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Stem cells continue to inspire the imagination of the entire world, as almost every day, a new breakthrough highlights the healing and curing power of these amazing cells. In my lifetime, I fully expect that stem cells will play a major role in treatments and possibly even cures for cancer, Alzheimer disease, Parkinson disease, and other debilitating diseases and disorders that currently have limited treatment options and no cures. One of the most profound scientific questions of our time is what source of stem cells will be the most effective and utilized in future medical settings.

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) generate significant media attention and hype for their potential but we have yet to see any real cures or treatments from these cell sources. The main reason for the lack of therapeutic options with these two cell sources is that ESCs and iPSCs have been shown to form tumors in many studies [Knoepfler, 2009]. Since we do not currently understand how tumors form, this is a gigantic barrier to overcome before real therapies can be developed. However, it is critically important that research continue in both academic and industry settings on both ESCs as well as on iPSCs. ESC research and iPSCs research should be funded, if for no other reason than to provide tools for the scientific community to learn more about stem cells and the mechanism of action of stem cells. Furthermore, we may potentially learn how or what causes tumors through the study of ESCs and iPSCs. ESCs and iPSCs are especially powerful tools for the study of stem cells because ESCs are the earliest stem cells, forming around day 3 or 4 of embryonic life, and the potential of iPSCs deals with the ability to be reprogrammed into earlier cell lines. By understanding the earliest formation of life through the study of ESCs and also how cellular life subsequently develops into more complex cell systems through the study of iPSCs, the scientific community will learn volumes about how to better utilize stem cells for treatments and eventually cures.

Mesenchymal stem cells (MSCs) have recently begun to garner the support and interest from the scientific community that they deserve. MSCs have many properties that suggest that they are the ideal cell for regenerative medicine applications. The MSC can form all three germ layers and has been shown to be immune privileged, which means that these cells can be used without human leukocyte antigen (HLA) matching and are suitable for allogeneic or “off the shelf” therapeutic applications [Weiss et al., 2008]. Many of the regenerative medicine companies utilize MSCs for their therapeutic stem cell products. The majority of the MSC regenerative medicine products that are being developed derive stem cells from bone marrow or from adipose tissue. Although bone marrow-derived MSCs have shown regenerative medicine potential, they do have drawbacks. Bone marrow MSCs have been shown to senescence around passage 10 or 12 [Karahuseyinoglu, 2007; Zimmermann et al., 2003]. Because of senescence around passage 10, some regenerative medicine companies pool donor MSCs to develop products with enough stem cells to be effective in treatments that require billions of cells. Obviously, pooling donors opens up a Pandora’s box and raises significant questions about the stem cell product. Another drawback is that
the recruitment of qualified donors is expensive and requires the donor to undergo a painful bone marrow aspirate. Usually, a donor also has to be in his early twenties in order to have MSCs in his bone marrow that are potent enough to be expanded for a large number of passages or doublings. This suggests that as a person ages, the MSCs present in their bone marrow become less potent [Campagnoli et al., 2001; Clarke and McCann, 1989]. As many autologous bone marrow MSC products are developed for diseases and disorders that usually occur later in life, such as cardiac disease, the question can be raised as to the effectiveness of autologous bone marrow-derived MSC regenerative medicine treatments.

Stem cells from perinatal tissue sources, such as the umbilical cord tissue/Wharton’s jelly, umbilical cord blood, placental blood and placental tissue, amnion and amniotic fluid, represent the most primitive sources of MSCs. In contrast to bone marrow-derived MSCs, MSCs from perinatal sources do not have the same challenges to overcome. MSCs from perinatal stem cell sources express markers such as OCT-4, Nanog, and SOX-2. These markers are commonly associated with ESCs [Carlin et al., 2006; La Rocca et al., 2009]. These markers are generally believed to indicate greater expansion potential [Karahuseynoglu et al., 2007; La Rocca et al., 2009; Weiss et al., 2006]. MSCs from the Wharton’s jelly have faster and greater expansion potential than bone marrow MSCs [Baksh et al., 2007]. Additionally, MSCs from perinatal stem cell sources can easily be collected post-delivery and offer an abundant resource for developing large donor banks, as the perinatal tissues are simply thrown away in 99% of all deliveries. It is for these reasons as well as many others that are highlighted throughout Perinatal Stem Cells, Second Edition, that I believe perinatal stem cell sources represent the ideal starting point for regenerative medicine therapeutic applications.

