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Placental Stem Cells from Domestic Animals: Translational Potential and Clinical Relevance

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Abstract

The field of regenerative medicine is moving toward clinical practice in veterinary science. In this context, placenta-derived stem cells isolated from domestic animals have covered a dual role, acting both as therapies for patients and as a valuable cell source for translational models. The biological properties of placenta-derived cells, comparable among mammals, make them attractive candidates for therapeutic approaches. In particular, stemness features, low immunogenicity, immunomodulatory activity, multilineage plasticity, and their successful capacity for long-term engraftment in different host tissues after autotransplantation, allotransplantation, or xenotransplantation have been demonstrated. Their beneficial regenerative effects in domestic animals have been proven using preclinical studies as well as clinical trials starting to define the mechanisms involved. This is, in particular, for amniotic-derived cells that have been thoroughly studied to date. The regenerative role arises from a mutual tissue-specific cell differentiation and from the paracrine secretion of bioactive molecules that ultimately drive crucial repair processes in host tissues (e.g., anti-inflammatory, antifibrotic, angiogenic, and neurogenic factors). The knowledge acquired so far on the mechanisms of placenta-derived stem cells in animal models represent the proof of concept of their successful use in some therapeutic treatments such as for musculoskeletal disorders. In the next future, legislation in veterinary regenerative medicine will be a key element in order to certify those placenta-derived cell-based protocols that have already demonstrated their safety and efficacy using rigorous approaches and to improve the degree of standardization of cell-based treatments among veterinary clinicians.

Keywords

placenta stem cells, regenerative medicine, cell-based therapy, domestic animals

Introduction

Stem cell–based regenerative medicine represents one of the most relevant challenges in the biomedical sciences. The scientific expectation toward regenerative medicine is related to its potential in producing a paradigm shift in medicine. With few exceptions (i.e., antimicrobials and hormone replacement therapy), traditional medicine has been concerned with treatment of symptoms of disease but rarely the correction or reversal of the pathology itself.

However, whenever possible, medical therapy should also contain a component of disease correction. Correction of a disease process can be accomplished through several mechanisms. The therapy applied can itself replace or reverse the disease-causing process. The cell-based approach, if effective, would directly replace the degenerated tissues of the patient by providing an immediate and direct functional effect. One of the mechanisms of action prompted by cell transplantation is to stimulate disease correction by facilitating the body's innate regenerative pathways. Another way to enhance repair of damage is via inhibition of events that actually prevent natural

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regeneration from occurring. Cell-induced regeneration may, indeed, proceed through many processes even if 2 major mechanisms are recognized: the in situ transdifferentiation toward the lineage of host damaged tissues and/or the improvement of paracrine factors that modulate the proliferation/differentiation of resident progenitor cells. In both cases, the repair of the damaged tissues and the restoration of organ functions can be achieved. Since increasing evidence supports the hypothesis that stem cell therapy has the capacity to provide the complex processes involved in tissue regeneration it is now moving from translation to clinical practice[1–3]. However, there are still knowledge gaps and safety concerns regarding stem cell–based therapies. Improving the research value of large animal models may represent one key challenge to favor the progress of regenerative medicine and to facilitate eventual use of them in medical clinical translational research.

Veterinary Stem Cell–Based Therapy: Domestic Animals between Translational Models and Patients

Veterinary medicine has an important role in the translational process offering the missing link between basic science and human clinical applications. Many diseases encountered in humans also pose a problem in veterinary patients with similar pathology and etiopathogenesis. These diseases certainly raise the interest in regenerative medical treatments on the veterinary side but, at the same time, offer a perfect model for human patients, much better than laboratory animals that do not accurately reproduce in full the complexity of disease conditions.

Tight cooperation between basic science and human and veterinary medicine would therefore not only be beneficial for veterinary patients but would drive the field of regenerative medicine forward for the benefit of human patients. This concept is clearly supported from past preclinical studies performed on canine models that have been instrumental in advancing hematopoietic stem cell protocols for human oncology. Translational preclinical studies have allowed the development of bone marrow (BM)-based therapy[4–6] and optimization of this technique over 4 decades[7,8]. Success in numerous animal models of disease and emerging achievements in human clinical trials allow scientists to realize that stem cell–based regenerative medicine will soon be possible. A remaining challenge is collaboration among different experts such as veterinarians, biologists, geneticists, physicians, and other scientific health and environmental professionals operating using a "One Health" approach[9].

