AMNIOTIC FLUID AND CHORIONIC VILLI STEM CELLS

SCIENTIFIC PAPER REVIEW

APRIL 2013

Biocell Center S.p.A. - Viale Stelvio 125, 21052, Busto Arsizio (Va) _ Italy- Tel. +39 0331 386028 - Fax +39 0331 367321 - N. Verde 800 042433 - www.biocellcenter.it - info@biocellcenter.it

Biocell Center Corporation – 200, Boston Av. – 02155, Medford, MA, USA - Tel. +1 3912040 - Fax +1 7813950302 – Toll Free 866 BIO 2720– www.biocellcenter.com - info@biocellcenter.com

Introduction

This issue represents a collection of the most important articles related on amniotic fluid and chorionic villi stem cells. The review includes articles published in 2013 and 2012. The summary carries title, and then, for each article is reported the scientific journal, publication date, authors and the abstract.

Introduzione

In questo fascicolo sono stati raccolti gli articoli più significativi relativi alle cellule staminali da liquido amniotico e da villi coriali. La rassegna copre i primi mesi del 2013 e la seconda metà del 2012; l'indice generale riporta i titoli delle ricerche. Nel dettaglio vengono riportati la rivista scientifica sulla quale è stato pubblicato l'articolo, la data di pubblicazione, gli autori ed il sommario.

SUMMARY

1. AF-MSCs fate can be regulated by culture conditions. 13

2. Human amniotic fluid stem cells protect rat lungs exposed to moderate hyperoxia. 16

3. Neurorescue effects and stem properties of chorionic villi and amniotic progenitor cells. 18

4. Fetal mesenchymal stem cells in cancer therapy... 21

5. Therapeutic potential of amniotic fluid stem cells. 23

6. Human amniotic fluid stem cells as an attractive tool for clinical applications. 25

7. The contribution of stem cell therapy to skeletal muscle remodeling in heart failure. . 27

8. Isolation, Culture, and Identification of Amniotic Fluid-Derived Mesenchymal Stem Cells. 29

9. Comparison of the Neural Differentiation Potential of Human Mesenchymal Stem Cells

from Amniotic Fluid and Adult Bone Marrow. 31

10. The use of human amniotic fluid mesenchymal stem cells as the feeder layer to establish human embryonic stem cell lines.. 33

11. Generation of Induced Pluripotent Stem Cells from Human Amniotic Fluid Cells by Reprogramming with two Factors in Feeder-free Conditions. 35

12. Modeling neurogenesis impairment in Down syndrome with induced pluripotent stem cells from Trisomy 21 amniotic fluid cells. ... 37

13. The amniotic fluid as a source of neural stem cells in the setting of experimental neural tube defects... 39

14. In vitro osteogenic differentiation of human amniotic fluid-derived stem cells on a poly(lactide-co-glycolide) (PLGA)-bladder submucosa matrix (BSM) composite scaffold for bone tissue engineering. 41

15. Phenotypic and functional characterization of mesenchymal stem cells from chorionic villi of human term placenta. 43

16. Human mid-trimester amniotic fluid stem cells cultured under embryonic stem cell conditions with valproic acid acquire pluripotent characteristics. 46

17. Amniotic fluid-derived cells for cardiovascular tissue engineering applications. 48

18. Amniotic fluid stem cells and fetal cell microchimerism. 50

19. Genetic Modification of Primate Amniotic Fluid-Derived Stem Cells Produces Pancreatic Progenitor Cells in vitro. 51

20. Complex heterogeneous tissue constructs containing multiple cell types prepared by inkjet printing technology. 53

21. Molecular Signature of Human amniotic Fluid Stem Cells During Fetal Development. 55

22. Isolation of canine mesenchymal stem cells from amniotic fluid and differentiation into hepatocyte-like cells. 57

23. Positive C4d immunostaining of placental villous syncytiotrophoblasts supports

host-versus-graft rejection in villitis of unknown etiology. 59

24. Spindle shaped human mesenchymal stem/stromal cells from amniotic fluid promote neovascularization. 61

25. Genome-wide analysis reveals the unique stem cell identity of human amniocytes. 63

26. Amniotic fluid and placental membranes: unexpected sources of highly multipotent cells. 65

27. Epigenetic analysis and suitability of amniotic fluid stem cells for research and therapeutic purposes. 67

28. Craniofacial repair with fetal bone grafts engineered from amniotic mesenchymal stem cells. 69

29. Effects of mesenchymal stem cells isolated from amniotic fluid and platelet-rich plasma gel on severe decubitus ulcers in a septic neonatal foal. 72

30. Chondrogenic differentiation of amniotic fluid stem cells and their potential for regenerative therapy. 73

31. Amniotic fluid-derived stem cells as a cell source for bone tissue engineering. 75

32. Pdx1 and controlled culture conditions induced differentiation of human amniotic fluid-derived stem cells to insulin-producing clusters. 77

33. Human amniotic fluid stem cell-derived muscle progenitor cell therapy for stress urinary incontinence. 79

34. Cell sources for trachea tissue engineering: past, present and future. 81

35. Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. 83

36. Efficient direct reprogramming of mature amniotic cells into endothelial cells by ETS factors and TGFβ suppression. 85

37. Simvastatin induces osteogenic differentiation in human amniotic fluid mesenchymal stem cells (AFMSC) 87

38. Role of amniotic fluid mesenchymal cells engineered on MgHA/collagen-based scaffold allotransplanted on an experimental animal study of sinus augmentation......................... 89

39. Amniotic Fluid Stem Cells Rescue Both In Vitro And In Vivo Growth, Innervation And Motility In Nitrofen-Exposed Hypoplastic Rat Lungs Through Paracrine Effects. 92

40. Xenografted human amniotic fluid-derived stem cell as a cell source in therapeutic angiogenesis... 95

41. Valproic acid confers functional pluripotency to human amniotic fluid stem cells in a transgene-free approach. 98

42. Protective effects of human amniotic fluid stem cells in a model of aorta allograft vasculopathy in rats. 100

43. The effect of differentiation stage of amniotic fluid stem cells on bone regeneration. 103

44. Potential of placenta-derived mesenchymal stem cells as seed cells for bone tissue engineering: preliminary study of

osteoblastic differentiation and immunogenicity. 105

45. Human amniotic fluid stem cell injection therapy for urethral sphincter regeneration in an animal model. 106

46. Generation, characterization, and potential therapeutic applications of cardiomyocytes from various stem cells. 109

47. High efficiency of reprogramming CD34^{+} cells derived from human amniotic fluid into induced pluripotent stem cells with Oct4. 111

48. Proangiogenic soluble factors from amniotic fluid stem cells mediate the recruitment of endothelial progenitors in a model of ischemic fasciocutaneous flap. 113

49. Potential antitumor therapeutic strategies of human amniotic membrane and amniotic fluid-derived stem cells. 115

50. Clone-derived human AF-amniotic fluid stem cells are capable of skeletal myogenic differentiation in vitro and in vivo. 117

51. Amniotic fluid stem cells restore the muscle cell niche in a HSA-Cre, Smn(F7/F7) mouse model. 119

52. Establishment of an internal control for directed differentiation using pluripotent stem cell lines derived from heterozygotic twins. 121

53. Human amniotic fluid stem cell preconditioning improves their regenerative potential. 123

54. Stem cells for heart valve regeneration. 125

55. Bilayered constructs aimed at osteochondral strategies: the influence of medium supplements in the osteogenic and chondrogenic differentiation of amniotic fluid-derived stem cells. 127

56. Stem cells from umbilical cord and placenta for musculoskeletal tissue engineering. 130

57. Tissue engineering of reproductive tissues and organs. 132

58. CD117(+) amniotic fluid stem cells: state of the art and future perspectives. 134

59. Congenital anomalies: treatment options based on amniotic fluid-derived stem cells. 136

60. Prenatally engineered autologous amniotic fluid stem cell-based heart valves in the fetal circulation. 138

61. Injectable PLGA porous beads cellularized by hAFSCs for cellular cardiomyoplasty. 140

62. Human amniotic fluid-derived stem cells expressing cytosine deaminase and thymidine kinase inhibits the growth of breast cancer cells in cellular and xenograft mouse models. 142

63. Evaluation of a low cost cryopreservation system on the biology of human amniotic fluid-derived mesenchymal stromal cells. 145

64. Human chorionic villus mesenchymal stromal cells reveal strong endothelial conversion properties. 147

65. Renal differentiation of amniotic fluid stem cells: perspectives for clinical application and for studies on specific human genetic diseases. 149

66. Therapeutic potential of a distinct population of human amniotic fluid mesenchymal stem cells and their secreted molecules in mice with acute hepatic failure. 151

67. Comparison of human amniotic fluid-derived and umbilical cord Wharton's Jelly-derived mesenchymal stromal cells: Characterization and myocardial differentiation capacity............................. 153

68. Dual regeneration of muscle and nerve by intravenous administration of human amniotic fluid-derived mesenchymal stem cells regulated by stromal cell-derived factor-1α in a sciatic nerve injury model......................... 156

69. Prenatal tracheal reconstruction with a hybrid amniotic mesenchymal stem cells-engineered construct derived from decellularized airway. 159

70. Evaluation of endothelial cells differentiated from amniotic fluid-derived stem cells. 161

INDICE DEI TITOLI IN ITALIANO 164

1. AF-MSCs fate can be regulated by culture conditions.

Zagoura DS, Trohatou O, Bitsika V, Makridakis M, Pappa KI, Vlahou A, Roubelakis MG, Anagnou NP.

Cell Death Dis. 2013 Apr 4.

Human mesenchymal stem cells (hMSCs) represent a population of multipotent adherent cells able to differentiate into many lineages. In our previous studies, we isolated and expanded fetal MSCs from second-trimester amniotic fluid (AF) and characterized them based on their phenotype, pluripotency and proteomic profile. In the present study, we investigated the plasticity of these cells based on their differentiation, dedifferentiation and transdifferentiation potential in vitro. To this end, adipocyte-like cells (AL cells) derived from AF-MSCs can regain, under certain culture conditions, a more primitive phenotype through the process of dedifferentiation. Dedifferentiated AL cells derived from AF-MSCs (DAF-MSCs), gradually lost the expression of adipogenic

markers and obtained similar morphology and differentiation potential to AF-MSCs, together with regaining the pluripotency marker expression. Moreover, a comparative proteomic analysis of AF-MSCs, AL cells and DAF-MSCs revealed 31 differentially expressed proteins among the three cell populations. Proteins, such as vimentin, galectin-1 and prohibitin that have a significant role in stem cell regulatory mechanisms, were expressed in higher levels in AF-MSCs and DAF-MSCs compared with AL cells. We next investigated whether AL cells could transdifferentiate into hepatocyte-like cells (HL cells) directly or through a dedifferentiation step. AL cells were cultured in hepatogenic medium and 4 days later they obtained a phenotype similar to AF-MSCs, and were termed as transdifferentiated AF-MSCs (TRAF-MSCs). This finding, together with the increase in pluripotency marker expression, indicated the adaption of a more primitive phenotype before transdifferentiation. Additionally, we observed that AF-, DAF- and TRAF-MSCs

displayed similar clonogenic potential, secretome and proteome profile. Considering the easy access to this fetal cell source, the plasticity of AF-MSCs and their potential to dedifferentiate and transdifferentiate, AF may provide a valuable tool for cell therapy and tissue engineering applications.

2. Human amniotic fluid stem cells protect rat lungs exposed to moderate hyperoxia.

Grisafi D, Pozzobon M, Dedja A, Vanzo V, Tomanin R, Porzionato A, Macchi V, Salmaso R, Scarpa M, Cozzi E, Fassina A, Navaglia F, Maran C, Onisto M, Caenazzo L, De Coppi P, De Caro R, Chiandetti L, Zaramella P.

Pediatr Pulmonol. 2013 Mar 26.

BACKGROUND: Treatment of bronchopulmonary dysplasia (BPD) remains as yet an unmet clinical need and recently stem cells have been proposed as a therapeutic tool in animal models. We investigated the role of amniotic fluid stem cells (AFS) in an adult rat model of hyperoxia lung injury.

METHODS: Fifty Sprague-Dawley rats were, at birth, randomly exposed to moderate hyperoxia or room air for 14 days and a single dose of human amniotic fluid stem (hAFS) or human Fibroblasts (hF), cells was delivered intratracheally (P21). At P42 animals were euthanized and lung tissue examined using

histology, immunohistochemistry, PCR, and ELISA. hAFS cells characterization and homing were studied by immunofluorescence.
RESULTS: In rats treated with hAFS and hF cells 16S human rRNA fragment was detected. Despite a low level of pulmonary hAFS cell retention ($1.43 \pm 0.2\%$ anti-human-mitochondria-positive cells), the lungs of the treated animals revealed higher secondary crest numbers and lower mean linear intercept and alveolar size, than those exposed to hyperoxia, those left untreated or treated with hF cells. Except for those treated with hAFS cells, moderate hyperoxia induced an increase in protein content of IL-6, IL-1β, as well as IF-γ and TGF-1β in lung tissues. High VEGF expression and arrangement of capillary architecture in hAFS cell group were also detected.
CONCLUSIONS: Treatment with hAFS cells has a reparative potential through active involvement of cells in alveolarization and angiogenesis. A downstream paracrine action was also taken into account, in order to understand the immunodulatory response.

3. Neurorescue effects and stem properties of chorionic villi and amniotic progenitor cells.

Calzarossa C, Bossolasco P, Besana A, Manca MP, De Grada L, De Coppi P, Giardino D, Silani V, Cova L.

Neuroscience. 2013 Mar 27;234:158-72.

The capability to integrate into degenerative environment, release neurotrophic cytokines, contrast oxidative stress and an inherent differentiation potential towards site appropriate phenotypes are considered crucial for the use of stem cells in tissue repair and regeneration. Naïve human chorial villi- (hCVCs) and amniotic fluid- (hAFCs) derived cells, whose properties and potentiality have not been extensively investigated, may represent two novel foetal cell sources for stem cell therapy. We previously described that long-term transplantation of hAFCs in the lateral ventricles of wobbler and healthy mice was feasible and safe. In the present study we examine the in vitro intrinsic stem

potential of hCVCs and hAFCs for future therapeutic applications in neurodegenerative disorders. Presence of stem lineages was evaluated assessing the expression pattern of relevant candidate markers by flow cytometry, reverse transcription-polymerase chain reaction (RT-PCR) and immunocytochemistry. Release of cytokines that may potentialy sustain endogenous neurogenesis and/or activate neuroprotective pathways was quantified by enzyme-linked immunosorbent assays (ELISAs). We also performed an in vitro neurorescue assay, wherein a neuroblastoma cell line damaged by 6-hydroxydopamine (6-OHDA) was treated with hCVC/hAFC-derived conditioned medium (CM). Naïve hCVCs/hAFCs show a neurogenic/angiogenic predisposition. Both cell types express several specific neural stem/progenitor markers, such as nestin and connexin 43, and release significant amounts of brain-derived neurotrophic factor, as well as vascular

endothelial growth factor. hCVC and hAFC populations comprise several interesting cell lineages, including mesenchymal stem cells (MSCs) and cells with neural-like phenotypes. Moreover, although CMs obtained from both cell cultures actively sustained metabolic activity in a 6-OHDA-induced Parkinson's disease (PD) cell model, only hCVC-derived CMs significantly reduced neurotoxin-induced apoptosis. In conclusion, this study demonstrates that naïve hAFCs and hCVCs may enhance cell-recovery following neuronal damage through multiple rescue mechanisms, and may provide a suitable means of stem cell therapy for neurodegenerative disorders including PD.

4. Fetal mesenchymal stem cells in cancer therapy.

Bitsika V, Vlahou A, Roubelakis MG.

Curr Stem Cell Res Ther. 2013 Mar 1;8(2):133-43.

There is compelling evidence that mesenchymal stem cells (MSCs) can be utilized as delivery vehicles for cancer therapeutics. During the last decade, bone marrow MSCs have been used as delivery vehicles for the local production of therapeutic proteins in multiple tumor types, taking advantage of their innate tropism to the tumor site and their low immunogenicity. More recently, MSCs have been isolated from fetal tissues during gestation or after birth. Fetal MSCs derived from amniotic fluid, amniotic membrane, umbilical cord matrix (Wharton's jelly) and umbilical cord blood are more advantageous than adult MSCs, as they can be isolated noninvasively in large numbers without the ethical reservations associated with embryo research. Several

studies have documented that fetal MSCs harbor a therapeutic potential in cancer treatment, as they can home to the tumor site and reduce tumor burden. This natural tumor tropism together with their low immunogenicity renders fetal MSCs as powerful therapeutic tools in gene therapy-based cancer therapeutic schemes. This review summarizes various approaches where the tumor-homing capacity of fetal MSCs has been employed for the localized delivery of anti-tumor therapeutic agents.