Perinatal Stem Cells, Second Edition is a selection of chapters that feature a wide array of research topics and reviews written by some of the world’s leading scientists working in the perinatal stem cell field. It is patently clear in the second edition that in the last 3 years since Perinatal Stem Cells, First Edition was published, the perinatal stem cell field has made great strides towards the clinic.

In Chapter 1, Atala and Murphy focus on stem cells found in the amniotic fluid (AFSC). The authors discuss isolation techniques as well as review the literature and accomplishments of others working in the field with a particular emphasis on the differentiation potential of AFSC and the future clinical applications of AFSCs.

In Chapter 2, Haspel and Ballen describe the clinical practice of cord blood transplantation. The authors provide a review of the collection, processing, and utility of cord blood in comparison with adult hematopoietic sources, such as bone marrow and peripheral blood, as well as present the challenges and the advantages of single and double cord blood transplantation. They also provide an extensive bibliography on the subject.

In Chapter 3, Mikkola provides an update to her chapter in the first edition of Perinatal Stem Cells, and describes hematopoietic stem cell (HSC) development in the placenta. This chapter provides evidence that the placenta is capable of de novo hematopoiesis and protects the HSCs from premature differentiation, a unique concept that suggests a novel role of the placenta as a fetal HSC niche.

In Chapter 4, Taghizadeh provides a review of the challenges faced in hematopoietic transplantation and discusses a novel strategy of utilizing the cord tissue/Wharton’s jelly-derived stem cells in a co-transplantation model.

In Chapter 5, Klingemann discusses the use of MSCs to prevent and treat complications after transplantation of both HSCs as well as transplantation of solid organs. His extrapolated results suggest that early MSCs such as found in umbilical cord tissue/Wharton’s jelly may have a similar spectrum of events to bone marrow MSCs.
In Chapter 6, La Rocca and coworkers provide a report on the regenerative medicine properties of cord tissue/Wharton’s jelly-derived stem cells, with special emphasis on the immune regulation features from MSCs from cord tissue.

In Chapter 7, Lutton and Duran-Struuck discuss the current literature surrounding the immunogenicity and immunomodulatory effects of MSCs from perinatal stem cell sources.

In Chapter 8, Cetrulo and coworkers discuss the use of MSCs to treat and prevent graft-versus-host disease in hematopoietic transplantation, as well as provide a review of the future possible uses of MSCs in regenerative medicine.

In Chapter 9, Boyd and coworkers provide a comprehensive overview of the amnion and the regenerative medicine applications. This chapter includes discussion of the amnion membrane as well as amnion cells and MSCs derived from the amnion.

In Chapter 10, Semenov and Breymann present an overview of the role of stem cells in regenerative medicine and then narrow in on the potential role stem cells from perinatal sources will play in regenerative medicine via cell therapy and tissue regeneration.

In Chapter 11, Cox and coworkers provide a thorough review of cellular therapy for the treatment of traumatic brain injury and the use of perinatal stem cell sources in this field.

In Chapter 12, Parolini and coworkers provide an excellent overview of the amniotic membrane. This chapter includes isolation techniques, current established clinical uses of the amniotic membrane, as well as discusses preclinical studies that are ongoing that may lead to new clinical applications.

In Chapter 13, Strom and coworkers discuss the use of the human amnion to manage liver disease and provide an update to the research they presented in the first edition of *Perinatal Stem Cells*.

In Chapter 14, Borlongan and coworkers discuss the use of amnion-derived cells for treatments for stroke therapy.

In Chapter 15, Khosrotehrani and coworkers describe the phenomenon of fetal stem cells (fetal microchimeric) in maternal circulation and the possibility of these cells acting as a naturally occurring stem cell therapy.

**INDUSTRY REVIEW**

In Chapter 16, Cetrulo provides insight on the stem cell banking industry, as well as the regenerative medicine industry.

