrup SP. J Heart Valve Dis. 2008 Jul;17(4):446-55; discussion 455.

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BACKGROUND AND AIM OF THE STUDY: Fetal stem cells represent a promising cell source for heart valve tissue engineering. In particular, amniotic fluid-derived cells (AFDC) have been shown to lead to autologous fetal-like heart valve tissues in vitro for pediatric application. In order to expand the versatility of these cells also for adult application, cryopreserved AFDC were investigated as a potential life-long available cell source for heart valve tissue engineering. METHODS: Human AFDC were isolated using CD133 magnetic beads, and then differentiated and analyzed. After expansion of CD133- as well as CD133+ cells up to passage 7, a part of the cells was cryopreserved. After four months, the cells were re-cultured and phenotyped by flow cytometry and immunohistochemistry, including expression of CD44, CD105, CD90, CD34, CD31, CD141, eNOS and vWF, and compared to their non-cryopreserved counterparts. The stem cell potential was investigated in differentiation assays. The viability of cryopreserved AFDC for heart valve tissue engineering was assessed by creating heart valve leaflets in vitro. RESULTS: After cryopreservation, amniotic fluid-derived CD133- and CD133+ cells retained their stem cell-like phenotype, expressing mainly CD44, CD90 and CD105. This staining pattern was comparable to that of their non-cryopreserved counterparts. Moreover, CD133- cells demonstrated differentiation potential into osteoblast-like and adipocyte-like cells. CD133+ cells showed characteristics of endothelial-like cells by eNOS, CD141 and beginning vWF expression. When used for the fabrication of heart valve leaflets, cryopreserved CD133- cells produced extracellular matrix elements comparable to their non-cryopreserved counterparts. Moreover, the resulting tissues showed a cellular layered tissue formation covered by functional endothelia. The mechanical properties were similar to those of tissues fabricated from non-cryopreserved cells. CONCLUSION: The study results suggest that the use of cell bank technology fetal amniotic fluid-derived stem cells might represent a life-long available autologous cell source for heart valve tissue engineering, and also for adult application.

Human amniotic fluid stem cells can integrate and differentiate into epithelial lung lineages.

Carraro G, Perin L, Sedrakyan S, Giuliani S, Tiozzo C, Lee J, Turcatel G, De Langhe SP, Driscoll B, Bellusci S, Minoo P, Atala A, De Filippo RE, Warburton D. Stem Cells. 2008 Nov;26(11):2902-11.

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A new source of stem cells has recently been isolated from amniotic fluid; these amniotic fluid stem cells have significant potential for regenerative medicine. These cells are multipotent, showing the ability to differentiate into cell types from each embryonic germ layer. We investigated the ability of human amniotic fluid stem cells (hAFSC) to integrate into murine lung and to differentiate into pulmonary lineages after injury. Using microinjection into cultured mouse embryonic lungs, hAFSC can integrate into the epithelium and express the early human differentiation marker thyroid transcription factor 1 (TTF1). In adult nude mice, following hyperoxia injury, tail vein-injected hAFSC localized in the distal lung and expressed both TTF1 and the type II pneumocyte marker surfactant protein C. Specific damage of Clara cells through naphthalene injury produced integration and differentiation of hAFSC at the bronchioalveolar and bronchial positions with expression of the specific Clara cell 10-kDa protein. These results illustrate the plasticity of hAFSC to respond in different ways to different types of lung damage by expressing specific alveolar versus bronchiolar epithelial cell lineage markers, depending on the type of injury to recipient lung. Disclosure of potential conflicts of interest is found at the end of this article.

Combination of G-CSF administration and human amniotic fluid mesenchymal stem cell transplantation promotes peripheral nerve regeneration.

Pan HC, Chen CJ, Cheng FC, Ho SP, Liu MJ, Hwang SM, Chang MH, Wang YC. Neurochem Res. 2009 Mar;34(3):518-27.