5. Therapeutic potential of amniotic fluid stem cells.

Abdulrazzak H, De Coppi P, Guillot PV.

Curr Stem Cell Res Ther. 2013 Mar 1;8(2):117-24.

Human amniotic fluid cells have been used traditionally as a diagnostic tool for genetic anomalies. More recently it has been recognized that amniotic fluid contains populations of stem cells. Mesenchymal stem cells (AFMSC) were first to be described. These cells are able to differentiate towards mesodermal lineages. More recently cells with broader potential, defined as amniotic fluid stem cells (AFSC), were also isolated. They have intermediate characteristics between embryonic and adult stem cells and are able to differentiate into lineages representative of all three germ layers but unlike ES cells they do not form tumours in vivo. Furthermore, AFSC have been reverted to functional pluripotency in a transgene-free approach using an epigenetics modifier.

These characteristics, together with absence of ethical issues concerning their employment, have made stem cells from amniotic fluid a promising candidate for cell therapy and tissue engineering.

6. Human amniotic fluid stem cells as an attractive tool for clinical applications.

Trohatou O, Anagnou NP, Roubelakis MG.

Curr Stem Cell Res Ther. 2013 Mar 1;8(2):125-32.

Recent studies support cell based therapies for several diseases. Human fetal stem cells have received much attention for developing new therapeutic strategies. Recently, our group and others have successfully isolated and expanded karyotypically normal stem cells from an alternative fetal source, the human second trimester amniotic fluid (AF) and performed a systematic phenotypic and molecular analysis. The main characteristics of amniotic fluid stem cells (hAFSCs) are their fetal origin, the high number of isolated cells, their wide differentiation properties and their rapid expansion in vitro. These characteristics render hAFSCs as a very attractive tool for clinical applications based on cell therapy. The use of hAFSC transplantation has been studied in a variety

of disease animal models related to bone regeneration, myocardial infarction, acute kidney injury, acute hepatic failure, skin injury, ischemic hind limb or cancer. The major aim of this review is to summarize the advent of hAFSCs capabilities into novel therapeutic modalities and discuss their potential use in future pre-clinical and clinical studies.

7. The contribution of stem cell therapy to skeletal muscle remodeling in heart failure.

Castellani C, Vescovo G, Ravara B, Franzin C, Pozzobon M, Tavano R, Gorza L, Papini E, Vettor R, De Coppi P, Thiene G, Angelini A.

Int J Cardiol. 2013 Feb 28.

BACKGROUND: The aim of our study was to investigate whether stem cell (SC) therapy with human amniotic fluid stem cells (hAFS, fetal stem cells) and rat adipose tissue stromal vascular fraction cells-GFP positive cells (rSVC-GFP) was able to produce favorable effects on skeletal muscle (SM) remodeling in a well-established rat model of right heart failure (RHF). METHODS: RHF was induced by monocrotaline (MCT) in Sprague-Dawley rats. Three weeks later, four millions of hAFS or rSVC-GFP cells were injected via tail vein. SM remodeling was assessed by Soleus muscle fiber cross sectional area (CSA), myocyte apoptosis, myosin heavy chain (MHC) composition, satellite cells pattern, and SC immunohistochemistry. RESULTS: hAFS and

rSVC-GFP injection produced significant SC homing in Soleus (0.68±1.0 and 0.67±0.75% respectively), with a 50% differentiation toward smooth muscle and endothelial cells. Pro-inflammatory cytokines were down regulated to levels similar to those of controls. SC-treated (SCT) rats showed increased CSA ($p<0.004$ vs MCT) similarly to controls with a reshift toward the slow MHC1 isoform. Apoptosis was significantly decreased (11.12.±8.8cells/mm3 hAFS and 13.1+7.6 rSVC-GFP) ($p<0.001$ vs MCT) and similar to controls (5.38±3.0cells/mm3). RHF rats showed a dramatic reduction of satellite cells(MCT 0.2±0.06% Pax7 native vs controls 2.60±2.46%, $p<0.001$), while SCT induced a repopulation of both native and SC derived satellite cells ($p<0.005$). CONCLUSIONS: SC treatment led to SM remodeling with satellite cell repopulation, decreased atrophy and apoptosis. Modulation of the cytokine milieu might play a crucial pathophysiological role with a possible scenario for autologous transplantation of SC in pts with CHF myopathy.

8. Isolation, Culture, and Identification of Amniotic Fluid-Derived Mesenchymal Stem Cells.

Fei X, Jiang S, Zhang S, Li Y, Ge J, He B, Goldstein S, Ruiz G.

Cell Biochem Biophys. 2013 Mar 19.

Amniotic fluid-derived mesenchymal stem cells (AF-MSC) are newly described, excellent seed cells that have good differentiation capability and are convenient to obtain. However, it is important to develop a method to isolate and culture AF-MSC efficiently. Amniotic fluid samples were obtained from rabbits and the adherence method was used for AF-MSC culture. Flow cytometry, western blot, and immunofluorescence studies were used to analyze the phenotypic characteristics of the cultured AF-MSC. Amniotic fluid-derived mesenchymal stem cells were successfully isolated and cultured from amniotic fluid. Flow cytometric analysis demonstrated that these cells expressed CD29 and CD44, while

they did not express CD34. The expression of transcription factor Oct-4 was confirmed by western blot and immunofluorescence analysis. Using the adherence method, we developed a successful, reproducible protocol for the isolation of AF-MSC from amniotic fluid. The results of our phenotypic analysis revealed that the AF-MSC isolated in the present study were multipotent cells.

9. Comparison of the Neural Differentiation Potential of Human Mesenchymal Stem Cells from Amniotic Fluid and Adult Bone Marrow.

Yan ZJ, Hu YQ, Zhang HT, Zhang P, Xiao ZY, Sun XL, Cai YQ, Hu CC, Xu RX.

Cell Mol Neurobiol. 2013 Mar 12.

Human mesenchymal stem cells (MSCs) are considered a promising tool for cell-based therapies of nervous system diseases. Bone marrow (BM) has been the traditional source of MSCs (BM-MSCs). However, there are some limitations for their clinical use, such as the decline in cell number and differentiation potential with age. Recently, amniotic fluid (AF)-derived MSCs (AF-MSCs) have been shown to express embryonic and adult stem cell markers, and can differentiate into cells of all three germ layers. In this study, we isolated AF-MSCs from second-trimester AF by limiting dilution and compared their proliferative capacity, multipotency, neural differentiation ability, and secretion of

neurotrophins to those of BM-MSCs. AF-MSCs showed a higher proliferative capacity and more rapidly formed and expanded neurospheres compared to those of BM-MSCs. Both immunocytochemical and quantitative real-time PCR analyses demonstrated that AF-MSCs showed higher expression of neural stemness markers than those of BM-MSCs following neural stem cell (NSC) differentiation. Furthermore, the levels of brain-derived growth factor and nerve growth factor secreted by AF-MSCs in the culture medium were higher than those of BM-MSCs. In addition, AF-MSCs maintained a normal karyotype in long-term cultures after NSC differentiation and were not tumorigenic in vivo. Our findings suggest that AF-MSCs are a promising and safe alternative to BM-MSCs for therapy of nervous system diseases.

10. The use of human amniotic fluid mesenchymal stem cells as the feeder layer to establish human embryonic stem cell lines.

Soong YK, Huang SY, Yeh CH, Wang TH, Chang KH, Cheng PJ, Shaw SW.

J Tissue Eng Regen Med. 2013 Mar 4.

Human embryonic stem cells (hESCs) are pluripotent cells that have the potential to differentiate into the three germ layers and possibly all tissues of the human body. To fulfil the clinical potentials for cell-based therapy, banks of hESC lines that express different combinations of the major histocompatibility genes should be established, preferably without exposing such cells to animal cells and proteins. In this study, we tested human amniotic fluid mesenchymal stem cells (AFMSCs) as feeder cells to support the growth of hESCs. Our results indicated that mitomycin-treated AFMSCs were able to support the newly established hESC lines CGLK-1 and CGLK-

2. The hESC colonies cultured on AFMSCs expressed alkaline phosphatase (ALK-P), SSEA-4, TRA-1-60, TRA-1-81, Oct-4, Nanog and Sox-2, which are markers for undifferentiated hESCs. Chromosomal analyses of both hESC lines, CGLK-1 and CGLK-2, which were cultured on AFMSC feeders for 22 and 14 passages, respectively, were confirmed to be normal karyotypes (46, XX). The ability of AFMSCs as feeder cells to maintain the undifferentiated growth and pluripotency of hESCs was confirmed by in vivo formation of teratomas derived on AFMSC hESCs in severe combined immune-compromised mice. The use of AFMSCs for feeder cells to culture hESCs has several advantages, in that AFMSCs are not tumourigenic and can be expanded extensively with a short doubling time.

11. Generation of Induced Pluripotent Stem Cells from Human Amniotic Fluid Cells by Reprogramming with two Factors in Feeder-free Conditions.

Li Q, Fan Y, Sun X, Yu Y.

J Reprod Dev. 2013 Feb 20;59(1):72-7.

The ectopic expression of transcription factors for reprogramming human somatic cells to a pluripotent state represents a valuable resource for the development of in vitro-based models for human disease and has great potential in regenerative therapies. However, the majority of studies have used skin fibroblasts to generate induced pluripotent stem cells (iPSCs) that typically require the enforced expression of several transcription factors, thereby posing a mutagenesis risk by the insertion of viral transgenes. To reduce this risk, iPSCs have been generated with OCT4 and KLF4 from human neural stem cells that endogenously express the remaining reprogramming factors. However, human neural stem cells are rare

and difficult to obtain. Here, we show that iPSCs can be generated from human amniotic fluid cells (hAFCs) with two transcription factors: OCT4 and KLF4. Furthermore, iPSCs can be readily derived from hAFCs in a feeder-free conditions, thereby eliminating the potential variability caused by using feeder cells. Our results indicate that hAFCs represent an accessible source of cells that can be reprogrammed into pluripotent stem cells with two Yamanaka factors. Therefore, hAFCs may become a preferred cell type in the future for safe reprogramming without any exogenous genetic material.

12. Modeling neurogenesis impairment in Down syndrome with induced pluripotent stem cells from Trisomy 21 amniotic fluid cells.

Lu HE, Yang YC, Chen SM, Su HL, Huang PC, Tsai MS, Wang TH, Tseng CP, Hwang SM.

Exp Cell Res. 2013 Feb 15;319(4):498-505.

Down syndrome (DS), or Trisomy 21 (T21) syndrome, one of the most common chromosomal abnormalities, is caused by an extra duplication of chromosome 21. In studies of neuron development, experimental models based on human cells are considered to be the most desired and accurate for basic research. The generation of diseased induced pluripotetn stem (iPS) cell is a critical step in understanding the developmental stages of complex neuronal diseases. Here, we generated human DS iPS cell lines from second trimester amniotic fluid (AF) cells with T21 by co-expressing Yamanaka factors through lentiviral delivery and subsequently differentiated them into neuronal

progenitor cells (NPCs) for further analyses. T21 AF-iPS cells were characterized for the expression of pluripotent markers and for their ability to differentiate into all three germ layers by forming embryoid bodies in vitro and teratomas in vivo. The T21 AF-iPS cells maintained their unique pattern of chromosomal karyotypes: three pairs of chromosome 21. The level of amyloid precursor protein was significantly increased in NPCs derived from T21 AF-iPS cells compared with NPCs from normal AF-iPS cells. The expression levels of miR-155 and miR-802 in T21 AF-iPS-NPCs were highly elevated in the presence of low expression of MeCP2. We observed that T21 iPS-NPCs generated fewer neurons compared with controls. T21 iPS-NPCs exhibit developmental defects during neurogenesis. Our findings suggest that T21 AF-iPS cells serve as a good source to further elucidate the impairment neurogenesis of DS and the onset of Alzheimer's disease.

13. The amniotic fluid as a source of neural stem cells in the setting of experimental neural tube defects.

Turner CG, Klein JD, Wang J, Thakor D, Benedict D, Ahmed A, Teng YD, Fauza DO.

Stem Cells Dev. 2013 Feb 15;22(4):548-53.

We sought to determine whether neural stem cells (NSCs) can be isolated from the amniotic fluid in the setting of neural tube defects (NTDs), as a prerequisite for eventual autologous perinatal therapies. Pregnant Sprague-Dawley dams (n=62) were divided into experimental (n=42) and control (n=20) groups, depending on prenatal exposure to retinoic acid for the induction of fetal NTDs. Animals were killed before term for analysis (n=685 fetuses). Amniotic fluid samples from both groups underwent epigenetic selection for NSCs, followed by exposure to neural differentiation media. Representative cell samples underwent multiple morphological and phenotypical analyses at different time points. No control fetus (n=267) had any

structural abnormality, whereas at least one type of NTD developed in 52% (217/418) of the experimental fetuses (namely, isolated spina bifida, n=144; isolated exencephaly, n=24; or a combination of the two, n=49). Only amniotic samples from fetuses with a NTD yielded cells with typical neural progenitor morphology and robust expression of both Nestin and Sox-2, primary markers of NSCs. These cells responded to differentiation media by displaying typical morphological changes, along with expression of beta-tubulin III, glial fibrillary acidic protein, and/or O4, markers for immature neurons, astrocytes, and oligodendrocytes, respectively. This was concurrent with downregulation of Nestin and Sox-2. We conclude that the amniotic fluid can harbor disease-specific stem cells, for example, NSCs in the setting of experimental NTDs. The amniotic fluid may be a practical source of autologous NSCs applicable to novel forms of therapies for spina bifida.

14. In vitro osteogenic differentiation of human amniotic fluid-derived stem cells on a poly(lactide-co-glycolide) (PLGA)-bladder submucosa matrix (BSM) composite scaffold for bone tissue engineering.

Kim J, Jeong SY, Ju YM, Yoo JJ, Smith TL, Khang G, Lee SJ, Atala A.

Biomed Mater. 2013 Feb;8(1):014107.

Stem cells have become an important component of tissue regeneration, as they are able to differentiate into various cell types if guided appropriately. It is well known that cellular differentiation is greatly influenced by the surrounding microenvironment. We have developed a composite scaffold system using a collagen matrix derived from porcine bladder submucosa matrix (BSM) and poly(lactide-co-glycolide) (PLGA). In this study, we investigated whether a composite scaffold composed of naturally derived matrix combined with synthetic polymers would provide a microenvironment to facilitate the induction of osteogenic differentiation. We first showed that

human amniotic fluid-derived stem cells (hAFSCs) adhered to the composite scaffolds and proliferated over time. We also showed that the composite scaffolds facilitated the differentiation of hAFSCs into an osteogenic lineage. The expression of osteogenic genes, including RUNX2, osteopontin (OPN) and osteocalcin (OCN) was upregulated in cells cultured on the composite scaffolds incubated in the osteogenic medium compared with ones without. Increased alkaline phosphatase (ALP) activity and calcium content indicates that hAFSCs seeded on 3D porous BSM-PLGA composite scaffolds resulted in higher mineralization rates as the duration of induction increased. This was also evidenced by the mineralized matrix within the scaffolds. The composite scaffold system provides a proper microenvironment that can facilitate osteogenic differentiation of AFSCs. This scaffold system may be a good candidate material for bone tissue engineering.

15. Phenotypic and functional characterization of mesenchymal stem cells from chorionic villi of human term placenta.

Abumaree MH, Al Jumah MA, Kalionis B, Jawdat D, Al Khaldi A, Altalabani AA, Knawy BA.

Stem Cell Rev. 2013 Feb;9(1):16-31.

BACKGROUND: Bone marrow derived mesenchymal stem cells (BM-MSCs) are used extensively in transplantation but their use is associated with many problems including low abundance in BM, low overall number, decreased differentiation potential with age and the invasive isolation procedures needed to obtain BM. We report a novel method of isolating placental MSCs (pMSCs) from chorionic villi, which exhibit the phenotypic and functional characteristics that will make them an attractive source of MSCs for cell-based therapy. METHODS: A novel explant approach was used to isolate pMSCs from chorionic villi of human placentae.