In Chapter 17, Wetherell provides an explanation of how patents can and are used to protect stem cell innovations.

In Chapter 18, Cetrulo interviews Frances Verter, the founder of the nonprofit organization, Parents Guide to Cord Blood.

In Chapter 19, Perlow provides a perspective from a practicing OB/GYN on the cord blood banking industry.

It is with great excitement that I head to work each day knowing that the scientific community is on one of the most exciting journeys in the history of mankind. We are learning about the most fundamental building blocks of our species and of life. With this edition of *Perinatal Stem Cells, Second Edition*, it is the goal of the editors to provide a snapshot in time of what we currently know about perinatal stem cells in 2012. The most amazing aspect of working with these cells is that although we know a great deal, the full potential of these perinatal stem cells may never be fully reached or realized.

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AMNIOTIC FLUID STEM CELLS

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INTRODUCTION

Human amniotic fluid can be obtained during amniocentesis at the second trimester. This procedure is already performed in many pregnancies in which the fetus has a congenital abnormality or to determine characteristics such as sex [Hoehn et al., 1975]. Amniotic fluid may have more utility than only as a diagnostic tool and may be a source of a powerful therapy for a multitude of congenital and adult disorders.

Gestational tissues, such as the placenta, amniotic fluid, and umbilical cord, are a rich source of highly multipotent stem cells with potent immunosuppressive properties. These stem cell sources are providing the field of regenerative medicine with an exciting new tool for the treatment of disease [De Coppi et al., 2007; Friedman et al., 2007; Murphy et al., 2011; Serikov et al., 2009]. Gestational tissue offers a considerable advantage as a stem cell source over “traditional sources,” such as bone marrow or embryo-derived cells. Such tissue is often discarded following birth so is readily available without an invasive biopsy or the destruction of a human embryo [Murphy et al., 2010; Serikov et al., 2009; Troyer and Weiss, 2008]. This means that there are minimal ethical and legal considerations associated with their collection and use.

Recently, researchers have isolated and characterized highly multipotent cells from the amniotic fluid, called amniotic fluid-derived stem cells (AFSCs) [De Coppi et al.,
Cell culture experiments with these types of cells have demonstrated that they have the potential to differentiate into various cell lineages, including hematopoietic, adipogenic, osteogenic, myogenic, endothelial, hepatic, chondrocytic, pulmonary, cardiac and neurogenic [De Coppi et al., 2007; in ‘t Anker et al., 2003]. The highly multipotent and anti-inflammatory properties of these cells suggest potential clinical applications of these cells to treat diseases, such as bone defects, lung disease, neurological disorders, kidney disease, and heart disease [Delo et al., 2008; Furth and Atala, 2009; Murphy et al., 2011, 2012; Perin et al., 2007; Shaw et al., 2011].

DEVELOPMENT OF GESTATIONAL STEM CELLS

Shortly after fertilization, the zygote undergoes a series of cell divisions to form a solid ball of cells known as the morula [Swartz, 1983]. The morula develops into a fluid-filled sphere (the blastocoele), which then compacts, forming an inner cell mass, which subsequently forms the embryo, and the outer cell mass (the trophoblast), which develops into placental tissue. At embryonic day 4–5, the inner cell mass becomes differentiated into two tissues: the hypoblast, which will form most extraembryonic structures, and the epiblast, from which the embryo will develop. The hypoblast and epiblast form a bilayered disk, dividing the blastocyst into two chambers: a yolk sac and a fluid-filled amniotic cavity. Originally, this fluid is isotonic, containing proteins, carbohydrates, lipids, phospholipids, urea, and electrolytes. Later, urine excreted by the fetus increases its volume and changes its composition [Bartha et al., 2000; Heidari et al., 1996; Sakuragawa et al., 1999; Srivastava et al., 1996].