Animal Models for Stem Cell–Based Regenerative Medicine: Mice versus Domestic Animals

It is generally accepted that use of companion (dog, cat, and horse) and farm animals (sheep, goat, bovine, and pig) has an enhanced ability over laboratory mammals for predicting clinical efficacy of new medical devices, pharmacological therapies, and cell organ–based surgery[10]. It should be noted that the nomenclature regarding animals is quite confusing. In this review, companion and farm animals will be referred to as domestic animal models whether they are used as clinical patients or in preclinical/translational settings.

Although the use of rodents, especially genetically altered mice, maintains a central role in the study of stem cell biology, no rodent models have better advantages compared to domestic animals with regard to system physiology and anatomy, which are more similar to humans. Indeed, domestic animals and humans share a similar basal cellular metabolism rate of cells, a longer life span, comparable organs in size, and physiology[11–15]. Moreover, unlike laboratory rodents, the health conditions of domestic animals are determined by lifestyle influences. They are outbred and thereby continually exposed to environmental factors that underlie several diseases (cancer, diabetes, etc.) or traumatic defects. Similar to humans, many dogs and horses are expected to undertake an athletic (e.g., sport horses and agility dogs) or a working career (service dogs). This increases the incidence of chronic musculoskeletal disorders that continue to be a therapeutic, diagnostic, and clinical challenge in medicine. Another relevant aspect that increases the scientific interest in nonrodent animal models is that they suffer from a variety of spontaneously occurring diseases that reproduce the human phenotype and etiology. In particular, some domestic animals are currently used to study human genetic diseases. Indeed, they may be spontaneously affected by genetic disorders induced by a single gene defect or due to the complex interaction between gene expression and environmental conditions[16–18]. Alternatively, genetically induced conditions are also inducible in nonrodent animals to reproduce debilitating degenerative disorders (e.g., Alzheimer and Huntington diseases, cystic fibrosis, and muscular dystrophy) by targeting specific genomic sites[19]. Thus, the use of domestic animals has a tremendous potential for validating and advancing the crucial field of regenerative medicine.

Even if domestic animals offer numerous advantages, major limitations remain. For example, specie-specific reagents are less available, such as antibodies, growth factors, and fully annotated expression microarrays. There is a lack of centralized resources where cells are characterized and stored, reagents made available, and databases maintained for the wider biomedical community. If these drawbacks still represent a barrier for researchers, several strategies can be implemented to overcome these limitations.

Important research institutions like the National Institutes of Health (NIH) are trying to overcome these limitations by developing a publicly available website and annual meetings that provide investigators and program officers with an online resource to disseminate information such as what species are available from which particular centers, service/expertise available at each site, and so on in order to

facilitate collaboration. Indeed, having better integrative and informatics tools will likely have a greater and more innovative impact on biomedical research.

All of these efforts can be strengthened by the awareness that research using domestic animals will complement the use of mice, leading to more comprehensive studies that can then be applied to humans.

Stem Cell–Based Therapy Application in Veterinary Medicine

The impact of stem cell-based regenerative medicine in veterinary clinics offers interesting insights. Several private companies, spin-offs, and university departments (Vet-stem; VetBiologics; UCDAVIS; Riddle and Rood; RENOVOCyte; Biologics Medivet; Celavet; Therapies, ART Advanced Regenerative; Laboratories, Fat-Stem, etc.) are engaged in a widespread stem cell service at providing cells isolated from patient's tissue samples with or without an amplification step, or, alternatively, to sell kits that allow for in-house cell isolation from tissues. Such a service, originated in North America, has now extended to Asia (Histostem Co., Ltd., South Korea) and Europe (Belgium Fat-Stem Laboratories) and supports cell-based treatments for thousands of animals[20,21]. However, this empirical use of cell products applied in a variety of pathological conditions mainly in horses, dogs, and cats has not really enhanced knowledge on the properties and mechanisms of these innovative therapeutic procedures for the care of animals. The major clinical outcomes generated by this widespread practice are mainly represented by anecdotal and case reports. Although the animal cell products have been commercially available since 2003, few studies have documented the scientific improvement promoted by the injection of autologous cells collected by adipose tissue (AT). As an example, 2 double-blinded controlled and multicenter studies performed on 21[22] and 39 dogs[23] affected by coxofemoral osteoarthritis (OA) and 2 clinical trials involving 14 recruited dogs with humeroradial joint OA[24] and 10 dogs with severe hip OA[25] have been published.