These pMSCs were characterized by flow cytometry and were differentiated into adipocytes, osteocytes and chondrocytes using differentiation medium as demonstrated by cytochemical staining. The gene and protein expression profiles of pMSCs were also characterized using real time polymerase chain reaction (PCR) and flow cytometry, respectively. In addition, cytokine secretion by pMSCs was also analysed using sandwich enzyme-linked immunosorbent assay (ELISA) technique. Moreover, the migration and proliferation potentials of pMSCs were also determined. RESULTS: pMSCs were isolated from fetal part of the chorionic villi and these pMSCs expressed CD44, CD90, CD105, CD146, CD166 and HLA-ABC but not CD14, CD19, CD40, CD45, CD80, CD83, CD86 and HLA-DR. In addition, these pMSCs differentiated into osteocytes, chondrocytes and adipocytes and they also expressed several adhesion molecules, chemokines/receptors, growth factor

receptors and cytokines/receptors. Moreover, they secreted many cytokines (IL-1Ra, IL6, IL8, IL10, IL11 and IL15) and they were able to proliferate. Furthermore, they migrated in response to chemotactic factors including stromal cell-derived factor-1 (SDF-1), platelet derived growth factor (PDGF), hepatocyte growth factor (HGF), and monocyte chemotactic protein-1 (MCP-1). CONCLUSIONS: We devised a novel explant method of isolating pMSCs that expressed many biological factors responsible for mediating cellular processes such as migration/homing, immune modulation and angiogenesis. Therefore, we suggest that pMSCs prepared from human term placental chorionic villous explants are an attractive source of MSCs for cell therapy.

16. Human mid-trimester amniotic fluid stem cells cultured under embryonic stem cell conditions with valproic acid acquire pluripotent characteristics.

Moschidou D, Mukherjee S, Blundell MP, Jones GN, Atala AJ, Thrasher AJ, Fisk NM, De Coppi P, Guillot PV.

Stem Cells Dev. 2013 Feb 1;22(3):444-58.

Human mid-trimester amniotic fluid stem cells (AFSC) have promising applications in regenerative medicine, being broadly multipotent with an intermediate phenotype between embryonic (ES) and mesenchymal stem cells (MSC). Despite this propluripotent phenotype, AFSC are usually cultured in adherence in a serum-based expansion medium, and how expansion in conditions sustaining pluripotency might affect their phenotype remains unknown. We recently showed that early AFSC from first trimester amniotic fluid, which endogenously express Sox2 and Klf4, can be reprogrammed to pluripotency without viral vectors using the histone deacetylase inhibitor valproic acid (VPA). Here, we show

that mid-trimester AFSC cultured under MSC conditions contained a subset of cells endogenously expressing telomerase, CD24, OCT4, C-MYC, and SSEA4, but low/null levels of SOX2, NANOG, KLF4, SSEA3, TRA-1-60, and TRA-1-81, with cells unable to form embryoid bodies (EBs) or teratomas. In contrast, AFSC cultured under human ESC conditions were smaller in size, grew faster, formed colonies, upregulated OCT4 and C-MYC, and expressed KLF4 and SOX2, but not NANOG, SSEA3, TRA-1-60, and TRA-1-81. Supplementation with VPA for 5 days further upregulated OCT4, KLF4, and SOX2, and induced expression of NANOG, SSEA3, TRA-1-60, and TRA-1-81, with cells now able to form EBs and teratomas. We conclude that human mid-trimester AFSC, which may be isolated autologously during pregnancy without ethics restriction, can acquire pluripotent characteristics without the use of ectopic factors. Our data suggest that this medium-dependant approach to pluripotent mid-trimester AFSC reflects true reprogramming and not the selection of prepluripotent cells.

17. Amniotic fluid-derived cells for cardiovascular tissue engineering applications.

Petsche JJ, Camci-Unal G, Khademhosseini A, Jacot J.

Tissue Eng Part B Rev. 2013 Jan 25.

Recent research has demonstrated that a population of stem cells can be isolated from amniotic fluid removed by amniocentesis that are broadly multipotent and non-tumorogenic. These amniotic fluid-derived stem cells (AFSC) could potentially provide an autologous cell source for treatment of congenital defects identified during gestation, particularly cardiovascular defects. In this review, the various methods of isolating, sorting and culturing AFSC are compared, along with techniques for inducing differentiation into cardiac myocytes and endothelial cells. Though research has not demonstrated complete and high yield cardiac differentiation, AFSC have been shown to effectively differentiate into endothelial cells

and can effectively support cardiac tissue. Additionally, several tissue engineering and regenerative therapeutic approaches for the use of these cells in heart patches, injection after myocardial infarction, heart valves, vascularized scaffolds and blood vessels are summarized. These applications show great promise in the treatment of congenital cardiovascular defects, and further studies of isolation, culture, and differentiation of AFSC will help to develop their use for tissue engineering, regenerative medicine, and cardiovascular therapies.

18. Amniotic fluid stem cells and fetal cell microchimerism.

Rosner M, Hengstschläger M.

Trends Mol Med. 2013 Jan 18.

Fetal cells (and cell-free, fetal DNA used for non-invasive prenatal diagnosis) are known to exist in the circulation of pregnant women. These cells exhibit stem cell properties when they differentiate at the site of injured maternal tissue, but the origin of these fetal, natural, and probably reparative cells is unknown. During pregnancy, mobilized pluripotent fetal stem cells of yet unidentified in vivo significance float in the amniotic fluid, and we argue that circulating fetal cells and the pluripotent amniotic fluid cells might share a common origin.

19. Genetic Modification of Primate Amniotic Fluid-Derived Stem Cells Produces Pancreatic Progenitor Cells in vitro.

Zhou Y, Mack DL, Williams JK, Mirmalek-Sani SH, Moorefield E, Chun SY, Wang J, Lorenzetti D, Furth M, Atala A, Soker S.

Cells Tissues Organs. 2013 Jan 8.

Insulin therapy for type 1 diabetes does not prevent serious long-term complications including vascular disease, neuropathy, retinopathy and renal failure. Stem cells, including amniotic fluid-derived stem (AFS) cells – highly expansive, multipotent and nontumorigenic cells - could serve as an appropriate stem cell source for β-cell differentiation. In the current study we tested whether nonhuman primate (nhp)AFS cells ectopically expressing key pancreatic transcription factors were capable of differentiating into a β-cell-like cell phenotype in vitro. nhpAFS cells were obtained from Cynomolgus monkey amniotic fluid by immunomagnetic selection for a CD117 (c-kit)-

positive population. RT-PCR for endodermal and pancreatic lineage-specific markers was performed on AFS cells after adenovirally transduced expression of PDX1, NGN3 and MAFA. Expression of MAFA was sufficient to induce insulin mRNA expression in nhpAFS cell lines, whereas a combination of MAFA, PDX1 and NGN3 further induced insulin expression, and also induced the expression of other important endocrine cell genes such as glucagon, NEUROD1, NKX2.2, ISL1 and PCSK2. Higher induction of these and other important pancreatic genes was achieved by growing the triply infected AFS cells in media supplemented with a combination of B27, betacellulin and nicotinamide, as well as culturing the cells on extracellular matrix-coated plates. The expression of pancreatic genes such as NEUROD1, glucagon and insulin progressively decreased with the decline of adenovirally expressed PDX1, NGN3 and MAFA. Together, these experiments suggest that forced expression of pancreatic transcription factors in primate AFS cells induces them towards the pancreatic lineage.

20. Complex heterogeneous tissue constructs containing multiple cell types prepared by inkjet printing technology.

Xu T, Zhao W, Zhu JM, Albanna MZ, Yoo JJ, Atala A.

Biomaterials. 2013 Jan;34(1):130-9.

This study was designed to develop a versatile method for fabricating complex and heterogeneous three-dimensional (3D) tissue constructs using simultaneous ink-jetting of multiple cell types. Human amniotic fluid-derived stem cells (hAFSCs), canine smooth muscle cells (dSMCs), and bovine aortic endothelial cells (bECs), were separately mixed with ionic cross-linker calcium chloride (CaCl(2)), loaded into separate ink cartridges and printed using a modified thermal inkjet printer. The three cell types were delivered layer-by-layer to pre-determined locations in a sodium alginate-collagen composite located in a chamber under the printer. The reaction between CaCl(2) and sodium alginate resulted in a

rapid formation of a solid composite gel and the printed cells were anchored in designated areas within the gel. The printing process was repeated for several cycles leading to a complex 3D multi-cell hybrid construct. The biological functions of the 3D printed constructs were evaluated in vitro and in vivo. Each of the printed cell types maintained their viability and normal proliferation rates, phenotypic expression, and physiological functions within the heterogeneous constructs. The bioprinted constructs were able to survive and mature into functional tissues with adequate vascularization in vivo. These findings demonstrate the feasibility of fabricating complex heterogeneous tissue constructs containing multiple cell types using inkjet printing technology.

21. Molecular Signature of Human amniotic Fluid Stem Cells During Fetal Development.

Moschidou D, Drews K, Eddaoudi A, Adjaye J, De Coppi P, Guillot PV.

Curr Stem Cell Res Ther. 2013 Jan 1;8(1):73-81.

Mid-gestation c-KIT+ amniotic fluid stem cells (AFSC) have an intermediate phenotype between embryonic and adult stem cells and are easy to reprogram to pluripotency. We previously showed that 1st trimester AFSC can be reprogrammed to functional pluripotency in a transgene-free approach. Despite both parental populations sharing a common phenotype, expressing CD29, CD44, CD73, CD90, CD105, SSEA4 and OCT4, 2nd trimester AFSC, contrary to 1st trimester cells, do not express NANOG, SSEA3, TRA-1-60 and TRA-1-81, and have slower growth kinetics. Here, we used the Illumina Beadstudio microarray platform to analyse the transcriptome of 1st and 2nd trimester

AFSC and show a unique 1st trimester AFSC-specific gene expression signature consisting of 366 genes and a larger set of 603 genes common with hESC compared to 496 genes overlapping between 2nd trimester AFSC and hESC. We conclude that both populations are related but distinct to each other as well as to hESC.

22. Isolation of canine mesenchymal stem cells from amniotic fluid and differentiation into hepatocyte-like cells.

Choi A, Choi S, Kim J, Lee S, Lee H, Park Y, Kim Y, Li X, Oh Y, Lee S, Kim K.

In Vitro Cell Dev Biol Anim. 2013 Jan;49(1):42-51.

Recent findings have demonstrated that amniotic fluid cells are an interesting and potential source of mesenchymal stem cells (MSCs). In this study, we isolated MSCs from canine amniotic fluid and then characterized their multilineage differentiation ability. Canine amniotic fluid stem (cAFS) cells at passage 5 had a fibroblast-like morphology instead of forming colonies and were positive for pluripotent stem cell markers such as OCT4, NANOG, and SOX2. Flow cytometry analysis showed the expression of MSC surface markers CD44, CD29, and CD90 on the cAFS cells. In addition, these cells were cultured under conditions favorable for adipogenic, chondrogenic, and osteogenic induction. The

results of this experiment confirmed the mesenchymal nature of cAFS cells and their multipotent potential. Interestingly, although the cells exhibited a fibroblast-like morphology after hepatogenic induction, reverse transcription-polymerase chain reaction revealed that the expression of several hepatic genes, such as albumin, tyrosine aminotransferase, and alpha-1 antiproteinase, increased following maturation and differentiation. These findings indicated that cAFS cells have functional properties similar to those of hepatocytes. Taken together, the results of our study demonstrated that cAFS cells with mesenchymal characteristics can be successfully isolated from canine amniotic fluid and possess functional properties characteristic of hepatocytes. The findings of our work suggest that cAFS cells have the potential to be a resource for cell-based therapies in a canine model of hepatic disease.

23. Positive C4d immunostaining of placental villous syncytiotrophoblasts supports host-versus-graft rejection in villitis of unknown etiology.

Rudzinski E, Gilroy M, Newbill C, Morgan T.

Pediatr Dev Pathol. 2013 Jan-Feb;16(1):7-13.

ABSTRACT Chronic villitis of unknown etiology (VUE) occurs in 5% of placentas submitted to pathology and is characterized by lymphohistiocytic infiltration of chorionic villi. VUE is associated with fetal growth restriction, preterm birth, and recurrent pregnancy loss. Accumulating evidence indicates that VUE may represent a host-versus-graft reaction analogous to transplant rejection. Pathologists routinely screen for antibody-mediated rejection in transplant biopsies by immunostaining for C4d, which highlights the recognition of donor cells by the host immune system. Since the hemochorial placenta is bathed in maternal blood, we hypothesized that cases of VUE may show C4d deposition onto villous syncytiotrophoblasts (STB). Chronic villitis was diagnosed in 82 of 1986 (4%) singleton

placentas submitted to our department from 2007 through 2011. Forty randomly selected cases were gestational age-matched with 40 negative controls. Patient charts were reviewed and representative placental sections were immunostained for C4d. A positive C4d result was defined as circumferential immunostaining of the STB around at least one villous, or strong staining of fetal endothelial cells in the chorionic plate or stem villi. Our data indicate that VUE usually occurs in the 3rd trimester (37 ± 0.5 weeks) and is associated with significantly reduced placental weight ($P = 0.006$). Positive C4d staining of STB was more common in VUE (35/40, 88%) compared with negative controls (2/40, 5%) ($P < 0.0001$). It was also more common in multiparous (35/66, 53%) than primiparous (2/14, 14%) women ($P < 0.01$). Although the precise mechanism remains to be determined, our data support the hypothesis that VUE may represent host-versus-graft rejection by the mother.

24. Spindle shaped human mesenchymal stem/stromal cells from amniotic fluid promote neovascularization.

Roubelakis MG, Tsaknakis G, Pappa KI, Anagnou NP, Watt SM.

PLoS One. 2013;8(1):e54747.

Human amniotic fluid obtained at amniocentesis, when cultured, generates at least two morphologically distinct mesenchymal stem/stromal cell (MSC) subsets. Of these, the spindle shaped amniotic fluid MSCs (SS-AF-MSCs) contain multipotent cells with enhanced adipogenic, osteogenic and chondrogenic capacity. Here, we demonstrate, for the first time, the capacity of these SS-AF-MSCs to support neovascularization by umbilical cord blood (UCB) endothelial colony forming cell (ECFC) derived cells in both in vitro and in vivo models. Interestingly, although the kinetics of vascular tubule formation in vitro were similar when the supporting SS-AF-MSCs were compared with the best

vasculogenic supportive batches of bone marrow MSCs (BMSCs) or human dermal fibroblasts (hDFs), SS-AF-MSCs supported vascular tubule formation in vivo more effectively than BMSCs. In NOD/SCID mice, the human vessels inosculated with murine vessels demonstrating their functionality. Proteome profiler array analyses revealed both common and distinct secretion profiles of angiogenic factors by the SS-AF-MSCs as opposed to the hDFs and BMSCs. Thus, SS-AF-MSCs, which are considered to be less mature developmentally than adult BMSCs, and intermediate between adult and embryonic stem cells in their potentiality, have the additional and very interesting potential of supporting increased neovascularisation, further enhancing their promise as vehicles for tissue repair and regeneration.

25. Genome-wide analysis reveals the unique stem cell identity of human amniocytes.

Maguire CT, Demarest BL, Hill JT, Palmer JD, Brothman AR, Yost HJ, Condic ML.

PLoS One. 2013;8(1):e53372.

Human amniotic fluid contains cells that potentially have important stem cell characteristics, yet the programs controlling their developmental potency are unclear. Here, we provide evidence that amniocytes derived from multiple patients are marked by heterogeneity and variability in expression levels of pluripotency markers. Clonal analysis from multiple patients indicates that amniocytes have large pools of self-renewing cells that have an inherent property to give rise to a distinct amniocyte phenotype with a heterogeneity of pluripotent markers. Significant to their therapeutic potential, genome-wide profiles are distinct at different gestational ages and times in culture, but do not differ between genders. Based on

hierarchical clustering and differential expression analyses of the entire transcriptome, amniocytes express canonical regulators associated with pluripotency and stem cell repression. Their profiles are distinct from human embryonic stem cells (ESCs), induced-pluripotent stem cells (iPSCs), and newborn foreskin fibroblasts. Amniocytes have a complex molecular signature, coexpressing trophoblastic, ectodermal, mesodermal, and endodermal cell-type-specific regulators. In contrast to the current view of the ground state of stem cells, ESCs and iPSCs also express high levels of a wide range of cell-type-specific regulators. The coexpression of multilineage differentiation markers combined with the strong expression of a subset of ES cell repressors in amniocytes suggests that these cells have a distinct phenotype that is unlike any other known cell-type or lineage

26. Amniotic fluid and placental membranes: unexpected sources of highly multipotent cells.

Murphy SV, Atala A.

Semin Reprod Med. 2013 Jan;31(1):62-8.