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Amniotic fluid mesenchymal stem cells (AFS) harbor the potential to improve peripheral nerve injury by inherited neurotrophic factor secretion, but present the drawback of the short-term survival after transplantation. Granulocyte-colony stimulating factor (G-CSF) has a diversity of functions, including anti-inflammatory and anti-apoptotic effects. This study was conducted to evaluate whether G-CSF could augment the neuroprotective effect of transplanted AFS against peripheral nerve injury. The potential involvement of anti-inflammation/anti-apoptosis effect was also investigated. Peripheral nerve injury was produced in Sprauge-Dawley rats by crushing left sciatic nerve using a vessel clamp. The AFS were embedded in fibrin glue and delivered to the injured site. G-CSF (50 microg/kg) was administrated by intra-peritoneal injection for 7 consecutive days. Cell apoptosis, inflammatory cytokines, motor function, and nerve regeneration were evaluated 7 or 28 days after injury. Crush injury induced inflammatory response, disrupted nerve integrity, and impaired nerve function in sciatic nerve. Crush injury-provoked inflammation was attenuated in groups receiving G-CSF but not in AFS only group. In transplanted AFS, marked apoptosis was detected and this event was reduced by G-CSF treatment. Increased nerve myelination and improved motor function were observed in AFS transplanted, G-CSF administrated, and AFS/G-CSF combined treatment groups. Significantly, the combined treatment showed the most beneficial effect. In conclusion, the concomitant treatment of AFS with G-CSF augments peripheral nerve regeneration which may involve the suppression of apoptotic death in implanted AFS and the attenuation of inflammatory response.

Stem/progenitor cells in lung development, injury repair, and regeneration.

Warburton D, Perin L, Defilippo R, Bellusci S, Shi W, Driscoll B. Proc Am Thorac Soc. 2008 Aug 15;5(6):703-6.

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At least two populations of epithelial stem/progenitor cells give rise to the lung anlage, comprising the laryngo-tracheal complex versus the distal lung below the first bronchial bifurcation. Amplification of the distal population requires FGF9-FGF10-FGFR2b-Sprouty signaling. Residual pools of adult stem cells are hypothesized to be the source of lung regeneration and repair. These pools have been located within the basal layer of the upper airways, within or near pulmonary neuroendocrine cell rests, at the bronchoalveolar junction as well as within the alveolar epithelial surface. Rapid repair of the denuded alveolar surface after injury is clearly key to survival. Strategies to enhance endogenous alveolar epithelial repair could include protection of epithelial progenitors from injury and/or stimulation of endogenous progenitor cell function. Protection with inosine or FGF signaling are possible small molecule therapeutic options. Alternatively, exogenous stem/progenitor cells can be delivered into the lung either intravenously, intratracheally, or by direct injection. Sources of exogenous stem/progenitor cells that are currently under evaluation in the context of acute lung injury repair include embryonic stem cells, bone marrow- or fat-derived mesenchymal stem cells, circulating endothelial progenitors, and, recently, amniotic fluid stem/progenitor cells. Further work will be needed to translate stem/progenitor cell therapy for the lung.

Oxytocin receptor- and Oct-4-expressing cells in human amniotic fluid.

Stefanidis K, Loutradis D, Anastasiadou V, Bletsa R, Kiapekou E, Drakakis P, Beretsos P, Elenis E, Mesogitis S, Antsaklis A. Gynecol Endocrinol. 2008 May;24(5):280-4.

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BACKGROUND/AIMS: The present clinical and molecular study aimed at investigating the presence of the genes encoding oxytocin receptor (OT-R) and Oct-4 in human amniotic fluid cells. METHODS: Amniotic fluid samples were obtained from amniocentesis. Cells from human amniotic fluid samples were analyzed for mRNA expression of OT-R and Oct-4 via reverse transcription-polymerase chain reaction (RT-PCR). Immunocytochemistry was also performed with OT-R and Oct-4 antibodies. RESULTS: RT-PCR from 10 independent amniocentesis samples demonstrated the expression of OT-R and Oct-4 mRNA. The cells also showed strong immunoreactivity for molecular markers of OT-R and Oct-4. CONCLUSION: OT-R and Oct-4 are expressed in human amniotic fluid cells. The role of oxytocin in the physiology and pathophysiology of amniotic fluid cells remains to be settled.