A widespread use of stem cells in veterinary clinics is the consequence of a complete absence of legislation. For this reason, cell therapy may be adopted using an empirical approach without holding a solid scientific basis. Veterinarians can either prepare the necessary cell-based therapy products themselves or, alternatively, obtain the stocks from suppliers located in their respective country or from abroad. Although, at least in Europe, stem cell–based pharmaceuticals for veterinary use have been widely ignored by legislators, no special regulations have been issued compared to stem cell–based pharmaceuticals for human use. The existing legislation is incomplete and leaves too many loopholes for unproven stem cell–based pharmaceuticals for veterinary use. It is likely that in the future, regulations on veterinary use of cell therapy will be modeled after the established legislation for cell therapy for humans. However, in the meantime, the popular appeal of stem cell–based therapies, and their widespread commercialization, has led to their application for many conditions in veterinary patients for which there are little to no evidence-based preclinical animal studies or even supporting in vitro data. Therefore, enthusiasm for stem cell therapies as a powerful treatment strategy for the repair and regeneration of tissue injury and disease must be tempered until experimental evidence is sufficient to supersede anecdotal reports. In the absence of any regulations, however, no such cell-based therapy protocol has been certified so far and we are missing out on the opportunity to expand the evidence-based preclinical and clinical trials on veterinary patients, which may accelerate the advancement of regenerative medicine applications for several mammalian species[26].

The current European Union (EU) and national legislations on veterinary medicine needs to be reformed in order to bring about legislative improvements, which can facilitate the development of cell-based pharmaceuticals for human use. The legal requirements of manufacturing, marketing, and application of cell-based veterinary pharmaceuticals are not as well developed as the requirements for chemical pharmaceuticals. Stem cell–based veterinary pharmaceuticals are medicinal products in the sense of the pharmaceutical laws of the EU. For that reason, such medicinal products principally require official approval for their manufacture and an official marketing authorization for their placement on the market before being used by the veterinarian. The manufacture, marketing, and use of cell-based veterinary pharmaceuticals without manufacturing approval and marketing authorization, is permitted only in certain exceptional cases determined by EU and individual Member State law. Violations of this requirement may have consequences for the respective veterinarian under criminal law and under the code of professional conduct in the respective Member States[26].

Therefore, legislation is desirable in order to be able to certify the safety and efficacy of cell-based treatments, to standardize protocols, and to make comparable clinical outcomes between centers. Obviously, legislation such as that applied to stem cell–based pharmaceuticals in medicine may completely hinder veterinary research. Animal health, indeed, does not receive public funding and does not affect the economy the way human medicine does. However, the lack of any legislation limits the ability to control the clinical effects of innovative therapeutic approaches and it neutralizes the positive effects that could arise from dynamic market competitiveness.

Stem/Progenitor Cell Sources for Veterinary Regenerative Medicine

Several preclinical and clinical studies have been performed on domestic animals, offering important insights on cell-based tissue regenerative mechanisms. Most of the information has been derived from canine and equine models which have been chosen based on their impact on veterinary

Table 1. Cell-Based Regenerative Medicine in Dog.