Gestational tissue such as the placenta, placental membranes, and amniotic fluid are usually discarded following birth. Recently, researchers have identified gestational tissue as an untapped source of stem cells that are highly multipotent and possess potent immunosuppressive properties. Placental mesenchymal stem cells (MSCs), human amnion epithelial cells (hAECs), and amniotic fluid-derived stem cells (AFSCs) have been shown to differentiate into various cell types including adipogenic, osteogenic, myogenic, endothelial, pulmonary, neurogenic, hepatogenic, cardiac, and pancreatic lineages. Their immunomodulatory properties suggest that gestational stem cells may have an important application in the treatment of various inflammatory diseases

such as graft versus host and autoimmune diseases. In clinical and preclinical studies, gestational stem cells have shown efficacy in the treatment of Crohn disease, lung disease, diabetes, repair of bone defects, heart disease, kidney disease, neural degeneration, and blood disorders. Stem cells derived from the placenta, placental membranes, and amniotic fluid are a valuable resource for the field of regenerative medicine.

27. Epigenetic analysis and suitability of amniotic fluid stem cells for research and therapeutic purposes.

Phermthai T, Suksompong S, Tirawanchai N, Issaragrisil S, Julavijitphong S, Wichitwiengrat S, Silpsorn D, Pokathikorn P.

Stem Cells Dev. 2012 Dec 18.

Abstract Amniotic fluid stem cells (AFSs) are interesting mesenchymal stem cells (MSCs) characterized by great potential for cell proliferation and differentiation compared to other types of MSCs identified to date. However, MSCs in prolonged culture have been found to exhibit defects in genetic stability and differentiation capacity. Epigenetic anomalies have been hypothesized to be a cause of these defects. Here, we investigated the genomic methylation and genetic imprinting in AFSs during prolonged in vitro culture. Four human imprinted genes, IGF2, H19, SNRPN and MEST, were evaluated for expression levels and methylation statuses in AFS cell lines. The

data revealed epigenetic instability in high passage number AFS cultures. The real time-PCR analysis showed that the expression levels of the imprinted genes gradually increased with increased time in culture. The loss of parental allele-specific imprinting for at least one gene among IGF2, H19 and SNRPN was observed in every AFS line after passage 8 using allelic expression analysis. The imprinting control regions (ICRs) of the IGF2 and H19 genes were assayed for site-specific methylation using bisulfate sequencing. This assay revealed a variable level of methylated CpG sites in the ICRs of IGF2 and H19. This variable level of CpG methylation is related to the aberrant expression of the IGF2 and H19 genes in late-passage AFSs. Our results did not reveal any irregularity in the epigenetic control system in the early-passage AFSs, indicating that the standard in vitro culturing of AFSs used in medical treatments should be limited to eight passages.

28. Craniofacial repair with fetal bone grafts engineered from amniotic mesenchymal stem cells.

Turner CG, Klein JD, Gray FL, Ahmed A, Zurakowski D, Fauza DO.

J Surg Res. 2012 Dec;178(2):785-90.

BACKGROUND: Ethically acceptable applications of fetal tissue engineering as a perinatal therapy can be expanded beyond life-threatening anomalies by amniotic fluid cell-based methods, in which cell procurement poses no additional risk to the mother. We sought to start to determine whether osseous grafts engineered from amniotic mesenchymal stem cells (aMSCs) could be an adjunct to craniofacial repair. METHODS: New Zealand rabbits (n = 12) underwent creation of a full-thickness diploic nasal bone defect. We then equally divided animals into two groups based on how the defect was repaired: namely, size-matched implants of electrospun biodegradable nanofibers with or without nuclear labeled,

allogeneic aMSCs maintained in osteogenic medium. We killed animals 8 wk post-implantation for multiple analyses. Statistical analysis included analysis of variance, post-hoc Bonferroni adjusted comparisons, and Levene's F-test, as appropriate ($P < 0.05$), with significance set at $P < 0.05$. RESULTS: Micro-computed tomography scanning (two- and three-dimensional) showed no significant differences in defect radiodensity between groups. However, extracellular calcium levels were significantly higher in engineered grafts than in acellular implants ($P = 0.003$). There was significantly greater variability in mineralization in acellular implants than in engineered grafts by both direct calcium ($P = 0.008$) and micro-computed tomography measurements ($P = 0.032$). There were no significant differences in alkaline phosphatase activity or variance between groups. We documented labeled cells in the engineered grafts. CONCLUSIONS: Craniofacial repair with osseous grafts engineered from aMSCs

lead to enhanced and more consistent mineralization compared with an equivalent acellular prosthetic repair. Amniotic fluid-derived engineered bone may become a practical adjunct to perinatal craniofacial reconstruction.

29. Effects of mesenchymal stem cells isolated from amniotic fluid and platelet-rich plasma gel on severe decubitus ulcers in a septic neonatal foal.

Iacono E, Merlo B, Pirrone A, Antonelli C, Brunori L, Romagnoli N, Castagnetti C.

Res Vet Sci. 2012 Dec;93(3):1439-40.

This paper documents the treatment of severe decubitus ulcers with amniotic fluid mesenchymal stem cells and platelets rich plasma (PRP) gel in a septic neonatal foal. The colt needed 25 days of hospitalization: during this period ulcers were treated for 15 days with mesenchymal stem cells (MSCs) plus PRP, PRP gel alone, or aloe gel. Healing was faster using MSCs+PRP, and at 7 months an ulcer treated with aloe gel was still not completely healed.

30. Chondrogenic differentiation of amniotic fluid stem cells and their potential for regenerative therapy.

Preitschopf A, Zwickl H, Li K, Lubec G, Joo G, Rosner M, Hengstschläger M, Mikula M.

Stem Cell Rev. 2012 Dec;8(4):1267-74.

Chronic articular cartilage defects are the most common disabling conditions of humans in the western world. The incidence for cartilage defects is increasing with age and the most prominent risk factors are overweight and sports associated overloading. Damage of articular cartilage frequently leads to osteoarthritis due to the aneural and avascular nature of articular cartilage, which impairs regeneration and repair. Hence, patients affected by cartilage defects will benefit from a cell-based transplantation strategy. Autologous chondrocytes, mesenchymal stem cells and embryonic stem cells are suitable donor cells for regeneration approaches and most recently the discovery of amniotic fluid stem cells has opened a

plethora of new therapeutic options. It is the aim of this review to summarize recent advances in the use of amniotic fluid stem cells as novel cell sources for the treatment of articular cartilage defects. Molecular aspects of articular cartilage formation as well as degeneration are summarized and the role of growth factor triggered signaling pathways, scaffolds, hypoxia and autophagy during the process of chondrogenic differentiation are discussed.

31. Amniotic fluid-derived stem cells as a cell source for bone tissue engineering.

Rodrigues MT, Lee SJ, Gomes ME, Reis RL, Atala A, Yoo JJ.

Tissue Eng Part A. 2012 Dec;18(23-24):2518-27.

In tissue engineering, stem cells have become an ideal cell source that can differentiate into most human cell types. Among the stem cells, bone marrow-derived stem cells (BMSCs) have been widely studied, and there is strong evidence that these cells can be differentiated into cells of the osteogenic lineage. Thus, BMSCs have become the gold standard for studies of tissue engineering in orthopedics. However, novel stem cell sources, such as amniotic fluid-derived stem cells (AFSCs) have been identified, and these have important and unique features that may lead to novel and successful applications toward the regeneration of bone tissue. This study was designed to originally compare the osteogenic potential of both BMSCs and

AFSCs under distinct culture environments to determine whether the osteogenic differentiation process of both types of stem cells is related to the origin of the cells. Osteogenic differentiation was carried out in both two and three dimensions using a tissue culture plate and by means of seeding the cells onto microfibrous starch and poly(ε-caprolactone) scaffolds (a blend of starch and polycaprolactone), respectively. BMSCs and AFSCs were successfully differentiated into the osteogenic cell type, as cells derived from them produced a mineralized extracellular matrix. Nevertheless, the two types of cells presented different expression patterns of bone-related markers as well as different timing of differentiation, indicating that both cell origin and the culture environment have a significant impact on the differentiation into the osteogenic phenotype in AFSCs and BMSCs.

32. Pdx1 and controlled culture conditions induced differentiation of human amniotic fluid-derived stem cells to insulin-producing clusters.

Chun SY, Mack DL, Moorefield E, Oh SH, Kwon TG, Pettenati MJ, Yoo JJ, Coppi PD, Atala A, Soker S.

J Tissue Eng Regen Med. 2012 Nov 13.

This study investigated the differentiation of human amniotic fluid-derived stem cells (hAFSCs) into insulin-producing clusters in vitro. Adenovirally-delivered mouse Pdx1 (Ad-Pdx1) induced human Pdx1 expression in hAFSCs and enhanced the coordinated expression of downstream β-cell markers. When Ad-Pdx1-transduced hAFSCs were sequentially treated with activin A, bFGF and nicotinamide and the culture plate surface coated with poly-l-ornithine, the expression of islet-associated human mRNAs for Pdx1, Pax6, Ngn3 and insulin was increased. C-peptide ELISA confirmed that Ad-Pdx1-transduced hAFSCs processed and secreted

insulin in a manner consistent with that pathway in pancreatic β-cells. To sustain the β-cell-like phenotype and investigate the effect of three-dimensional (3D) conformation on the differentiation of hAFSCs, Pdx1-transduced cells were encapsulated in alginate and cultured long-term under serum-free conditions. Over 2 weeks, partially differentiated hAFSC clusters increased in size and increased insulin secretion. Taken together, these data demonstrate that ectopic Pdx1 expression initiates pancreatic differentiation in hAFSCs and that a β-cell-like phenotype can be augmented by culture conditions that mimic the stromal components and 3D geometry associated with pancreatic islets.

33. Human amniotic fluid stem cell-derived muscle progenitor cell therapy for stress urinary incontinence.

Chun SY, Cho DH, Chae SY, Choi KH, Lim HJ, Yoon GS, Kim BS, Kim BW, Yoo JJ, Kwon TG.

J Korean Med Sci. 2012 Nov;27(11):1300-7.

The most promising treatment for stress urinary incontinence can be a cell therapy. We suggest human amniotic fluid stem cells (hAFSCs) as an alternative cell source. We established the optimum in vitro protocol for the differentiation from hAFSCs into muscle progenitors. These progenitors were transplanted into the injured urethral sphincter and their therapeutic effect was analyzed. For the development of an efficient differentiation system in vitro, we examined a commercial medium, co-culture and conditioned medium (CM) systems. After being treated with CM, hAFSCs were effectively developed into a muscle lineage. The progenitors were integrated into the host urethral sphincter and

the host cell differentiation was stimulated in vivo. Urodynamic analysis showed significant increase of leak point pressure and closing pressure. Immunohistochemistry revealed the regeneration of circular muscle mass with normal appearance. Molecular analysis observed the expression of a larger number of target markers. In the immunogenicity analysis, the progenitor group had a scant CD8 lymphocyte. In tumorigenicity, the progenitors showed no teratoma formation. These results suggest that hAFSCs can effectively be differentiated into muscle progenitors in CM and that the hAFSC-derived muscle progenitors are an accessible cell source for the regeneration of injured urethral sphincter.

34. Cell sources for trachea tissue engineering: past, present and future.

He X, Fu W, Zheng J.

Regen Med. 2012 Nov;7(6):851-63.

Trachea tissue engineering has been one of the most promising approaches to providing a potential clinical application for the treatment of long-segment tracheal stenosis. The sources of the cells are particularly important as the primary factor for tissue engineering. The use of appropriate cells seeded onto scaffolds holds huge promise as a means of engineering the trachea. Furthermore, appropriate cells would accelerate the regeneration of the tissue even without scaffolds. Besides autologous mature cells, various stem cells, including bone marrow-derived mesenchymal stem cells, adipose tissue-derived stem cells, umbilical cord blood-derived mesenchymal stem cells, amniotic fluid stem cells, embryonic stem cells and induced pluripotent stem cells, have received extensive attention in the field of

trachea tissue engineering. Therefore, this article reviews the progress on different cell sources for engineering tracheal cartilage and epithelium, which can lead to a better selection and strategy for engineering the trachea.

35. Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds.

Skardal A, Mack D, Kapetanovic E, Atala A, Jackson JD, Yoo J, Soker S.

Stem Cells Transl Med. 2012 Nov;1):792-802.

Stem cells obtained from amniotic fluid show high proliferative capacity in culture and multilineage differentiation potential. Because of the lack of significant immunogenicity and the ability of the amniotic fluid-derived stem (AFS) cells to modulate the inflammatory response, we investigated whether they could augment wound healing in a mouse model of skin regeneration. We used bioprinting technology to treat full-thickness skin wounds in nu/nu mice. AFS cells and bone marrow-derived mesenchymal stem cells (MSCs) were resuspended in fibrin-collagen gel and "printed" over the wound site. At days 0, 7, and 14, AFS cell- and MSC-driven wound closure and re-epithelialization were significantly greater than

closure and re-epithelialization in wounds treated by fibrin-collagen gel only. Histological examination showed increased microvessel density and capillary diameters in the AFS cell-treated wounds compared with the MSC-treated wounds, whereas the skin treated only with gel showed the lowest amount of microvessels. However, tracking of fluorescently labeled AFS cells and MSCs revealed that the cells remained transiently and did not permanently integrate in the tissue. These observations suggest that the increased wound closure rates and angiogenesis may be due to delivery of secreted trophic factors, rather than direct cell-cell interactions. Accordingly, we performed proteomic analysis, which showed that AFS cells secreted a number of growth factors at concentrations higher than those of MSCs. In parallel, we showed that AFS cell-conditioned media induced endothelial cell migration in vitro. Taken together, our results indicate that bioprinting AFS cells could be an effective treatment for large-scale wounds and burns.

36. Efficient direct reprogramming of mature amniotic cells into endothelial cells by ETS factors and TGFβ suppression.

Ginsberg M, James D, Ding BS, Nolan D, Geng F, Butler JM, Schachterle W, Pulijaal VR, Mathew S, Chasen ST, Xiang J, Rosenwaks Z, Shido K, Elemento O, Rabbany SY, Rafii S.

Cell. 2012 Oct 26;151(3):559-75.

ETS transcription factors ETV2, FLI1, and ERG1 specify pluripotent stem cells into induced vascular endothelial cells (iVECs). However, iVECs are unstable and drift toward nonvascular cells. We show that human midgestation c-Kit(-) lineage-committed amniotic cells (ACs) can be reprogrammed into vascular endothelial cells (rAC-VECs) without transitioning through a pluripotent state. Transient ETV2 expression in ACs generates immature rAC-VECs, whereas coexpression with FLI1/ERG1 endows rAC-VECs with a vascular repertoire and morphology matching mature endothelial

cells (ECs). Brief TGFβ-inhibition functionalizes VEGFR2 signaling, augmenting specification of ACs into rAC-VECs. Genome-wide transcriptional analyses showed that rAC-VECs are similar to adult ECs in which vascular-specific genes are expressed and nonvascular genes are silenced. Functionally, rAC-VECs form stable vasculature in Matrigel plugs and regenerating livers. Therefore, short-term ETV2 expression and TGFβ inhibition with constitutive ERG1/FLI1 coexpression reprogram mature ACs into durable rAC-VECs with clinical-scale expansion potential. Banking of HLA-typed rAC-VECs establishes a vascular inventory for treatment of diverse disorders.

37. Simvastatin induces osteogenic differentiation in human amniotic fluid mesenchymal stem cells (AFMSC).

de Lara Janz F, Favero GM, Bohatch MS Jr, Aguiar Debes A, Bydlowski SP.

Fundam Clin Pharmacol. 2012 Oct 24.

Amniotic fluid is a complex mixture composed of water, salts and different cells types derived from embryo exfoliation. Some of these cells present similar characteristics to mesenchymal stem cells as adherent properties, typical surface antigens and differentiation capacity. These cells are called amniotic fluid-derived mesenchymal stem cells (AFMSCs) and are easily obtained by amniocentesis, propagated in culture and differentiated in several cell types with specific inductions. In this study, we observe the ability of simvastatin, a 3-HMG-CoA reductase inhibitor, to induce AFSMCs osteogenic differentiation. When AFSMCs were incubated with medium containing simvastatin, it was observed morphological

changes, calcium deposits formation confirmed by Alizarin Red stain. Differentiated cells also expressed typical osteogenic genes, as osteopontin and osteocalcin. In conclusion, simvastatin could be used as an optional osteogenic induction agent for amniotic fluid-derived mesenchymal stem cells.