Amniotic fluid also contains a mixture of different cell types. A number of different origins have been suggested for these cells [Medina-Gomez and del Valle, 1988], with identification of cells derived from the developing fetus, sloughed from the fetal amnion membrane and skin, as well as the alimentary, respiratory, and urogenital tracts. The cell population found within the amniotic fluid changes with time and reflects the changes in the developing fetus [Torricelli et al., 1993]. Due to the origin of the amniotic fluid and placental membranes, these tissues are a rich source of cells that maintain highly multipotent differentiation potential. The amniotic fluid develops prior to the process of gastrulation [Downs and Harmann, 1997; Snow and Bennett, 1978], so many cells found in the fluid do not undergo the process of lineage specialization observed in the developing embryo. Thus, the amniotic fluid is comprises a cell population that is reported to contain cells of all three germ layers [in ‘t Anker et al., 2003; Prusa and Hengstschlager, 2002].

ISOLATION AND CHARACTERIZATION OF AMNIOTIC FLUID STEM CELLS

As many pregnant women already undergo amniocentesis to screen for fetal abnormalities, cells can be isolated from this fluid and saved for future use. There is also the potential to collect amniotic fluid at term from routine cesarean sections. Theoretically, a bank with 100,000 specimens could supply 99% of the U.S. population with perfect genetic matches for transplantation. However, a major advantage of isolating cells from amniotic fluid is that it allows for autologous reimplantation, effectively bypassing the problems associated
with a technique called donor-recipient HLA matching and minimizing the chances of cell rejection [Tsai et al., 2004].

Two milliliters of amniotic fluid contains up to 20,000 cells [Kaviani et al., 2001], and a highly multipotent subpopulation of stem cells can be isolated through positive selection for cells expressing the membrane receptor c-kit (CD117) [De Coppi et al., 2007]. C-kit is a protein tyrosine-kinase receptor that specifically binds to the ligand stem cell factor (SCF) and has critical functions in gametogenesis, melanogenesis, and hematopoiesis [Fleischman, 1993]. Approximately 1% of cells present in amniotic fluid have been shown to be c-kit positive. AFSCs also express human embryonic stage-specific marker SSEA-4, and the stem cell marker Oct-4, as well as mesenchymal and neuronal markers CD29, CD44, CD73, CD90, and CD105. AFSCs are also characterized by the absence of a variety of surface molecules, such as the hematopoietic lineage marker CD45, hematopoietic stem cell markers CD34, CD133, and markers associated with embryonic stem (ES) cells, such as SSEA3 and Tra-1-81. This expression profile is of interest as it demonstrates expression of some key markers of the ES cell phenotype, but not the full complement of markers expressed by ES cells. Like embryonic stem cells, AFSCs form embryoid bodies in vitro, which stain positive for markers of all three germ layers. However, unlike embryonic stem cells, when implanted into immune-deficient mice in vivo, AFSCs do not form teratomas, an essential safety characteristic for a potential cell therapy. This indicates that these cells represent an intermediate stage between embryonic stem cells and adult stem cells.

Stem cells isolated from the amniotic fluid, maintain a round shape for 1 week when cultured in non-treated culture dishes. In this state, they demonstrate low proliferative capability. After the first week, the cells begin to adhere to the plate and change their morphology, becoming elongated and proliferating rapidly, reaching 80% confluence and a need for passage every 48–72 hours. Feeder layers are not required for maintenance or expansion. AFSCs show a high self-renewal capacity with over 250 population doublings. This far exceeds Hayflick’s limit, which is defined as 50 doublings for most cultured somatic cells. AFSCs maintain a normal karyotype at late passages, display normal G1 and G2 cell cycle checkpoints, and conserve a long telomere length due to continued telomerase activity (Fig. I.1). AFSCs have a high clonal capacity where a single cell can give rise to a population that differentiates into cells representative of all three primary germ layers.

MULTIPOTENCY OF AMNIOTIC FLUID STEM CELLS

The multipotent properties of stem cells isolated from gestational tissue, such as the amniotic fluid, allows researchers to generate large numbers of specialized cell types that can be applied in regenerative therapies (Fig. I.2). Regenerative cell therapies have the potential to treat a range of chronic diseases. The section below provides an overview of the specialized cell types derived from AFSCs in vitro.