Stem/progenitor cells source	Experimental and spontaneous diseases treated with cell-based protocols
Hematopietic Stem Cells - Bone marrow[19,118,119] - Peripheral blood cells[120,121]	**Hematologic cancer disorders** [120,149]
Mesenchymal Stem Cells - Bone marrow[68,80,122-125] - Adipose tissue[68,80,123,126-132] - Umbilical cord[68,78-80,133-139] - Skeletal muscle[139] -	**Musceleskeleton** - Bone[67,68,150-161] - Intrarticular[22-25,162,163] - Muscle[164,165]
Amniotic derived Cells[134,135,140-143]	**Neurogenic**[78-80,166-171]
Embryonic Stem Cells[144]	**Cardiac**[172-179]
induced Pluripotent Stem Cells[145-148]	**Ophmatology**[106,107]
	Urologic[180]

Table 2. Cell-Based Regenerative Medicine in Horse.

Stem/progenitor cells source	Experimental and spontaneous diseases treated with cell-based protocols
Mesenchymal stem cells - Bone marrow[94,181-191] - Peripheral blood cells[181,187,192] - Adipose tissue[93,181-185,189,191,193-196] - Synovial membrane[197-199] - Peridontal ligament[200-202] - Umbelical cord[75,185,203-218] - Tendon derived stem cells[219-221]	**Muscleskeleton** - Tendon[55,58,59,92,96,101,103,104,190,193,212,224,236-260] - Bone[261,262] - Osteoarticular[75,182,191,263-278] - Muscle[257]
Amniotic derived Cells[55,101,110,203,204,222-227]	**Wound**[110,279]
Embryonic Stem Cells[228-232]	**Ophmatology**[108,109]
induced Pluripotent Stem Cells[148,231,233-235]	

medicine (animal patients) and from ovine or porcine models mainly adopted as translational models. As detailed in Table 1, the stem/progenitor cells isolated so far in canine model are of various origins, but the most characterized are mesenchymal stem cells (MSCs) isolated from BM, AT, and umbilical cord (UBC). Additionally, other stem cell sources have been thoroughly investigated recently such as amniotic cells and to a lesser extent, embryonic and induced pluripotent stem (iPS) cells (Table 1). Scientists have focused mainly on treatment of canine musculoskeletal, cardiac, and nervous system disorders. Moreover, the dog has represented for decades the ideal translational model for optimization of cell transplantation in hematologic cancers, whereas, more sporadically, it has been adopted for the treatment of ophthalmologic and urologic disorders (Table 1).

The horse is another domestic animal in which stem cell therapy has been extensively studied and applied either to treat experimental or spontaneous diseases (Table 2).

MSCs derived from BM and AT are the chief cell types used in equine research and clinic trials, however many other stem/progenitor cells sources have been taken into account and relevant results have been obtained to date (Table 2). Stem cell–based protocols have been mostly developed so far to deal with pathologies related to sports medicine such as tendon, ligament, and cartilage/joint disorders or, to a lesser extent, bone defects. It is not surprising that musculoskeletal disorders represent the main clinical target in horses, a species largely involved in athletic competition.

Sheep represent another domestic animal model for designing translational experiments aimed at verifying the effectiveness of stem cell–based therapy for musculoskeletal disorders. Sheep, indeed, are considered a valuable medium-sized translational mammal. In this model, evidence of regenerative potential of amniotic cells, MSCs isolated from BM and from UBC, as well as the embryonic stem cells have been documented (Table 3). Importantly, the ovine model offers the opportunity for investigating prenatal surgical stem-based treatments. Many rigorous preclinical studies of in utero stem cell transplantation have confirmed that the clinical use of stem cells can be adopted to ameliorate prenatal congenital diseases, thereby offering new innovative therapeutic approaches (Table 3).

In addition, animal iPS cells represent powerful biological models for assessing human iPS therapeutic applications. There was an innovative breakthrough in the field of stem cell research with the isolation of iPS cells from humans and mice. Several studies on various animal cellular systems (Tables 1–3) suggest that the basic pluripotency network appears to be conserved among different species, allowing derivation of iPS cells from a variety of domestic animal species.