38. Role of amniotic fluid mesenchymal cells engineered on MgHA/collagen-based scaffold allotransplanted on an experimental animal study of sinus augmentation.

Berardinelli P, Valbonetti L, Muttini A, Martelli A, Peli R, Zizzari V, Nardinocchi D, Vulpiani MP, Tetè S, Barboni B, Piattelli A, Mattioli M.

Clin Oral Investig. 2012 Oct 14.

OBJECTIVES: The present research has been performed to evaluate whether a commercial magnesium-enriched hydroxyapatite (MgHA)/collagen-based scaffold engineered with ovine amniotic fluid mesenchymal cells (oAFMC) could improve bone regeneration process in vivo. MATERIALS AND METHODS: Bilateral sinus augmentation was performed on eight adult sheep in order to compare the tissue regeneration process at 45 and 90 days after implantation of the oAFMC-engineered scaffold (Test Group) or of the scaffold alone (Ctr Group). The process

of tissue remodeling was analyzed through histological, immunohistochemical, and morphometric analyses by calculating the proliferation index (PI) of oAFMC loaded on the scaffold, the total vascular area (VA), and vascular endothelial growth factor (VEGF) expression levels within the grafted area. RESULTS: MgHA/collagen-based scaffold showed high biocompatibility preserving the survival of oAFMC for 90 days in grafted sinuses. The use of oAFMC increased bone deposition and stimulated a more rapid angiogenic reaction, thus probably supporting the higher cell PI recorded in cell-treated sinuses. A significantly higher VEGF expression (Test vs. Ctr Group; $p = 0.0004$) and a larger total VA ($p = 0.0006$) were detected in the Test Group at 45 days after surgery. The PI was significantly higher ($p = 0.027$) at 45 days and became significantly lower at 90 days ($p = 0.0007$) in the Test Group sinuses, while the PI recorded in the Ctr Group continued to increase

resulting to a significantly higher PI at day 90 (CTR day 45 vs. CTR day 90; $p = 0.022$). CONCLUSIONS: The osteoinductive effect of a biomimetic commercial scaffold may be significantly improved by the presence of oAFMC. CLINICAL RELEVANCE: The amniotic fluid mesenchymal cell (AFMC) may represent a novel, largely and easily accessible source of mesenchymal stem cells to develop cell-based therapy for maxillofacial surgery.

39. Amniotic Fluid Stem Cells Rescue Both In Vitro And In Vivo Growth, Innervation And Motility In Nitrofen-Exposed Hypoplastic Rat Lungs Through Paracrine Effects.

Pederiva F, Ghionzoli M, Pierro A, De Coppi P, Tovar JA.

Cell Transplant. 2012 Oct 8.

Background: Lung hypoplasia can be prevented in vitro by retinoic acid (RA). Recent evidence suggests that amniotic fluid stem (AFS) cells may integrate injured lungs and influence their recovery. We tests the hypothesis that AFS cells might improve lung growth and motility by paracrine mechanisms. Material and methods: Pregnant rats received either nitrofen or vehicle on E9.5. In vitro E13 embryonic lungs were cultured in presence of culture medium alone or with RA, basophils or AFS cells. In vivo GFP+rat-AFS-cells were transplanted in nitrofen-exposed rats on E10.5. E13 lung explants were cultured before analysis. The

surface, the number of terminal buds and the frequency of bronchial contractions were assessed. PGP 9.5 and α-actin protein levels were measured. The lung explants transplanted with AFS cells were stained for α-actin, PGP9.5 and TTF-1. FGF10, VEGFα, and TGFβ1 levels secreted by the AFS cells in the culture medium were measured. Comparison between groups was made by Anova tests.Results: In vitro The surface, the number of terminal buds and the bronchial peristalsis were increased in nitrofen+AFS-cells explants in comparison with nitrofen-exposed lungs. Whilenitrofen+RA lungs were similar to nitrofen+AFS ones, basophils did not normalize these measurements. PGP9.5 protein was decreased in nitrofen lungs, but after adding AFS cells, the value was similar to controls. No differences were found in the expression of α-actin. In vivo Surface, number of terminal buds and peristalsis were similar to control after injection of AFS cells in nitrofen-exposed rats. Colocalization with

TTF-1-positive cells was found. The levels of FGF10 and VEGFα were increased in nitrofen+AFS-cells explants setting, while the levels of TGFβ1 were similar to controls.Conclusions: Lung growth, bronchial motility and innervation are decreased in nitrofen explants and rescued by AFS cells both in vitro and in vivo, similarly to what observed before with RA. AFS cells beneficial effect was probably related to paracrine action of growth factors secretion.

40. Xenografted human amniotic fluid-derived stem cell as a cell source in therapeutic angiogenesis.

Liu YW, Roan JN, Wang SP, Hwang SM, Tsai MS, Chen JH, Hsieh PC.

Int J Cardiol. 2012 Oct 6. S0167-5273(12)01178-3.

BACKGROUND: Amniotic fluid-derived stem cells (AFSCs) are pluripotent with high renewal capacity and are not tumorigenic. We tested whether AFSCs can function as a cell source for therapeutic angiogenesis in a mouse hindlimb ischemia model. METHODS: Using a defined culture medium for endothelial lineage cells (ECs), we differentiated human AFSCs into AFSC-derived ECs (AFSC-ECs) in vitro, as evidenced by expression of EC markers, and capillary-like network formation on Matrigel. We assessed the in vivo therapeutic angiogenesis efficacy of AFSC-ECs in an athymic nude mouse model of hindlimb ischemia. One day after high ligation of the

external iliac artery in athymic nude mice, AFSC-ECs were intramuscularly injected into ischemic limbs. RESULTS: The AFSC-ECs demonstrated endothelial cell characteristics in vitro. Four weeks later, AFSC-ECs transplantation significantly increased limb salvage (85%), compared to AFSCs (56%), human umbilical vein endothelial cells (HUVECs; 25%), or medium (0%). Laser Doppler perfusion analysis revealed that the ischemic/normal limb blood perfusion ratio significantly improved in the AFSC-EC group. AFSC-EC transplantation significantly increased capillary and arteriole densities as compared to AFSCs, HUVECs, and medium. Transplanted AFSC-ECs were incorporated into vessels in the ischemic region, as confirmed by immunofluorescent staining for human smooth muscle 22α or von Willebrand factor. Matrix metalloproteinase (MMP)-3 and MMP-9 expressions were significantly higher in AFSC-ECs. MMP-9 might activate angiogenesis by regulation of vascular

endothelial growth factor. CONCLUSIONS: Our study indicated that AFSC-EC transplantation improved limb salvage and blood perfusion by promoting neovascularization. Therefore, AFSC-ECs possess the potential for therapeutic angiogenesis.

41. Valproic acid confers functional pluripotency to human amniotic fluid stem cells in a transgene-free approach.

Moschidou D, Mukherjee S, Blundell MP, Drews K, Jones GN, Abdulrazzak H, Nowakowska B, Phoolchund A, Lay K, Ramasamy TS, Cananzi M, Nettersheim D, Sullivan M, Frost J, Moore G, Vermeesch JR, Fisk NM, Thrasher AJ, Atala A, Adjaye J, Schorle H, De Coppi P, Guillot PV.

Mol Ther. 2012 Oct;20(10):1953-67.

Induced pluripotent stem cells (iPSCs) with potential for therapeutic applications can be derived from somatic cells via ectopic expression of a set of limited and defined transcription factors. However, due to risks of random integration of the reprogramming transgenes into the host genome, the low efficiency of the process, and the potential risk of virally induced tumorigenicity, alternative methods have been developed to generate pluripotent cells using nonintegrating systems, albeit with limited success. Here, we show that c-KIT+ human first-trimester amniotic fluid

stem cells (AFSCs) can be fully reprogrammed to pluripotency without ectopic factors, by culture on Matrigel in human embryonic stem cell (hESC) medium supplemented with the histone deacetylase inhibitor (HDACi) valproic acid (VPA). The cells share 82% transcriptome identity with hESCs and are capable of forming embryoid bodies (EBs) in vitro and teratomas in vivo. After long-term expansion, they maintain genetic stability, protein level expression of key pluripotency factors, high cell-division kinetics, telomerase activity, repression of X-inactivation, and capacity to differentiate into lineages of the three germ layers, such as definitive endoderm, hepatocytes, bone, fat, cartilage, neurons, and oligodendrocytes. We conclude that AFSC can be utilized for cell banking of patient-specific pluripotent cells for potential applications in allogeneic cellular replacement therapies, pharmaceutical screening, and disease modeling.

42. Protective effects of human amniotic fluid stem cells in a model of aorta allograft vasculopathy in rats.

Santana AC, Dellê H, Cavaglieri RC, Lopes MA, Francisco RP, Zugaib M, Bydlowski SP, Noronha IL.

Transplant Proc. 2012 Oct;44(8):2490-4.

BACKGROUND: Chronic allograft vasculopathy (CAV) is an important cause of graft loss. Considering the immune inflammatory events involved in the development of CAV, therapeutic approaches to target this process are of relevance. Human amniotic fluid-derived stem cells (hAFSCs), a class of fetal, pluripotent stem cells with intermediate characteristics between embryonic and adult stem cells, display immunomodulatory properties. hAFSCs express mesenchymal and embryonic markers, show high proliferation rates; however, they do not induce tumor formation, and their use does not raise ethical issues. Thus, we sought to investigate the effect of

hAFSC on CAV in a model of aorta transplantation. METHODS: Orthotopic aorta transplantation was performed using Fisher (F344) rats as donors and Lewis rats as recipients. Rats were divided into three groups: syngeneic (SYNG), untreated F344 receiving aorta from F344 (n = 8); allogeneic (ALLO), Lewis rats receiving allogeneic aorta from F344 (n = 8); and ALLO +hAFSC, ALLO rats treated with hAFSC (10(6) cells; n = 8). Histological analysis and immunohistochemistry were performed 30 days posttransplantation. RESULTS: The ALLO group developed a robust aortic neointimal formation (208.7 ± 25.4 μm) accompanied by a significant high number of ED1+ (4845 ± 841 cells/mm2) and CD43+ cells (4064 ± 563 cells/mm2), and enhanced expression of α-smooth muscle actin in the neointima (25 ± 6%). Treatment with hAFSC diminished neointimal thickness (180.7 ± 23.7 μm) and induced a significant decrease of ED1+ (1100 ± 276 cells/mm2), CD43+

cells (1080 ± 309 cells/μm2), and α-smooth muscle actin expression 8 ± 3% in the neointima. CONCLUSIONS: These preliminary results showed that hAFSC suppressed inflammation and myofibroblast migration to the intima, which may contribute to ameliorate vascular changes in CAV.

43. The effect of differentiation stage of amniotic fluid stem cells on bone regeneration.

Rodrigues MT, Lee BK, Lee SJ, Gomes ME, Reis RL, Atala A, Yoo JJ.

Biomaterials. 2012 Sep;33(26):6069-78.

Bone tissue engineering strategies require cells with high proliferative and osteogenic potential as well as a suitable scaffold to support the development of these as they form new bone tissue. In this study, we evaluated whether the differentiation stage of amniotic fluid stem cells (AFSC) could enhance the regeneration of critical sized femoral defects in a rat model. For this purpose, AFSC were seeded onto a starch-poly(ε-caprolactone) (SPCL) scaffold and were cultured in vitro in osteogenic culture media for different periods of time in order to obtain: i) undifferentiated cells, ii) cells committed to the osteogenic phenotype and iii) "osteoblast-like" cells. In vitro results indicate that AFSC were considered to be osteogenically committed by

the end of week 2 and osteoblastic-like after week 3 in culture. Constructs composed of AFSC-SPCL scaffolds from each differentiation stage were implanted into critical sized femoral defects. The quality of new tissue formed in the defects was evaluated based on micro-CT imaging and histological analysis of constructs retrieved at 4 and 16 weeks after implantation. In vivo formation of new bone was observed under all conditions. However, the most complete repair of the defect was observed after 16 weeks in the animals receiving the SPCL scaffolds seeded with osteogenically committed AFSC. Furthermore, the presence of blood vessels was noted in the inner sections of the scaffolds suggests that these cells could potentially be used to induce bone regeneration and angiogenesis in non-union bone defects.

44. Potential of placenta-derived mesenchymal stem cells as seed cells for bone tissue engineering: preliminary study of osteoblastic differentiation and immunogenicity.

Zhong ZN, Zhu SF, Yuan AD, Lu GH, He ZY, Fa ZQ, Li WH.

Orthopedics. 2012 Sep;35(9):779-88.

Mesenchymal stem cells (MSCs) have been isolated from a variety of human tissues (eg, bone marrow, peripheral blood, muscle, fat, umbilical blood, amniotic fluid, embryonic tissues, and placenta). Placenta-derived MSCs (PDMSCs) have received considerable interest because of their wide availability and absence of ethical concerns. The authors characterized the biological properties, ultrastructure, growth factor production, and osteoblastic differentiation of PDMSCs and investigated their potential as seed cells for bone tissue engineering.

45. Human amniotic fluid stem cell injection therapy for urethral sphincter regeneration in an animal model.

Kim BS, Chun SY, Lee JK, Lim HJ, Bae JS, Chung HY, Atala A, Soker S, Yoo JJ, Kwon TG.

BMC Med. 2012 Aug 21;10:94.

BACKGROUND: Stem cell injection therapies have been proposed to overcome the limited efficacy and adverse reactions of bulking agents. However, most have significant limitations, including painful procurement, requirement for anesthesia, donor site infection and a frequently low cell yield. Recently, human amniotic fluid stem cells (hAFSCs) have been proposed as an ideal cell therapy source. In this study, we investigated whether periurethral injection of hAFSCs can restore urethral sphincter competency in a mouse model. METHODS: Amniotic fluids were collected and harvested cells were analyzed for stem cell characteristics and in vitro myogenic

differentiation potency. Mice underwent bilateral pudendal nerve transection to generate a stress urinary incontinence (SUI) model and received either periurethral injection of hAFSCs, periurethral injection of Plasma-Lyte (control group), or underwent a sham(normal control group).For in vivo cell tracking, cells were labeled with silica-coated magnetic nanoparticles containing rhodamine B isothiocyanate (MNPs@SiO2 (RITC)) and were injected into the urethral sphincter region (n = 9). Signals were detected by optical imaging. Leak point pressure and closing pressure were recorded serially after injection.Tumorigenicity of hAFSCs was evaluated by implanting hAFSCs into the subcapsular space of the kidney, followed two weeks later by retrieval and histologic analysis. RESULTS: Flow activated cell sorting showed that hAFSCs expressed mesenchymal stem cell (MSC) markers, but no hematopoietic stem cell markers. Induction of myogenic differentiation in the

hAFSCs resulted in expression of PAX7 and MYOD at Day 3, and DYSTROPHIN at Day 7. The nanoparticle-labeled hAFSCs could be tracked in vivo with optical imaging for up to 10 days after injection. Four weeks after injection, the mean LPP and CP were significantly increased in the hAFSC-injected group compared with the control group. Nerve regeneration and neuromuscular junction formation of injected hAFSCs in vivo was confirmed with expression of neuronal markers and acetylcholine receptor. Injection of hAFSCs caused no in vivo host CD8 lymphocyte aggregation or tumor formation. CONCLUSIONS: hAFSCs displayed MSC characteristics and could differentiate into cells of myogenic lineage. Periurethral injection of hAFSCs into an SUI animal model restored the urethral sphincter to apparently normal histology and function, in absence of immunogenicity and tumorigenicity.

46. Generation, characterization, and potential therapeutic applications of cardiomyocytes from various stem cells.

Liu J, Zhang Z, Liu Y, Guo C, Gong Y, Yang S, Ma M, Li Z, Gao WQ, He Z.

Stem Cells Dev. 2012 Aug 10;21(12):2095-110.

Heart failure is one of the leading causes of death worldwide. Myocardial cell transplantation emerges as a novel therapeutic strategy for heart failure, but this approach has been hampered by severe shortage of human cardiomyocytes. We have recently induced mouse embryonic stem cells to differentiate into embryoid bodies and eventually, cardiomyocytes. Here, we address recent advancements in cardiomyocyte differentiation from cardiac stem cells and pluripotent stem cells. We highlight the methodologies, using growth factors, endoderm-like cell cocultures, small molecules, and biomaterials, in directing the differentiation of pluripotent stem cells into

cardiomyocytes. The characterization and identification of pluripotent stem cell-derived cardiomyocytes by morphological, phenotypic, and functional features are also discussed. Notably, increasing evidence demonstrates that cardiomyocytes may be generated from the stem cells of several tissues outside the cardiovascular system, including skeletal muscles, bone marrow, testes, placenta, amniotic fluid, and adipose tissues. We further address the potential applications of cardiomyocytes derived from various kinds of stem cells. The differentiation of stem cells into functional cardiomyocytes, especially from an extra-cardiac stem cell source, would circumvent the scarcity of heart donors and human cardiomyocytes, and, most importantly, it would offer an ideal and promising cardiomyocyte source for cell therapy and tissue engineering in treating heart failure.