**Hematopoietic**

Ditadi and coworkers demonstrated the hematopoietic potential of AFSCs in vitro [Ditadi et al., 2009]. To induce hematopoietic differentiation, the authors cocultured AFSCs with a confluent stroma of OP9 cells in alpha medium with Glutamax I supplemented with 20% defined fetal calf serum, SCF, Flt3-L, and IL-7 (for T-cell differentiation) or in RPMI 1640 supplemented with 10% defined fetal calf serum, SCF, and IL-15 (for NK-cell differentiation).
Under these culture conditions, AFSCs formed colony-forming units (CFUs) and expressed surface markers and genes typically associated with a hematopoietic potential, including; CD4, CD5, CD7, CD8, CD16, and CD56 suggesting generation of T- and NK-cell phenotypes. These experiments demonstrate that AFSCs display multilineage hematopoietic differentiation potential in vitro, suggesting that AFSC may be an important source of cells to regenerate the hematopoietic system.

**Adipocytes**

The induction of AFSCs into an adipogenic phenotype in vitro can be achieved by maintaining cells in media containing dexamethasone, 3-isobutyl-1-methylxanthine, insulin, and indomethacin [De Coppi et al., 2007]. Maintenance in culture with adipogenic supplements induced morphological changes in AFSC from elongated to rounded within 8 days. These morphological changes coincided with the accumulation of intracellular lipid droplets. Following 16 days in culture, the majority of AFSCs contain cytoplasmic lipid-rich vacuoles. Maintenance in under these conditions also induces the expression of adipogenic markers, including peroxisome proliferation-activated receptor γ-2 (PPAR-γ2), a transcription factor that regulates adipogenesis, and lipoprotein lipase (LPL). Expression of these genes is only detected in progenitor cells under adipogenic conditions and not in undifferentiated cells. Chen and coworkers generated an AFSC line from porcine amniotic fluid, and confirmed adipogenic differentiation of this cell type, inducing AFSCs into adipose-like cells containing lipid droplets and expressing adipose-specific markers PPARγ and C/EBPα [Chen et al., 2011].

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**Figure I.1.** Consistent phenotype of AFSCs following long-term culture. (A) Clonal human AFSCs maintain a normal karyotype after 250 population doublings. (B) AFSCs passaged in culture show normal cell cycle control. (C) Telomere length is conserved in AFSCs between early passage (lane 3) and late passage (lane 4). Lane 1: short length telomere standards. Lane 2: high length telomere standards. (D) AFSCs express markers characteristic of embryonic stem cells, Oct4 and SSEA4. (E) AFSCs express markers characteristic of mesenchymal stem cells, CD73, CD90, and CD105.
### Figure 1.2: Multilineage Differentiation of AFSCs in Vitro.

(A) RT-PCR analysis of differentiation. U: Undifferentiated cells. D: Cells maintained under conditions for differentiation to; osteocytes (8 days), myocytes (8 days), adipocytes (16 days), endothelial cells (8 days), hepatocytes (45 days), and neurons (2 days). (B) Phase-contrast microscopy of undifferentiated AFSCs. (C) AFSC-derived osteocytes: histochemical staining for alkaline phosphatase. (D) AFSC-derived myocytes: multinucleated myotube-like cells. (E) AFSC-derived adipocytes: intracellular oil aggregation. (F) AFSC-derived endothelial cells: capillary-like structures. (G) AFSC-derived hepatocytes: immunofluorescent staining for albumin. (H) AFSC-derived neurons: immunofluorescent staining for nestin. (See insert for color representation of the figure.)
Osteocytes

Stem cell-derived osteocytes would be a powerful tool to treat craniofacial bone defects, spinal or major bone injuries. AFSCs can generate an osteogenic phenotype following maintenance in culture media supplemented with dexamethasone, beta-glycerophosphate, and ascorbic acid 2-phosphate. AFSCs maintained in osteogenic induction medium demonstrate a loss of their spindle-shape phenotype and development of an osteoblast-like appearance with finger-like excavations into the cytoplasm [De Coppi et al., 2007]. AFSCs form cell aggregates, producing typical lamellar bone-like structures, with calcium precipitation and production of alkaline phosphatase (AP), a major feature of osteoblasts. These AFSC aggregates also express specific genes implicated in mammalian bone development (AP, core-binding factor A1 [CBFA1], and osteocalcin), with a chronology consistent with the physiological analog. Maraldi and coworkers utilized three-dimensional (3D) culture surfaces to induce osteogenic differentiation of AFSCs [Maraldi et al., 2011]. This group maintained AFSCs on a 3D fibrin scaffold in osteogenic induction media, and found that this resulted in a significant increase in osteogenic marker expression and mineralized ECM production by AFSCs. Similar work demonstrated that culture of AFSCs on nanofibrous scaffolds significantly enhanced AP activity, calcium content, and osteogenic gene expression [Sun et al., 2010]. These studies also highlighted the importance of bone morphogenetic protein 7 (BMP-7) in the osteogenic induction of AFSCs.