Placenta-Derived Stem Cell Application in Domestic Animal Models

In addressing the complex scenario described above, increasing attention has been focused on the placenta as a possible source of progenitor/stem cells. The embryonic origin of placenta-derived cells (PCs) explains the evidence of their retained high plasticity, with the possibility of providing progenitor/stem cells that are capable of differentiating into multiple cell types[239]. Meanwhile, the fact that the placenta is fundamental for maintaining physiologically feto-maternal tolerance during pregnancy suggests that cells present in placental tissue may simultaneously have low immunogenicity and immunomodulatory activities. The confirmation for this is the absence of MHC class I molecules and the low expression of MHC class II, allowing these cells to be effectively employed in immunocompetent transplanted organisms[224,240]. Furthermore, these

Table 3. Cell-Based Regenerative Medicine in Ovine Translational Animal Model.

Stem/progenitor cells source	Experimental and spontaneous diseases treated with cell-based protocols
Mesenchymal stem cells - Bone marrow[280-282] - Adipose tissue[280] - Peridontal ligament[281] - Umbelical cord[283,284] - Skeletal muscle[220]	**Muscleskeleton** - Tendon[30,32,54,60,292] - Bone[69,70,74,280-281] - Osteoarticular[76]
Amniotic derived cells[29,41,56,58,70,87-89,91,285-289]	**Wound**[86]
Embryonic Stem Cells[290]	**Prenatal**[87-89,91]
induced Pluripotent Stem Cells[148,291]	

cells have also been shown to secrete soluble factors involved in pathophysiological processes that may aid tissue repair, such as cytokines which have immunomodulatory and anti-inflammatory effects[215,241] as well as angiogenic factors associated with wound healing[216,242], growth factors related to cell proliferation and differentiation[243], and antiapoptotic and antioxidative factors[244]. These key aspects make cells from placenta ideal candidates for developing cell therapy protocols that encourage PC allo-transplantation or xenotransplantation in different domestic animal models. Indeed, autologous transplantation is more feasible with other stem cell sources (i.e., BM-MSCs, AT-MSCs, etc.); hence, the patient can benefit from his or her own stem cells. With PCs, allotransplantation and xenogeneic transplantation are more realistic than autologous, but it has been well documented that PCs from different animal models possess high genetic stability and marked immunomodulatory properties. Furthermore, given that the placenta is generally discarded after birth or can be frequently collected at the slaughterhouse for several domestic animals, the derived tissues are largely available, thus the recovery of cells does not involve any invasive procedures and their use does not pose any ethical concerns[239,245–249].

According to cell origin, PCs can be distinguished in:

- amniotic-derived cells from which can be identified amniotic epithelial cells (AECs), amniotic MSCs (AMSCs), and amniotic fluid MSCs (AFMSCs);
- UBC-derived stem cells from which can be isolated umbilical cord blood (UCB), umbilical cord blood MSCs (UCBMSCs), and umbilical cord matrix MSCs (UCMMSCs).

Despite the extensive literature available, domestic animal PCs are not easily comparable, especially considering the current lack of common protocols and the different gestational stages that can highly affect the native biological properties. Indeed, gestational age is a key factor capable of influencing morphological and functional properties of PCs. For instance, Barboni et al.[225] demonstrated that gestation considerably affected the expression of surface markers, as well as the expression and localization of pluripotency markers of ovine AECs. Moreover, their differentiation ability changed with the gestational age, affecting cell plasticity and the degree of global DNA methylation, which increased in term gestation amnia, thus probably affecting the outcome of cell transplantation therapies. Therefore, innovative approaches on stem cell aging in preclinical models are essential before their application for clinical translation[250].

In addition, methodological aspects make animal cell research results frequently noncomparable[239]. The major criticisms are the absence of commercially species-specific reagents and of a complete protein/genome database that is required for methodological conception and comprehensive procedures for monitoring and sharing results. Moreover, all culture protocols result in mixed cell preparations and obtaining specialized cells in sufficient quantities and purity is still a challenge especially for PC-MSCs. For all these reasons and limitations, continuous and careful updates on research and breakthroughs using domestic animal PCs may help bring about reproducible results and allow for comparison among groups, to focus on the conserved biological properties among species, and to better understand the mechanisms underlying the regenerative efficacy.

Starting from this premise, this review aims to provide an overview of the contribution of PC-based therapies by considering the scientific evidence arising either from preclinical or clinical trials performed on domestic animals.