47. High efficiency of reprogramming CD34^{+} cells derived from human amniotic fluid into induced pluripotent stem cells with Oct4.

Liu T, Zou G, Gao Y, Zhao X, Wang H, Huang Q, Jiang L, Guo L, Cheng W.

Stem Cells Dev. 2012 Aug 10;21(12):2322-32.

Although many techniques can be used to generate multitype-induced pluripotent stem (iPS) cells from multitype seed cells, improving the efficiency and shortening the period of cell reprogramming remain troublesome issues. In this study, to generate iPS cells, CD34^{+} cells, isolated from human amniotic fluid cells (HuAFCs) by flow cytometry, were infected with retroviruses carrying only one reprogramming factor (Oct4) and cultured on human amniotic epithelial cell (HuAEC) feeder layers. Approximately 4 to 5 days after viral infection, some embryonic stem cell (ESC)-like colonies appeared among the feeder cells.

These colonies were positive for alkaline phosphatase and expressed high levels of ESC pluripotent markers (Nanog, Sox2, Oct4, CD133, and Rex1). Moreover, these iPS cells exhibited high levels of telomerase activity and had normal karyotypes. Additionally, these cells could differentiate into cell types from all 3 germ layers in vivo and in teratomas. In summary, we report a novel way of iPS generation that uses $CD34^+$ HuAFCs as seed cells. Using this method, we can generate human iPS cells with greater efficiency and safety (the oncogenic factors, c-Myc and Klf4, were not used), and using the minimum number of reprogramming factors (only one factor, Oct4). Besides, HuAECs were used as feeder layers to culture human iPS cells, which could not only avoid contamination with heterogeneous proteins, but also maintain iPS cells in a self-renewing and undifferentiated state for a long time.

48. Proangiogenic soluble factors from amniotic fluid stem cells mediate the recruitment of endothelial progenitors in a model of ischemic fasciocutaneous flap.

Mirabella T, Hartinger J, Lorandi C, Gentili C, van Griensven M, Cancedda R.

Stem Cells Dev. 2012 Aug 10;21(12):2179-88.

Skin flaps are routinely used in surgery for the functional and cosmetic repair of wounds or disfiguring scars. The recent concept of therapeutic angiogenesis has emerged as an attractive approach to overcome the problem of blood supply deficiency, often resulting in the flap grafting failure. In the present study, we embedded a gelatin membrane with amniotic fluid stem cells (AFSC) derived conditioned media (ACM) to topically deliver angiogenic growth factors and cytokines into a rat model of ischemic full-thickness skin flap elevated in the epigastric region. AFSC secretome triggered the endogenous repair by the recruitment of endothelial progenitor

cells. We studied the vascular perfusion rate, the vessel distribution, and the survival of ACM-treated flaps. In detail, the ischemic sectors of ACM-treated flaps showed at day 7 a perfusion level 50% higher than the preoperation baseline. The ensuing necrosis development was delayed and the histology analysis showed a normal arrangement of epidermal and dermal structures and a high density of vessels in subcutaneous tissues. Further, we found that ACM recruited $CD31^{+}/VEGFR2^{+}$ and $CD31^{+}/CD34^{+}$ cells into the ischemic subcutaneous tissues and that the isolated progenitors were capable to form clusters of von Willebrand factor-positive cells in culture. We propose ACM as a cell-free cocktail of chemokines and growth factors to be adopted for clinical applications.

49. Potential antitumor therapeutic strategies of human amniotic membrane and amniotic fluid-derived stem cells.

Kang NH, Hwang KA, Kim SU, Kim YB, Hyun SH, Jeung EB, Choi KC.

Cancer Gene Ther. 2012 Aug;19(8):517-22.

As stem cells are capable of self-renewal and can generate differentiated progenies for organ development, they are considered as potential source for regenerative medicine and tissue replacement after injury or disease. Along with this capacity, stem cells have the therapeutic potential for treating human diseases including cancers. According to the origins, stem cells are broadly classified into two types: embryonic stem cells (ESCs) and adult stem cells. In terms of differentiation potential, ESCs are pluripotent and adult stem cells are multipotent. Amnion, which is a membranous sac that contains the fetus and amniotic fluid and functions in protecting the developing embryo during gestation, is another stem cell source. Amnion-derived stem cells are classified as human amniotic membrane-derived epithelial stem

cells, human amniotic membrane-derived mesenchymal stem cells and human amniotic fluid-derived stem cells. They are in an intermediate stage between pluripotent ESCs and lineage-restricted adult stem cells, non-tumorigenic, and contribute to low immunogenicity and anti-inflammation. Furthermore, they are easily available and do not cause any controversial issues in their recovery and applications. Not only are amnion-derived stem cells applicable in regenerative medicine, they have anticancer capacity. In non-engineered stem cells transplantation strategies, amnion-derived stem cells effectively target the tumor and suppressed the tumor growth by expressing cytotoxic cytokines. Additionally, they also have a potential as novel delivery vehicles transferring therapeutic genes to the cancer formation sites in gene-directed enzyme/prodrug combination therapy. Owing to their own advantageous properties, amnion-derived stem cells are emerging as a new candidate in anticancer therapy.

50. Clone-derived human AF-amniotic fluid stem cells are capable of skeletal myogenic differentiation in vitro and in vivo.

Ma X, Zhang S, Zhou J, Chen B, Shang Y, Gao T, Wang X, Xie H, Chen F.

J Tissue Eng Regen Med. 2012 Aug;6(8):598-613.

Stem cell-based therapy may be the most promising method to cure skeletal muscle degenerative diseases such as Duchenne muscular dystrophy (DMD) and trauma in the future. Human amniotic fluid is enriched with early-stage stem cells from developing fetuses and these cells have cardiomyogenic potential both in vitro and in vivo. In the present study, we investigated the characteristics of human amniotic fluid-derived AF-type stem (HAF-AFS) cells by flow cytometry, immunofluorescence staining, reverse-transcription polymerase chain reaction, and osteogenic and adipogenic differentiation analysis. After confirming the stemness of

HAF-AFS cells, we tested whether HAF-AFS cells could differentiate into skeletal myogenic cells in vitro and incorporate into regenerating skeletal muscle in vivo. By temporary exposure to the DNA demethylation agent 5-aza-2'-deoxycytidine (5-Aza dC) or co-cultured with C2C12 myoblasts, HAF-AFS cells differentiated into skeletal myogenic cells, expressing skeletal myogenic cell-specific markers such as Desmin, Troponin I (Tn I) and α-Actinin. Four weeks after transplantation into cardiotoxin-injured and X-ray-irradiated tibialis anterior (TA) muscles of NOD/SCID mice, HAF-AFS cells survived, differentiated into myogenic precursor cells and fused with host myofibres. The findings that HAF-AFS cells differentiate into myogenic cells in vitro and incorporate in skeletal muscle regeneration in vivo hold the promise of HAF-AFS cell-based therapy for skeletal muscle degenerative diseases.

51. Amniotic fluid stem cells restore the muscle cell niche in a HSA-Cre, Smn(F7/F7) mouse model.

Piccoli M, Franzin C, Bertin E, Urbani L, Blaauw B, Repele A, Taschin E, Cenedese A, Zanon GF, André-Schmutz I, Rosato A, Melki J, Cavazzana-Calvo M, Pozzobon M, De Coppi P.

Stem Cells. 2012 Aug;30(8):1675-84.

Mutations in the survival of motor neuron gene (SMN1) are responsible for spinal muscular atrophy, a fatal neuromuscular disorder. Mice carrying a homozygous deletion of Smn exon 7 directed to skeletal muscle (HSA-Cre, Smn(F7/F7) mice) present clinical features of human muscular dystrophies for which new therapeutic approaches are highly warranted. Herein we demonstrate that tail vein transplantation of mouse amniotic fluid stem (AFS) cells enhances the muscle strength and improves the survival rate of the affected animals. Second, after cardiotoxin injury of the

Tibialis Anterior, only AFS-transplanted mice efficiently regenerate. Most importantly, secondary transplants of satellite cells (SCs) derived from treated mice show that AFS cells integrate into the muscle stem cell compartment and have long-term muscle regeneration capacity indistinguishable from that of wild-type-derived SC. This is the first study demonstrating the functional and stable integration of AFS cells into the skeletal muscle, highlighting their value as cell source for the treatment of muscular dystrophies.

52. Establishment of an internal control for directed differentiation using pluripotent stem cell lines derived from heterozygotic twins.

Luo YM, Fan Y, Chen XJ, Yue L, Li Q, He WZ, Ma XY, Zheng YH, Sun XF.

Zhonghua Yi Xue Yi Chuan Xue Za Zhi. 2012 Aug;29(4):398-403.

OBJECTIVE: To reprogram amniotic fluid cells into pluripotent stem cells in order to create an optimal internal control model for directed cell differentiation. METHODS: Human amniotic fluid-derived cells (hAFDCs) from heterozygotic twin fetuses were induced by retroviral vectors encoding Oct4, Sox2, c-Myc and Klf4. In vivo pluripotency, differentiation capacity and karyotype of hAFDCs induced pluripotent stem cells (hAFDCs-iPSCs) were determined. RESULTS: hAFDC-iPSCs derived from heterozygotic twins have maintained self renewal, with expression of high pluripotency marker gene detected at both mRNA and

protein levels. The cells have maintained their differentiation capacity both in vitro and vivo, and showed normal karyotypes after long-term culturing in vitro. CONCLUSION: hAFDCs-iPSCs derived from heterozygotic twins have good consistency in terms of genetic background, and can provide a good internal control for directed differentiation of iPSCs, and may be used an ideal source for autologous cell replacement therapy in the later life of the fetus.

53. Human amniotic fluid stem cell preconditioning improves their regenerative potential.

Rota C, Imberti B, Pozzobon M, Piccoli M, De Coppi P, Atala A, Gagliardini E, Xinaris C, Benedetti V, Fabricio AS, Squarcina E, Abbate M, Benigni A, Remuzzi G, Morigi M.

Stem Cells Dev. 2012 Jul 20;21(11):1911-23.

Human amniotic fluid stem (hAFS) cells, a novel class of broadly multipotent stem cells that share characteristics of both embryonic and adult stem cells, have been regarded as promising candidate for cell therapy. Taking advantage by the well-established murine model of acute kidney injury (AKI), we studied the proregenerative effect of hAFS cells in immunodeficient mice injected with the nephrotoxic drug cisplatin. Infusion of hAFS cells in cisplatin mice improved renal function and limited tubular damage, although not to control level, and prolonged animal survival. Human AFS cells engrafted injured kidney predominantly in peritubular region without

acquiring tubular epithelial markers. Human AFS cells exerted antiapoptotic effect, activated Akt, and stimulated proliferation of tubular cells possibly via local release of factors, including interleukin-6, vascular endothelial growth factor, and stromal cell-derived factor-1, which we documented in vitro to be produced by hAFS cells. The therapeutic potential of hAFS cells was enhanced by cell pretreatment with glial cell line-derived neurotrophic factor (GDNF), which markedly ameliorated renal function and tubular injury by increasing stem cell homing to the tubulointerstitial compartment. By in vitro studies, GDNF increased hAFS cell production of growth factors, motility, and expression of receptors involved in cell homing and survival. These findings indicate that hAFS cells can promote functional recovery and contribute to renal regeneration in AKI mice via local production of mitogenic and prosurvival factors. The effects of hAFS cells can be remarkably enhanced by GDNF preconditioning.

54. Stem cells for heart valve regeneration.

Weber B, Emmert MY, Hoerstrup SP.

Swiss Med Wkly. 2012 Jul 16;142:w13622.

Heart valve tissue engineering holds the potential to overcome limitations of currently used heart valve prostheses. It involves the isolation and expansion of autologous patient cells, the subsequent seeding of these cells onto an appropriate scaffold material, the in vitro incubation and the in vivo implantation of the derived tissue-engineered construct into the patient from whom the cells were taken. While vascular-derived cells require harvest of intact donor tissue and show limited expansion capacities, the use of stem or progenitor cells may overcome these limitations and expand the versatility of the concept of heart valve tissue engineering. Possible sources include cells isolated from blood, bone marrow, adipose tissue, amniotic fluid, chorionic villi, umbilical cord and induced pluripotent stem cells. Here we

review different stem cell sources with particular regard to cellular phenotypes and their suitability for application in heart valve tissue engineering.

55. Bilayered constructs aimed at osteochondral strategies: the influence of medium supplements in the osteogenic and chondrogenic differentiation of amniotic fluid-derived stem cells.

Rodrigues MT, Lee SJ, Gomes ME, Reis RL, Atala A, Yoo JJ.

Acta Biomater. 2012 Jul;8(7):2795-806.

The development of osteochondral tissue engineered interfaces would be a novel treatment for traumatic injuries and aging associated diseases that affect joints. This study reports the development of a bilayered scaffold, which consists of both bone and cartilage regions. On the other hand, amniotic fluid-derived stem cells (AFSCs) could be differentiated into either osteogenic or chondrogenic cells, respectively. In this study we have developed a bilayered scaffolding system, which includes a starch/polycaprolactone (SPCL) scaffold for osteogenesis and an agarose hydrogel for chondrogenesis. AFSC-seeded scaffolds were

cultured for 1 or 2 weeks in an osteochondral-defined culture medium containing both osteogenic and chondrogenic differentiation factors. Additionally, the effect of the presence or absence of insulin-like growth factor-1 (IGF-1) in the culture medium was assessed. Cell viability and phenotypic expression were assessed within the constructs in order to determine the influence of the osteochondral differentiation medium. The results indicated that, after osteogenic differentiation, AFSCs that had been seeded onto SPCL scaffolds did not require osteochondral medium to maintain their phenotype, and they produced a protein-rich, mineralized extracellular matrix (ECM) for up to 2 weeks. However, AFSCs differentiated into chondrocyte-like cells appeared to require osteochondral medium, but not IGF-1, to synthesize ECM proteins and maintain the chondrogenic phenotype. Thus, although IGF-1 was not essential for creating osteochondral constructs with

AFSCs in this study, the osteochondral supplements used appear to be important to generate cartilage in long-term tissue engineering approaches for osteochondral interfaces. In addition, constructs generated from agarose-SPCL bilayered scaffolds containing pre-differentiated AFSCs may be useful for potential applications in regeneration strategies for damaged or diseased joints.

56. Stem cells from umbilical cord and placenta for musculoskeletal tissue engineering.

Longo UG, Loppini M, Berton A, La Verde L, Khan WS, Denaro V.

Curr Stem Cell Res Ther. 2012 Jul;7(4):272-81.

Mesenchymal stem cells isolated from amnion/amniotic fluid, umbilical cord blood, placental tissue, umbilical cord vein and the Wharton's Jelly are promising candidates for musculoskeletal tissue engineering of bone and cartilage tissues. The extracorporeal nature of this source avoids the ethical concerns that plague the isolation of embryonic stem cells. Moreover, the harvesting does not require the invasive and discomfort extraction procedures as well as patient risks that attend adult stem cell isolation. Current preclinical studies support the application of these cell-based therapies for the regeneration of musculoskeletal tissues. We performed a review of the

literature to focus on actual knowledge and the future perspectives of the stem cells deriving from umbilical cord and placenta for musculoskeletal tissue engineering.

57. Tissue engineering of reproductive tissues and organs.
Atala A.
Fertil Steril. 2012 Jul;98(1):21-9.

Regenerative medicine and tissue engineering technology may soon offer new hope for patients with serious injuries and end-stage reproductive organ failure. Scientists are now applying the principles of cell transplantation, material science, and bioengineering to construct biological substitutes that can restore and maintain normal function in diseased and injured reproductive tissues. In addition, the stem cell field is advancing, and new discoveries in this field will lead to new therapeutic strategies. For example, newly discovered types of stem cells have been retrieved from uterine tissues such as amniotic fluid and placental stem cells. The process of therapeutic cloning and the creation of induced pluripotent cells provide still other potential sources of stem cells for cell-based tissue engineering applications.

Although stem cells are still in the research phase, some therapies arising from tissue engineering endeavors that make use of autologous adult cells have already entered the clinic. This article discusses these tissue engineering strategies for various organs in the male and female reproductive tract.