High transduction efficiency of human amniotic fluid stem cells mediated by adenovirus vectors.

Grisafi D, Piccoli M, Pozzobon M, Ditadi A, Zaramella P, Chiandetti L, Zanon GF, Atala A, Zacchello F, Scarpa M, De Coppi P, Tomanin R. Stem Cells Dev. 2008 Oct;17(5):953-62.

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In the last few years some studies have shown the possibility of deriving progenitors with various potential from the amniotic fluid. Amniocentesis is a widely accepted method for prenatal diagnosis; it is associated with low risk both for the mother and the fetus and overcomes the ethical problems commonly associated to other sources. Recently we have described that amniotic fluid stem (AFS) cells, for their ability to differentiate to various lineages, could represent a good candidate for therapeutic applications. For gene therapy purposes human AFS (hAFS) cells should be genetically modified with a therapeutic gene and delivered systematically or injected directly into the tissue of interest. The aim of this study was to investigate the feasibility of transducing hAFS cells with adenoviral vectors and to determine whether transduced stem cells retain the ability to differentiate into different lineages. Herein, we showed that hAFS cells could be efficiently infected by first generation adenovirus vectors. In addition, we demonstrated that infection and expression of two different marker genes, LacZ and EGFP, have no effect on cells phenotype and differentiation potential. In particular, on undifferentiated status, hAFS cells continued to express both the transgenes and stemness cell markers OCT4 and SSEA4. When cultured under mesenchymal conditions, infected cells could still differentiate into osteocytes and adipocytes expressing lineage specific genes. These preliminary findings suggest that adenovirus may be useful to engineer populations of pluripotent stem cells, which may be used in a wide range of gene therapy treatments.

Preclinical regulatory validation of a 3-stage amniotic mesenchymal stem cell manufacturing protocol.

Steigman SA, Armant M, Bayer-Zwirello L, Kao GS, Silberstein L, Ritz J, Fauza DO. J Pediatr Surg. 2008 Jun;43(6):1164-9.

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PURPOSE: Because of the 4 to 6-month interval between a diagnostic amniocentesis and birth, clinical application of amniotic mesenchymal stem cell (AMSC)-based therapies demands a 3-stage cell manufacturing process, including isolation/primary expansion, cryopreservation, and thawing/secondary expansion. We sought to determine the feasibility and cell yield of such a staged cell manufacturing process, within regulatory guidelines. METHODS: Human AMSCs isolated from diagnostic amniocentesis samples (n = 11) were processed under Food and Drug Administration-accredited good manufacturing practice. Expanded cells were characterized by flow cytometry and cryopreserved for 3 to 5 months. Cell release criteria included more than 90% CD29+, CD73+, and CD44+; less than 5% CD34+ and CD45+; negative mycoplasma quantitative polymerase chain reaction (QPCR) and endotoxin assay; and at least 70% viability. RESULTS: Isolation and ample expansion of AMSCs was achieved in 54.5% (6/11) of the samples. Early processing and at least a 2-mL sample were necessary for reliable cell manufacturing. Cell yield before cryopreservation was 223.2 +/- 65.4 x 10(6) cells (44.6-fold expansion), plus a 14.7 x 10(6)-cell backup, after 36.3 +/- 7.8 days. Cell viability postthaw was 88%. Expanded cells maintained a multipotent mesenchymal progenitor profile. CONCLUSIONS: Human amniotic mesenchymal stem cells can be manufactured in large numbers from diagnostic amniocentesis, by an accredited staged processing, under definite procurement guidelines. These data further support the viability of clinical trials of amniotic mesenchymal stem cell-based therapies.

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