Preclinical Studies to Test the Regenerative Properties of PCs in Domestic Animal Models

The properties displayed by PCs have led scientists to seek to take advantage of these types of stem cells by studying their therapeutic potential in domestic animal models of different diseases. In this regard, successful results on domestic animal preclinical models have been reported for the treatment of neurogenic disorders, myocardial infarction (MI), wound healing, prenatal diseases, and tendon bone and cartilage defects.

Several domestic animal models have been used to design preclinical experiments. Relevant for PCs is the ovine model where allo- and xenotransplantation settings have been performed. Ovine species play a major role in musculoskeletal regenerative and prenatal preclinical trials, because of its high translational value due to the similarities with humans in terms of weight, mechanical exertion, and reproductive gestational outcomes[11–14].

Musculoskeletal Preclinical Studies

Tendon Injuries

Tendon injuries are a common cause of disease in both human and veterinary medicine. Currently, in our society, more than 30 millions of musculoskeletal lesions occur and most of them involve tendons and ligaments. In the United States and in Europe, the economic impact of tendon and ligament morbidity is around €140 billion each year[251]. With the increase in life expectancy, tendon-related disorders will increase worldwide with a huge economic impact on the sanitary system[252]. The incidence is up to 25% considering the aging of the population, the increasing prevalence of metabolic disorders[253,254], and the increase in life expectancy[255]. Tendinopathies also have clinical relevance in veterinary medicine: 46% of racehorses suffer from tendon pathologies and their reduced sporting performance generate a negative economic impact estimated worldwide to be €400 billion[256,257]. Tendon injuries are among currently incurable diseases and the poor pronoses are often exacerbated by a high incidence of recurrences[258].

Tendons can be exposed to trauma during sports activities, but they can also be affected by overuse or aging. The most commonly injured are Achilles and patellar tendons in humans or superficial digital flexor tendons (SDFTs) in horses with pathologies ranging from degenerative tendinopathies, partial tears, up to complete ruptures[259–261]. These injuries are difficult to manage because adult tendons do not regenerate spontaneously but result in a fibrotic scar with poor tissue quality and mechanical properties, frequently resulting in long-term pain, discomfort, and disability[258]. Given the frequency and the increasing cost of treating injuries, as well as the relatively poor results obtained through surgical intervention, new and innovative strategies have become more appealing. In this context, in recent years, PCs have attracted increasing attention as a possible source of stem cells that may be useful for clinical application in tendon regenerative medicine[141–143,216,217,226,239,249,262]. In particular, our research group has increased the role of amniotic-derived cells[142,143,215–218,226,262] by carrying out preclinical studies adopting either allotransplantation or xenotransplantation approaches[218] on a validated ovine experimentally injured calcaneal tendon model (Fig. 1). These studies have demonstrated that ovine AECs have the ability to support tendon regeneration and an early recovery of the biomechanical properties of the tissue[216]. Through these studies, the mechanisms underlying tendon regeneration have begun to be elucidated. Transplanted AECs support tendon regeneration partly through a paracrine stimulation of the