Myocytes

AFSCs have shown promise as a cell therapy for cardiac disease and express several cardiac genes in their native state, including the transcription factor mef2, the gap junction connexin43, and H- and N-cadherin [Guan et al., 2011]. When cultured in myogenic induction medium AFSCs upregulate the expression of cardiac-specific genes cardiac troponin I and cardiac troponin T, redistribute connexin 43, and downregulate the stem cell marker SRY-box 2 (sox2). When co-cultured with neonatal rat cardiomyocytes (NRCs), AFSCs formed both mechanical and electrical connections with the NRCs. Bollini and coworkers supported these observations, observing expression of myocardial markers by AFSCs co-cultured with NRCs. After 9 days of co-culture, the authors detected the appearance of sarcomeric cTnT and cTnI, MyHC, and α-actinin in AFSCs. This group demonstrated that the direct cell-to-cell interaction with the beating NRCs is essential for the differentiation of the AFSC, as cells in noncontact culture, or cultured using conditioned medium, showed no cardiac differentiation and no increase in expression of cardiomyocyte markers. AFSCs can also be induced to form a myogenic phenotype by maintenance on a thin coat of Matrigel in medium supplemented with horse serum and chick embryo extract and in the presence of 5-azacytidine [Gekas et al., 2010]. Phenotypically, AFSCs under these conditions organize themselves into bundles that fuse to form multinucleated cells. These cells express myogenic factor 6 (Myf6), MyoD and desmin, which are essential for muscle development. The development profile of AFSC induction toward a myogenic phenotype closely follows a characteristic pattern of gene expression reflecting that seen with embryonic muscle development [Hinterberger et al., 1991]. These studies demonstrate that the AFSCs have the potential to be used as a cell therapy to treat cardiac and muscle-related diseases.

Endothelial Cells

De Coppi and coworkers have shown that AFSCs can be induced to form an endothelial phenotype by maintenance in medium supplemented with VEGF, hFGF-b, epidermal
growth factor, insulin-like growth factor-1, heparin, and ascorbic acid, and cultured on either gelatin or Matrigel-coated dishes [De Coppi et al., 2007]. Differentiated AFSCs stain positively for specific markers for endothelial cells, including human-specific endothelial cell surface marker (P1H12), factor VIII (FVIII), and kinase insert domain receptor (KDR). These cells also express CD31, von Willebrand factor (vWF), and vascular cell adhesion molecule (VCAM), which are important for cell adhesion and formation of junctions in epithelial cells. In response to physiological levels of shear force (12 dyne/cm²), AFSCs upregulate gene and protein levels of CD31 and vWF, suggesting that AFSCs acquire endothelial cell characteristics when stimulated by growth factors and shear force. AFSCs undergo morphological changes following culture in endothelial induction media for 1 week, including the formation of capillary-like structures. After 2 weeks of culture, clear cord structures are observed, and the length of these structures is increased following exposure to physiological levels of shear force. These studies demonstrate that AFSCs may have an important role as a cell therapy for the treatment of vascular diseases.