58. CD117(+) amniotic fluid stem cells: state of the art and future perspectives.
Cananzi M, De Coppi P.
Organogenesis. 2012 Jul-Sep;8(3):77-88.

Broadly multipotent stem cells can be isolated from amniotic fluid by selection for the expression of the membrane stem cell factor receptor c-Kit, a common marker for multipotential stem cells. They have clonogenic capability and can be directed into a wide range of cell types representing the three primary embryonic lineages. Amniotic fluid stem cells maintained for over 250 population doublings retained long telomeres and a normal karyotype. Clonal human lines verified by retroviral marking were induced to differentiate into cell types representing each embryonic germ layer, including cells of adipogenic, osteogenic, myogenic, endothelial, neuronal and hepatic lineages. AFS cells could be differentiate toward cardiomyogenic lineages, when co-cultured with neonatal cardiomyocytes, and have the

potential to generate myogenic and hematopoietic lineages both in vitro and in vivo. Very recently first trimester AFS cells could be reprogrammed without any genetic manipulation opening new possibilities in the field of fetal/neonatal therapy and disease modeling. In this review we are aiming to summarize the knowledge on amniotic fluid stem cells and highlight the most promising results.

59. Congenital anomalies: treatment options based on amniotic fluid-derived stem cells.

Kunisaki SM.

Organogenesis. 2012 Jul-Sep;8(3):89-95.

Over the past decade, amniotic fluid-derived stem cells have emerged as a novel, experimental approach for the treatment of a wide variety of congenital anomalies diagnosed either in utero or postnatally. There are a number of unique properties of amniotic fluid stem cells that have allowed it to become a major research focus. These include the relative ease of accessing amniotic fluid cells in a minimally invasive fashion by amniocentesis as well as the relatively rich population of progenitor cells obtained from a small aliquot of fluid. Mesenchymal stem cells, c-kit positive stem cells, as well as induced pluripotent stem cells have all been derived from human amniotic fluid in recent years. This article gives a pediatric surgeon's perspective on amniotic fluid stem cell

therapy for the management of congenital anomalies. The current status in the use of amniotic fluid-derived stem cells, particularly as they relate as substrates in tissue engineering-based applications, is described in various animal models. A roadmap for further study and eventual clinical application is also proposed.

60. Prenatally engineered autologous amniotic fluid stem cell-based heart valves in the fetal circulation.

Weber B, Emmert MY, Behr L, Schoenauer R, Brokopp C, Drögemüller C, Modregger P, tampanoni M, Vats D, Rudin M, Bürzle W, Farine M, Mazza E, Frauenfelder T, Zannettino AC, Zünd G, Kretschmar O, Falk V, Hoerstrup SP.

Biomaterials. 2012 Jun;33(16):4031-43.

Prenatal heart valve interventions aiming at the early and systematic correction of congenital cardiac malformations represent a promising treatment option in maternal-fetal care. However, definite fetal valve replacements require growing implants adaptive to fetal and postnatal development. The presented study investigates the fetal implantation of prenatally engineered living autologous cell-based heart valves. Autologous amniotic fluid cells (AFCs) were isolated from pregnant sheep between 122 and 128 days of gestation via transuterine

sonographic sampling. Stented trileaflet heart valves were fabricated from biodegradable PGA-P4HB composite matrices (n = 9) and seeded with AFCs in vitro. Within the same intervention, tissue engineered heart valves (TEHVs) and unseeded controls were implanted orthotopically into the pulmonary position using an in-utero closed-heart hybrid approach. The transapical valve deployments were successful in all animals with acute survival of 77.8% of fetuses. TEHV in-vivo functionality was assessed using echocardiography as well as angiography. Fetuses were harvested up to 1 week after implantation representing a birth-relevant gestational age. TEHVs showed in vivo functionality with intact valvular integrity and absence of thrombus formation. The presented approach may serve as an experimental basis for future human prenatal cardiac interventions using fully biodegradable autologous cell-based living materials.

61. Injectable PLGA porous beads cellularized by hAFSCs for cellular cardiomyoplasty.

Huang C, Wei J, Yeh C, Wang J, Lin W, Lee Y, Hwang M, Choi W, Xia Y, Chang Y, Sung W.

Biomaterials. 2012 Jun;33(16):4069-77.

Cellular cardiomyoplasty has been limited by poor graft retention after cell transplantation. To ensure good retention of the engrafted cells, a microfluidic device was used to fabricate spherical porous beads of poly(D,L-lactic-co-glycolic acid) as a platform for cell delivery. The beads thus obtained had a relatively uniform size, a highly porous structure, and a favorably interconnected interior architecture, to facilitate the transportation of oxygen and nutrients. These porous beads were loaded with human amniotic fluid stem cells (hAFSCs) to generate cellularized microscaffolds. Live/dead assay demonstrated that most of the cells in the porous constructs were viable. The hAFSCs that were grown in beads formed a complex three-dimensional organization with well-preserved extracellular matrices (ECM) according to their

porous structure. Retention of the administered beads was clearly identified at the site of engraftment following an experimentally induced myocardial infarction in a rat model. The results of echocardiography, magnetic resonance imaging, and histological analyses suggest that the transplantation of hAFSC beads into an infarcted heart could effectively maintain its gross morphology, prevent successive ventricular expansion, and thereby improve the post-infarcted cardiac function. Immunofluorescent staining revealed that the microenvironment that was provided by the infarcted myocardium might offer cues for the induction of the engrafted hAFSCs into angiogenic and cardiomyogenic lineages. Our results demonstrate that the cellularized beads with endogenously secreted ECM were of sufficient physical size to be entrapped in the interstitial tissues following transplantation, thereby benefiting the infarcted heart.

62. Human amniotic fluid-derived stem cells expressing cytosine deaminase and thymidine kinase inhibits the growth of breast cancer cells in cellular and xenograft mouse models.

Kang NH, Hwang KA, Yi BR, Lee HJ, Jeung EB, Kim SU, Choi KC.

Cancer Gene Ther. 2012 Jun;19(6):412-9.

As human amniotic fluid-derived stem cells (hAFSCs) are capable of multiple lineage differentiation, extensive self-renewal and tumor targeting, they may be valuable for clinical anticancer therapies. In this study, we used hAFSCs as vehicles for targeted delivery of therapeutic suicide genes to breast cancer cells. hAFSCs were engineered to produce AF2.CD-TK cells in order to express two suicide genes encoding bacterial cytosine deaminase (CD) and herpes simplex virus thymidine kinase (HSV-TK) that convert non-toxic prodrugs, 5-fluorocytosine (5-FC) and mono-phosphorylate ganciclovir (GCV-MP), into cytotoxic metabolites, 5-fluorouracil (5-

FU) and triphosphate ganciclovir (GCV-TP), respectively. In cell viability test in vitro, AF2.CD-TK cells inhibited the growth of MDA-MB-231 human breast cancer cells in the presence of the 5-FC or GCV prodrugs, or a combination of these two reagents. When the mixture of 5-FC and GCV was treated together, an additive cytotoxic effect was observed in the cell viability. In animal experiments using female BALB/c nude mouse xenografts, which developed by injecting MDA-MB-231 cells, treatment with AF2.CD-TK cells in the presence of 5-FC and GCV significantly reduced tumor volume and weight to the same extent seen in the mice treated with 5-FU. Histopathological and fluorescent staining assays further showed that AF2.CD-TK cells were located exactly at the site of tumor formation. Furthermore, breast tissues treated with AF2.CD-TK cells and two prodrugs maintained their normal structures (for example, the epidermis and reticular layers) while breast tissue structures

in 5-FU-treated mice were almost destroyed by the potent cytotoxicity of the drug. Taken together, these results indicate that AF2.CD-TK cells can serve as excellent vehicles in a novel therapeutic cell-based gene-directed prodrug system to selectively target breast malignancies.

63. Evaluation of a low cost cryopreservation system on the biology of human amniotic fluid-derived mesenchymal stromal cells.

Miranda-Sayago JM, Fernandez-Arcas N, Benito C, Reyes-Engel A, Herrero JR, Alonso A.

Cryobiology. 2012 Jun;64(3):160-6.

BACKGROUND: Human amniotic-derived mesenchymal stromal cells (hAMSC) are a novel population of multipotent stem cells that have been shown to have great potential for use in regenerative medicine. However, procedures to store and preserve hAMSC for future clinical applications have not been explored extensively. METHODS: In this study, we analyzed the influence of cryopreservation, using a protocol based on freezing rate of 1 °C/min, 10% dimethyl sulfoxide as cryoprotectant and a thawing rate >100 °C/min, on hAMSC morphology, proliferation rates, viability, cell cycle, karyotype, immune phenotype and

multilineage differentiation potential. RESULTS: This study found that this cryopreservation protocol does not affect the biological properties of hAMSC. DISCUSSION: This shows that this protocol is a viable system for banking hAMSC, with the associated advantages that has a low cost in terms of expense, time and personnel involved and is easy to implement.

64. Human chorionic villus mesenchymal stromal cells reveal strong endothelial conversion properties.

Meraviglia V, Vecellio M, Grasselli A, Baccarin M, Farsetti A, Capogrossi MC, Pompilio G, Coviello DA, Gaetano C, Di Segni M, Rossini A.

Differentiation. 2012 Jun;83(5):260-70.

Chorion, amnion and villi are reservoirs of mesenchymal stromal cells (StC) and the hypothesis that StC from fetal tissues retain higher plasticity compared to adult StC has been suggested. Aimed at investigating this aspect, a series of in vitro experiments were performed with StC isolated from first trimester human chorionic villi (CVStC). CVStC were cultured in: (i) standard mesenchymal medium (MM) and (ii) AmniomaxII® (AM), specifically designed to grow amnion-derived cells in prenatal diagnostic procedures. Cells were then exposed to distinct differentiation treatments and distinguished according to morphology, immunophenotype and molecular markers. Human StC obtained from adult bone marrow

(BMStC) were used as control. CVStC cultured either in MM or AM presented stromal morphology and immunophenotype, were negative for pluripotency factors (Nanog, Oct-4 and Sox-2), lacked detectable telomerase activity and retained high genomic stability. In AM, however, CVStC exhibited a faster proliferation rate compared to BMStC or CVStC kept in MM. During differentiation, CVStC were less efficient than BMStC in acquiring adipocytes and osteocytes features; the cardiomyogenic conversion occurred at low efficiency in both cell types. Remarkably, in the presence of pro-angiogenic factors, CVStC reprogrammed toward an endothelial-like phenotype at significantly higher efficiency than BMStC. This effect was particularly evident in CVStC expanded in AM. Mechanistically, the reduced CVStC expression of anti-angiogenic microRNA could support this process. The present study demonstrates that, despite of fetal origin, CVStC exhibit restricted plasticity, distinct from that of BMStC and predominantly directed toward the endothelial lineage.

65. Renal differentiation of amniotic fluid stem cells: perspectives for clinical application and for studies on specific human genetic diseases.

Rosner M, Schipany K, Gundacker C, Shanmugasundaram B, Li K, Fuchs C, Lubec G, Hengstschläger M.

Eur J Clin Invest. 2012 Jun;42(6):677-84.

BACKGROUND: Owing to growing rates of diabetes, hypertension and the ageing population, the prevalence of end-stage renal disease, developed from earlier stages of chronic kidney disease, and of acute renal failure is dramatically increasing. Dialysis and preferable renal transplantation are widely applied therapies for this incurable condition. However these options are limited because of morbidity, shortage of compatible organs and costs. Therefore, stem cell-based approaches are becoming increasingly accepted as an alternative therapeutic strategy. DESIGN: This review summarizes the current findings on the nephrogenic

potential of amniotic fluid stem (AFS) cells and their putative implications for clinical applications and for studies on specific human genetic diseases. RESULTS: Since their discovery in 2003, AFS cells have been shown to be pluripotent with the potential to form embryoid bodies. Compared to adult stem cells, induced pluripotent stem cells or embryonic stem cells, AFS cells harbour a variety of advantages, such as their high differentiation and proliferative potential, no need for ectopic induction of pluripotency and no somatic mutations and epigenetic memory of source cells, and no tumourigenic potential and associated ethical controversies, respectively. CONCLUSIONS: Recently, the results of different independent studies provided evidence that AFS cells could indeed be a powerful tool for renal regenerative medicine.

66. Therapeutic potential of a distinct population of human amniotic fluid mesenchymal stem cells and their secreted molecules in mice with acute hepatic failure.

Zagoura S, Roubelakis G, Bitsika V, Trohatou, Pappa I, Kapelouzou A, Antsaklis A, Anagnou.

Gut. 2012 Jun;61(6):894-906.

BACKGROUND: There is increasing interest in the therapeutic potential of human mesenchymal stem cells (hMSCs), especially in diseases such as acute hepatic failure (AHF) that are predominantly caused by a variety of drugs and viruses. In previous studies, a distinct population termed human spindle-shaped MSCs were isolated and expanded from second trimester amniotic fluid (AF-MSCs) and characterised based on their phenotype, pluripotency and differentiation potential. METHODS: AF-MSCs, hepatic progenitor-like (HPL) cells and hepatocyte-like (HL) cells derived from AF-MSCs were transplanted into CCl_4-injured NOD/SCID mice with the AHF phenotype in order to evaluate their therapeutic potential. Conditioned medium (CM) derived

from AF-MSCs or HPL cells was then delivered intrahepatically in order to determine whether the engraftment of the cells or their secreted molecules are the most important agents for liver repair. RESULTS: Both HPL cells and AF-MSCs were incorporated into CCl(4)-injured livers; HPL cell transplantation had a greater therapeutic effect. In contrast, HL cells failed to engraft and contribute to recovery. In addition, HPL-CM was found to be more efficient than CM derived from AF-MSCs in treatment of the liver. Proteome profile analysis of HPL-CM indicated the presence of anti-inflammatory factors such as interleukins IL-10, IL-1ra, IL-13 and IL-27 which may induce liver recovery. Blocking studies of IL-10 secretion from HPL cells confirmed the therapeutic significance of this cytokine in the AHF mouse model. CONCLUSIONS: Human spindle-shaped AF-MSCs or HPL cells might be valuable tools to induce liver repair and support liver function by cell transplantation. More importantly, the factors they release may also play an important role in cell treatment in diseases of the liver.

67. Comparison of human amniotic fluid-derived and umbilical cord Wharton's Jelly-derived mesenchymal stromal cells: Characterization and myocardial differentiation capacity.

Bai J, Hu Y, Wang YR, Liu LF, Chen J, Su SP, Wang Y.

J Geriatr Cardiol. 2012 Jun;9(2):166-71.

OBJECTIVE: To compare the characterization and myocardial differentiation capacity of amniotic fluid-derived mesenchymal stromal cells (AF MSCs) and umbilical cord Wharton's Jelly-derived mesenchymal stromal cells (WJ MSCs). METHODS: The human AF MSCs were cultured from amniotic fluid samples obtained by amniocentesis. The umbilical cord WJ MSCs were obtained from Wharton's Jelly of umbilical cords of infants delivered full-term by normal labor. The morphology, growth curves, and analyses by flow cytometry of cell surface markers were compared between the two types of cells.

Myocardial genes (GATA-4, c-TnT, α-actin, and Cx43) were detected by real-time PCR and the corresponding protein expressions were detected by Western blot analysis after myocardial induced in AF MSCs and WJ MSCs. RESULTS: Our findings revealed AF MSCs and WJ MSCs shared similar morphological characteristics of the fibroblastoid shape. The AF MSCs were easily obtained than the WJ MSCs and had a shorter time to reach adherence of 2.7 ± 1.6 days to WJ MSCs of 6.5 ± 1.8 days. The growth curves by MTT cytotoxic assay showed the AF MSCs had a similar proliferative capacity at passage 5 and passage 10. However, the proliferative capacities of WJ MSCs were decreased at 5 passage relative to 10 passage. Both AF stem cells and WJ stem cells had the characteristics of mesenchymal stromal cells with some characteristics of embryonic stem cells. They express CD29 and CD105, but not CD34. They were positive for Class I major

histocompatibility (MHC I) antigens (HLA-ABC), and were negative, or mildly positive, for MHC Class II (HLA-DR) antigen. Oct-4 was positive in all the two cells types. Both AF MSCs and WJ MSCs could differentiate along myocardium. The differentiation capacities were detected by the expression of GATA-4, c-TnT, α-actin, Cx43 after myocardial induction. CONCLUSIONS: Both AF MSCs and WJ MSCs have the potential clinical application for myogenesis in cardiac regenerative therapy.