damaged host tissue. AECs modulate the production of critical growth factors (i.e., vascular endothelial growth factor [VEGF] and transforming growth factor beta1 [TGFβ1])[216] and of the immunomodulatory cytokines[215,218] involved in healing processes. Amniotic-derived cells enhance, innate regenerative mechanisms, which was confirmed by greater recruitment of tenocytes involved in the organization of the extracellular matrix and a more active remodeling of supporting tissues such as blood and nervous system. Interesting, data obtained under allotransplantation and xenotransplantation settings are converging in order to confirm a direct role of AECs in the process of tendon regeneration exerted through their in situ transdifferentiation (Fig. 2). Indeed, the molecular chimerism obtained in xenotransplantation settings confirmed that the cells, which survived within the host tissue for 28 d, modulated their gene profile by upregulating 48 human species-specific genes. The functional analysis of these genes revealed that they are involved in epithelial-mesenchymal transition (*KDM6B, NR2F2*), inflammatory response (*CCRL2*) and extracellular matrix synthesis (*COL1A1*[218] as indicated in Fig. 2). The relevance of these genomic results has been reinforced by evidence that AECs differentiated toward tenocyte-like cell-synthesized collagen type I (COLI), thus contributing to tissue regeneration through a direct release of major tendon extracellular matrix proteins[142,216] (see Preclinical studies and Clinical Trials boxes in Fig. 2). Moreover, allotransplanted AECs not only modulated the phases of tissue regeneration but also guided a specific process of healing. In particular, cell injection was associated with a specific centripetal process of tissue regeneration that started close to the healthy portion of the tissue and progressively affected the core of the wound site. This dynamic process of tendon healing was accompanied by the migration of transplanted AECs that were always recorded in proximity to the front of extracellular matrix deposition. These cells enhanced collagen synthesis and participated in the process of cell and matrix alignment. Among AEC regenerating mechanisms, immunomodulatory effects seem to exert a central role: AEC tendon transplantation induced a reduction in leukocyte infiltration and modulation of the recruitment of macrophages (Mϕ) M1, pro-inflammatory, and M2, pro-regenerative, phenotype in favor of the latter ones[215] (see Preclinical Studies box in Fig. 2). The immunogenic role switched on in AEC-transplanted tendons may have a role in accelerating early healing and in preventing fibrosis[215] (Fig. 1). Altogether, these findings support the idea that AECs are one of the most promising stem cell sources for achieving tendon regeneration. They are indeed able to direct tendon healing by stimulating a prompt recovery of tissue function without any preliminary transfection.[263–265] Another clinical advantage offered by AECs is that they promoted tendon regeneration without any undesirable in situ cell differentiation (osteogenic or chondrogenic) as observed after MSC transplantation[263,266]. For both of these reasons, AECs may represent a source of progenitor/stem cells that can be quickly obtained for clinical practice. Tendon regeneration was also confirmed using green fluorescent protein (GFP)-nucleofected AFMSCs by Colosimo et al.[217] Notably, not only were limb tendons regenerated with AFMSCs but also diaphragmatic tendons. Indeed, Kunisaki et al.[267] demonstrated neonatal diaphragmatic tendon repair with the use of an AFMSC-based engineered tendon that led to improved structural outcomes when compared with equivalent fetal