Hepatocytes

Hepatocyte-like cells can be generated from AFSCs by maintaining cells on Matrigel or collagen-coated dishes in media supplemented with hepatocyte growth factor, insulin, oncostatin M, dexamethasone, fibroblast growth factor 4, and monothioglycerol [De Coppi et al., 2007]. After 7 days of culture, AFSCs shift from their elongated phenotype towards a more cobblestone-like appearance. AFSCs maintained under these conditions produce albumin and express the hepatocyte nuclear factor 4 (HNF4) transcription factor, the c-Met receptor, the multidrug resistance (MDR) membrane transporter, albumin, and alpha-fetoprotein. Further, following 45 days maintenance in hepatocyte induction media, AFSCs secrete urea, a characteristic liver-specific function that requires coordinated expression of multiple enzymes and specific mitochondrial amino acid transporters [Morris, 2002]. Zheng and coworkers confirmed the potential of AFSCs to be induced into a hepatic lineage, inducing the expression of liver-specific genes and protein for a-fetoprotein (AFP), albumin, cytokeratin-18 (CK18), hepatocyte-nuclear factor (HNF1α), CCAAT-enhancer binding protein (C/EBPα), and cytochrome P450 (CYP1A1). This group demonstrated that AFSCs induced toward the hepatic lineage secrete urea and have significant glycogen storage capacity, suggesting that AFSCs may prove to be a valuable and promising source of human hepatocytes for the treatment of liver disease [Zheng et al., 2008].

Chondrocytes

The ability to generate a readily available source of chondrocytes would greatly assist efforts to regenerate cartilage in patients with degenerative cervical spine disorders and those with severe joint injuries. Kolambkar and co-workers demonstrated that AFSC can be induced toward a chondrogenic phenotype in vitro by maintaining cells in a three-dimensional alginate hydrogels in medium supplemented with transforming growth factor-beta 1 (TGF-β1), bone morphogenetic protein 2 (BMP2), and insulin-like growth factor 1 (IGF1) [Kolambkar et al., 2007]. AFSCs maintained under these conditions synthesize sulfated glycosaminoglycan (sGAG) and produce type II collagen 3 weeks following induction. Synthesis of sGAG is an important marker for chondrocytes, as it is a key indicator of the formation of a connective tissue. Other methods have also shown promise in the generation of AFSC-derived chondrocytes. Encapsulation of AFSCs in a fibrin hydrogel supplemented with TGF-β3 induced the generation of a chondrogenic phenotype, the production of sGAG, type II collagen, as well as cartilage-specific proteins aggrecan,
COL II, and SOX9 [Park et al., 2011]. These studies demonstrated that TGF-β3 is a key factor in AFSC differentiation toward the chondrocyte phenotype.

**Neuronal Cells**

The generation of neural cell types from AFSCs would be valuable for the treatment of neurodegenerative diseases and spinal cord injuries. Mareschi and coworkers have generated cellular aggregates from AFSCs that are morphologically similar to neurospheres. This was achieved by maintaining cells for 24 hours in Neural Progenitor Maintenance Medium (NPMM) supplemented with recombinant hFGF-B, recombinant hEGF, and NSF-1 [Mareschi et al., 2009]. Following only 3 days in culture these floating neurosphere-like clusters stain positive for nestin, and after 3 weeks the neurosphere-like clusters are positive for nestin, GFAP, NSE, and MAP-2. Approximately 75% of total cells express both MAP-2 and GFAP, suggesting that the cell population may represent an early neural progenitor. Electrophysiological analysis demonstrated that AFSCs from these clusters also contain significant densities of functioning voltage-gated sodium channels.

**CLINICAL APPLICATION OF AMNIOTIC FLUID STEM CELLS**

The highly multipotent and immunosuppressive cell populations that can be isolated from the amniotic fluid and placental tissue are a valuable source of cells that can be utilized for the treatment of disease. Here we discuss current clinical and preclinical applications of amniotic fluid and placental stem cells.

**Bone Regeneration**

AFSC-based therapy for bone regeneration has the potential to be utilized to treat craniofacial bone defects, spinal, or major bone injury. De Coppi and coworkers performed preclinical studies investigating the potential for 3D scaffolds containing AFSCs to generate highly mineralized bone tissue [De Coppi et al., 2007]. Micro CT scanning analysis of the AFSC-seeded scaffolds 18 weeks after implantation into mice identified the presence of hard tissue within the AFSC-seeded constructs (Fig. I.3). The density of the tissue-engineered bone found at the sites of implantation was found to be somewhat greater than that of mouse femoral bone, suggesting the in vivo formation of AFSC-derived bone. In the future, scaffolds can be designed to produce bone to generate specific craniofacial shapes, or at densities to facilitate the replacement of major bones damaged by car accidents or battle injuries. Although early in development, these studies demonstrate that AFSCs are a valuable tool for future therapies for bone regeneration.