68. Dual regeneration of muscle and nerve by intravenous administration of human amniotic fluid-derived mesenchymal stem cells regulated by stromal cell-derived factor-1α in a sciatic nerve injury model.

Yang DY, Sheu ML, Su HL, Cheng FC, Chen YJ, Chen CJ, Chiu WT, Yiin JJ, Sheehan J, v Pan HC.

J Neurosurg. 2012 Jun;116(6):1357-67.

OBJECT: Human amniotic fluid-derived mesenchymal stem cells (AFMSCs) have been shown to promote peripheral nerve regeneration. The expression of stromal cell-derived factor-1α (SDF-1α) in the injured nerve exerts a trophic effect by recruiting progenitor cells that promote nerve regeneration. In this study, the authors investigated the feasibility of intravenous administration of AFMSCs according to SDF-1α expression time profiles to facilitate neural regeneration in a sciatic nerve crush injury model. METHODS: Peripheral nerve injury was induced in 63 Sprague-Dawley rats by

crushing the left sciatic nerve using a vessel clamp. The animals were randomized into 1 of 3 groups: Group I, crush injury as the control; Group II, crush injury and intravenous administration of AFMSCs (5 × 10(6) cells for 3 days) immediately after injury (early administration); and Group III, crush injury and intravenous administration of AFMSCs (5 × 10(6) cells for 3 days) 7 days after injury (late administration). Evaluation of neurobehavior, electrophysiological study, and assessment of regeneration markers were conducted every week after injury. The expression of SDF-1α and neurotrophic factors and the distribution of AFMSCs in various time profiles were also assessed. RESULTS: Stromal cell-derived factor-1α increased the migration and wound healing of AFMSCs in vitro, and the migration ability was dose dependent. Crush injury induced the expression of SDF-1α at a peak of 10-14 days either in nerve or muscle, and this increased expression paralleled the expression of its

receptor, chemokine receptor type-4 (CXCR-4). Most AFMSCs were distributed to the lung during early or late administration. Significant deposition of AFMSCs in nerve and muscle only occurred in the late administration group. Significantly enhanced neurobehavior, electrophysiological function, nerve myelination, and expression of neurotrophic factors and acetylcholine receptor were demonstrated in the late administration group. CONCLUSIONS: Amniotic fluid-derived mesenchymal stem cells can be recruited by expression of SDF-1α in muscle and nerve after nerve crush injury. The increased deposition of AFMSCs paralleled the expression profiles of SDF-1α and its receptor CXCR-4 in either muscle or nerve. Administration of AFMSCs led to improvements in neurobehavior and expression of regeneration markers. Intravenous administration of AFMSCs may be a promising alternative treatment strategy in peripheral nerve disorder.

69. Prenatal tracheal reconstruction with a hybrid amniotic mesenchymal stem cells-engineered construct derived from decellularized airway.

Gray FL, Turner CG, Ahmed A, Calvert CE, Zurakowski D, Fauza DO.

J Pediatr Surg. 2012 Jun;47(6):1072-9.

PURPOSE: This study was aimed at examining an airway construct engineered from autologous amniotic mesenchymal stem cells (aMSCs) and a xenologous decellularized airway scaffold as a means for tracheal repair. METHODS: Fetal lambs (N = 13) with a tracheal defect were divided into 2 groups. One group (acellular, n = 6) was repaired with a decellularized leporine tracheal segment. The other group (engineered, n = 7) received an identical graft seeded with expanded/labeled autologous aMSCs. Newborns were euthanized for multiple analyses. RESULTS: Eleven lambs survived to term, 10 of which could breathe at birth. Engineered grafts showed a significant

increase in diameter in vivo (P = .04) unlike acellular grafts (P = .62), although variable stenosis was present in all implants. Engineered constructs exhibited full epithelialization, compared with none of the acellular grafts (P = .002). Engineered grafts had a significantly greater degree of increase in elastin levels after implantation than acellular implants (P = .04). No such differences were noted in collagen and glycosaminoglycan contents. Donor cells were detected in engineered grafts, which displayed a pseudostratified columnar epithelium. CONCLUSIONS: Constructs engineered from aMSCs and decellularized airway undergo enhanced remodeling and epithelialization in vivo when compared with equivalent acellular implants. Amniotic mesenchymal stem cell-engineered airways may become an alternative for perinatal airway repair.

70. Evaluation of endothelial cells differentiated from amniotic fluid-derived stem cells.

Benavides OM, Petsche JJ, Moise KJ Jr, Johnson A, Jacot JG.

Tissue Eng Part A. 2012 Jun;18:1123-31.

Amniotic fluid holds great promise as a stem cell source, especially in neonatal applications where autologous cells can be isolated and used. This study examined chemical-mediated differentiation of amniotic fluid-derived stem cells (AFSC) into endothelial cells and verified the function of AFSC-derived endothelial cells (AFSC-EC). AFSC were isolated from amniotic fluid obtained from second trimester amnioreduction as part of therapeutic intervention from pregnancies affected with twin-twin transfusion syndrome. Undifferentiated AFSC were of normal karyotype with a subpopulation of cells positive for the embryonic stem cell marker SSEA4, hematopoietic stem cell marker c-kit,

and mesenchymal stem cell markers CD29, CD44, CD73, CD90, and CD105. Additionally, these cells were negative for the endothelial marker CD31 and hematopoietic differentiation marker CD45. AFSC were cultured in endothelial growth media with concentrations of vascular endothelial growth factor (VEGF) ranging from 1 to 100 ng/mL. After 2 weeks, AFSC-EC expressed von Willebrand factor, endothelial nitric oxide synthase, CD31, VE-cadherin, and VEGF receptor 2. Additionally, the percentage of cells expressing CD31 was positively correlated with VEGF concentration up to 50 ng/mL, with no increase at higher concentrations. AFSC-EC showed a decrease in stem cells markers c-kit and SSEA4 and were morphologically similar to human umbilical vein endothelial cells (HUVEC). In functional assays, AFSC-EC formed networks and metabolized acetylated low-density lipoprotein, also characteristic of HUVEC. Nitrate levels for AFSC-EC, an indirect

measure of nitric oxide synthesis, were significantly higher than undifferentiated controls and significantly lower than HUVEC. These results indicate that AFSC can differentiate into functional endothelial-like cells and may have the potential to provide vascularization for constructs used in regenerative medicine strategies.

Indice Dei Titoli In Italiano

1. Il destino delle cellule AF-MSCs viene regolato sulla base delle condizioni di coltura cellulare. 13

2. Le cellule staminali da liquido amniotico proteggono i polmoni di ratti esposti ad iperossia moderata. 16

3. Effetti neuroristorativi e proprietà staminali dei villi coriali e delle cellule progenitrici amniotiche. 18

4. Cellule staminali mesenchimali fetali nella terapia contro il cancro. 21

5. Potenziale terapeutico delle cellule staminali da liquido amniotico. 23

6. Le cellule staminali da liquido amniotico quale promettente risorsa per applicazioni cliniche. 25

7. Il contributo della terapia cellulare staminale per il rimodellamento del muscolo scheletrico nell’insufficienza cardiaca. 27

8. Isolamento, coltura ed identificazione delle cellule staminali mesenchimali derivate da liquido amniotico. 29

9. Confronto tra il potenziale differenziativo neurale delle cellule staminali mesenchimali umane da liquido amniotico e da midollo osseo. 31

10. Utilizzo delle cellule staminali mesenchimali umane da liquido amniotico come *feeder layer* per la messa a punto di linee cellulari staminali embrionali umane. 33

11. Cellule staminali pluripotenti ottenute per riprogrammazione delle cellule da liquido amniotico umano con due fattori di trascrizione, in condizioni *feeder-free*. 35

12. Cellule staminali pluripotenti ottenute per induzione di cellule da liquido amniotico affetto da trisomia 21 costituiscono un modello di deficit di neurogenesi associati alla sindrome di Down. 37

13. Il liquido amniotico come fonte di cellule staminali neurali utili per la messa a punto di modelli sperimentali di difetti del tubo neurale. 39

14. Differenziamento *in vitro* di cellule staminali umane da liquido amniotico su un supporto composito di poli(lattide-co-glicolide) (PLGA) e matrice submucosale vescicale (BSM) per ingegneria tissutale dell'osso. 41

15. Caratterizzazione fenotipica e funzionale delle cellule staminali mesenchimali da villi coriali umani ottenuti dalla placenta al parto. 43

16. Le cellule staminali umane ottenute dal liquido amniotico di secondo trimestre mostrano caratteristiche di pluripotenza quando coltivate secondo gli stessi protocolli impiegati per le cellule staminali embrionali. 46

17. Applicazioni di ingegneria tissutale delle cellule da liquido amniotico in ambito cardiovascolare. 48

18. Cellule staminali da liquido amniotico e microchimerismo cellulare fetale. 50

19. Produzione di cellule progenitrici pancreatiche tramite modificazioni genetiche di cellule staminali da liquido amniotico di primate. 51

20. Costrutti eterogenei tissutali complessi contenenti molteplici tipi cellulari ottenuti mediante tecnologia di *inkjet printing*. 53

21. Determinazione del profilo molecolare delle cellule staminali umane da liquido amniotico durante lo sviluppo fetale. 55

22. Isolamento delle cellule staminali mesenchimali canine da liquido amniotico e differenziamento in cellule simil-epatiche. 57

23. L'immunoreattività positiva dei sinciziotrofoblasti dei villi placentari per C4d supporta il rigetto del trapianto contro l'ospite di eziologia ignota. 59

24. Le cellule staminali mesenchimali/stromali a morfologia fusiforme ottenute dal liquido amniotico umano promuovono la neovascolarizzazione. 61

25. L'analisi dell'intero genoma rivela l'unicità sull'identità staminale degli amniociti umani. 63

26. Liquido amniotico e membrane placentari: un'inaspettata fonte di cellule ampiamente multipotenti. 65

27. Analisi epigenetica ed adeguatezza delle cellule staminali da liquido amniotico per la ricerca e per scopi terapeutici. 67

28. Ricostruzione cranio-facciale con impianti ossei fetali ingegnerizzati con l'utilizzo di cellule staminali mesenchimali amniotiche. 69

29. Effetti delle cellule staminali mesenchimali isolate da liquido amniotico e del gel plasmatico ricco di piastrine sulle ulcere da decubito gravi e infette nel puledro. 72

30. Differenziamento condrogenico delle cellule staminali da liquido amniotico e loro potenziale nelle terapie rigenerative. 73

31. Cellule staminali derivate da liquido amniotico come fonte per l'ingegneria tissutale ossea. 75

32. Pdx1 e condizioni di coltura controllate inducono il differenziamento delle cellule staminali amniotiche umane in *clusters* insulino-secernenti. 77

33. Progenitori muscolari ottenuti da cellule staminali umane da liquido amniotico nella terapia cellulare dell'incontinenza urinaria da sforzo. 79

34. Fonti cellulari per l'ingegnerizzazione tissutale della trachea: passato, presente e futuro. 81

35. Cellule staminali da liquido amniotico accelerano la guarigione di ampie ferite cutanee tramite *bioprinting*. 83

36. La soppressione del fattore trascrizionale EST e di TGFβ permette la riprogrammazione delle cellule amniotiche mature in cellule endoteliali in modo diretto ed efficiente. 85

37. La simvastatina induce il differenziamento osteogenico nelle cellule staminali mesenchimali da liquido amniotico (AFMSC). 87

38. Ruolo delle cellule mesenchimali da liquido amniotico ingegnerizzate su supporto a base di MgHA/collagene ed allotrapiantate in un modello animale. 89

39. Le cellule staminali da liquido amniotico ripristinano sia *in vitro* che *in vivo* la crescita, l'innervazione e la motilità

polmonare in ratti con ipoplasia esposti a nitrofen, attraverso effetti paracrini. 92

40. Cellule staminali umane da liquido amniotico quale fonte cellulare per xenotrapianti nell'angiogenesi terapeutica. 95

41. L'acido valproico conferisce pluripotenza funzionale alle cellule staminali da liquido amniotico con metodi non mediati da transgeni. 98

42. Effetti protettivi da parte delle cellule staminali da liquido amniotico nell'allotrapianto dell'aorta in un modello di vascolopatia nel ratto. 100

43. Effetto della fase di differenziamento delle cellule staminali da liquido amniotico nella rigenerazione ossea. 103

44. Potenziale delle cellule staminali mesenchimali di derivazione placentare quali cellule di semina per ingegneria tissutale ossea: uno studio preliminare di differenziazione osteoblastica e di immunogenicità. 105

45. Iniezione terapeutica di cellule staminali umane in un modello animale per la rigenerazione dello sfintere uretrale. 106

46. Generazione, caratterizzazione e potenziali terapeutici applicativi dei cardiomiociti da cellule staminali di diversa origine. 109

47. Elevata efficienza di riprogrammazione delle cellule CD34+ derivate da liquido amniotico umano in cellule staminali pluripotenti con Oct4. 111

48. Fattori solubili pro-angiogenici da cellule staminali da liquido amniotico mediano il reclutamento di progenitori endoteliali

in un modello ischemico di lembo fascio-cutaneo. 113

49. Potenziali terapie antitumorali della membrana amniotica umana e delle cellule staminali derivate dal liquido amniotico. 115

50. Cellule staminali da liquido amniotico di derivazione clonale hanno la capacità di differenziamento miogenico scheletrico *in vitro* ed *in vivo*. 117

51. Le cellule staminali da liquido amniotico ripristinano il microambiente cellulare muscolare in modelli HSA-CRE, Smn(F7/F7) murini. 119

52. Identificazione di un controllo interno da impiegare per il differenziamento con linee cellulari staminali pluripotenti derivate da gemelli eterozigoti. 121

53. Il precondizionamento delle cellule staminali umane da liquido amniotico aumenta il loro potenziale rigenerativo. 123

54. Cellule staminali per la rigenerazione di valvole cardiache. 125

55. Costrutti a doppio strato osteo-cartilaginei: influenza della supplementazione del terreno di coltura nel differenziamento delle cellule staminali da liquido amniotico. 127

56. Cellule staminali da cordone ombelicale e da placenta per ingegneria tissutale.130

57. Ingegneria tissutale di tessuti ed organi riproduttivi. 132

58. Cellule staminali da liquido amniotico CD117(+): stato dell'arte e prospettive future. 134

59. Anomalie congenite: alternative di trattamento basate sull'uso delle cellule staminali da liquido amniotico. 136

60. Ingegnerizzazione prenatale di valvole cardiache mediante cellule staminali autologhe da liquido amniotico nella circolazione fetale. 138

61. Biglie iniettabili in PLGA poroso cellularizzate con cellule staminali umane da liquido amniotico per applicazioni di cardiomioplastica. 140

62. Cellule staminali umane da liquido amniotico che esprimono citosina deaminasi e timidina chinasi inibiscono la crescita delle cellule del tumore al seno in modelli cellulari e di xenotrapianto murini. 142

63. Valutazione biologica di un sistema di criopreservazione a basso costo sulle

cellule staminali mesenchimali umane da liquido amniotico. 145

64. Le cellule stromali mesenchimali umane da villi coriali rivelano robuste proprietà di conversione endoteliale. 147

65. Differenziamento renale delle cellule staminali da liquido amniotico: prospettive per l'applicazione clinica e per gli studi di malattie genetiche specifiche nell'uomo. 149

66. Potenziale terapeutico di una distinta popolazione di cellule staminali mesenchimali umane da liquido amniotico e delle molecole da loro secrete, in topi con insufficienza epatica acuta. 151

67. Confronto tra cellule stromali mesenchimali umane da liquido amniotico e da gelatina di Wharton del

cordone ombelicale: caratterizzazione e capacità differenziativa miocardica. 153

68. La duplice rigenerazione di muscolo e nervo mediante somministrazione intravenosa di cellule staminali mesenchimali umane da liquido amniotico in un modello di lesione del nervo sciatico è regolata dal fattore cellulare stromale 1α. 156

69. Ricostruzione prenatale della trachea con un costrutto ibrido derivato da una via aerea decellularizzata ed ingegnerizzata con cellule staminali mesenchimali da liquido amniotico. 159

70. Analisi delle cellule endoteliali ottenute per differenziamento di cellule staminali da liquido amniotico. 164

Edited by Biocell Center group

Thanks to:

Prof. Giuseppe Simoni
Dr. Fabio Frattini
Dr. Emanuele Lantieri
Dr. Federico Maggi
Ing. Marco Reguzzoni
Dr. Edoardo Borgo
Dr. Giampaolo Leoni
Dr. Massimiliano Manganini
Dr. Maria Elena Colombo
Dr. Valentina Cavadini