**Myocardial Infarction**

Myocardial infarction (MI), commonly known as a heart attack, is a leading cause of death worldwide. MI results in myocardial tissue death, and the regeneration of this lost tissue is a major goal in the field of regenerative medicine. The therapeutic potential of AFSCs for acute myocardial infarction has been demonstrated by Bollini and coworkers in a study in which Wistar rats underwent 30 min of ischemia by ligation of the left anterior descending coronary artery, followed by administration of AFSCs [Bollini et al., 2011]. In this preclinical study, AFSC therapy was cardioprotective, improving myocardial cell survival
and decreasing the infarct size. Lee and coworkers xenogenically transplanted AFSC cell aggregates into the peri-infarct area of an immune-suppressed rat, via direct intra-myocardial injection [Lee et al., 2011]. The functional benefits of cell transplantation included the attenuation of the progression of heart failure, improved the global function, and increased the regional wall motion. The ability of AFSCs to directly contribute to the myocardium is important for the long-term outcomes for patients with MI. AFSCs have been shown to persist in the mouse heart up to 28 days following injection (Fig. 1.4) [Delo et al., 2008].

**Renal Disease**

Patients with end stage renal disease often require lifelong dialysis treatment as organ transplants are limited by donor shortages. Regenerative therapy has the potential to cure certain hereditary forms of renal disease and acute kidney injury. AFSCs are able to directly contribute to renal development both ex vivo and in vivo [Perin et al., 2007, 2010]. Acute tubular necrosis (ATN) is a kidney disorder involving damage to the tubule cells of the kidneys, which can lead to acute kidney failure. In a mouse model of ATN, AFSC
Amniotic Fluid Stem cell therapy provided a protective effect, integrating into the damaged tubules and ameliorating tubular necrosis in the acute injury phase. AFSC-treated animals showed a decreased creatinine and blood urea nitrogen blood levels and a decrease in the number of damaged tubules. AFSC administration also induced the proliferation of tubular epithelial cells, decreased cast formation, and decreased apoptosis of tubular epithelial cells. The authors demonstrated evidence of a direct contribution of AFSCs to renal regeneration. Engrafted AFSCs expressed renal markers PAX2, NPHS1, Dolicholus biflorus, and PA (Fig. I.5). These studies suggested that protective effect of AFSC administration was via immunomodulation of the local immune response to promote the resolution of tissue damage.

Figure I.4. AFSCs persist in the mouse heart up to 29 days following injection as shown by MRI and histology. One million cells MPIO-labeled AFSCs were injected in two different locations in the left ventricle of the mouse heart. (A) Cells were detected by MRI and engraftment confirmed using fluorescence microscopy (B and C). (D–E) Prussian blue iron staining indicated that AFSCs colocalized with the hypointense region seen by MRI. (F) It was confirmed by immunostaining for a human-specific nuclear matrix antibody (anti-NuMA) that MPIOs colocalized with injected AFSCs. (See insert for color representation of the figure.)

Neural Regeneration

Neurodegenerative disease, such as Parkinson, Alzheimer, and Huntington, are incurable and debilitating conditions that involve the progressive degeneration of nerve cells. A major goal of regenerative medicine is to provide a therapy to ameliorate this degeneration and prevent the loss of the neural structure and function. The potential of AFSC therapy as a therapy for neurodegenerative disease has been demonstrated in the “twitcher” mouse model of neurological disease [De Coppi et al., 2007]. These mice are deficient in the lysosomal enzyme galactocerebrosidase and undergo extensive neurodegeneration and neurological deterioration. AFSCs persist for up to 2 months following implantation directly into the lateral ventricles of the developing brain of newborn twitcher mice. Transplanted AFSCs integrated into the periventricular areas, the hippocampus, and the olfactory bulb. AFSCs have also shown promise in animal models of peripheral nerve