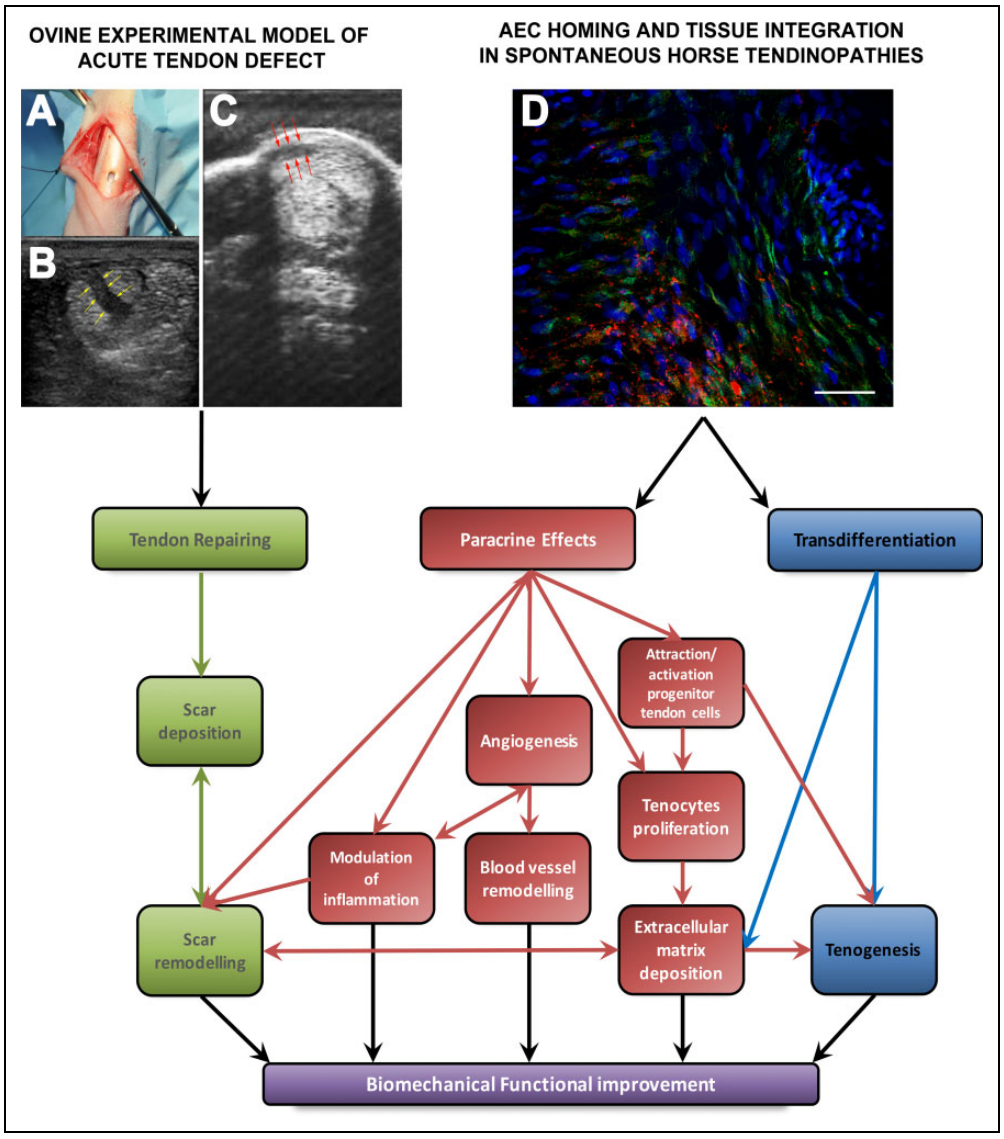

Figure 1. Potential mechanisms involved in tendon regeneration promoted by amniotic epithelial cell (AEC) transplantation. The figures and scheme described the potential mechanisms through which AEC-based therapies contribute to tendon regeneration as outlined in preclinical/translational studies and clinical trials performed on domestic animal models (i.e., sheep and horses). The scheme summarized that AEC transplantation induces an early functional recovery of the biomechanical properties of the damaged tendon by synchronizing angiogenesis, inflammation, and extracellular matrix deposition/remodeling. The early stage of tendon repair is also supported by the increased attraction and activation of resident progenitor cells as well as by the in situ transdifferentiation of transplanted AECs. The images on the left (A, B, and C) show the ovine experimental model of calcaneal tendon defect (equivalent to the Achilles tendon in human). (A) The defect was generated through the mechanical removal of a fixed volume of tissue. (B) Ultrasound image example of tendon immediately after the tissue removal (yellow arrows). (C) Ultrasound image example of the defect regeneration 14 d after AEC transplantation (red arrows). Inside of the defect, the deposition of new tendon fibers was evident through the improvement of the echogenicity score. The image on the right (D) displays the persistence of AECs injected into a spontaneous horse superficial digital flexor (SDFT) tendinopathy at day 60. The viability of AECs in host tissue has been demonstrated by loading the cells with a vital membrane dye (PKH26; Sigma-Aldrich, St. Louis, MO, USA). Cell labeling persisted after transplantation and it is identifiable as a red fluorescence localized on cell membrane around the blue nuclei (4′,6-diamidino-2-phenylindole [DAPI] counterstaining fluorescent dye [Sigma-Aldrich, St. Louis, MO, USA]). The host damaged tissue is visualized by the faint green fluorescence that indicated the low density of collagen type I (COLI, Chemicon Int., Billrerica, MA, USA) in the extracellular matrix. Scale bar = 50 μm.

myoblast–based and acellular grafts. Similar results were obtained by Turner et al.[268] who demonstrated tendon diaphragmatic repair with a clinically viable autologous tendon engineered with AMSCs with efficacy analyses performed up to ovine adulthood.

Bone Defects

Stem cell–based therapy for bone regeneration is an emerging treatment. PCs have the potential to be utilized to mainly treat craniofacial bone defects or major bone injuries. In particular, preclinical studies carried out on canine and

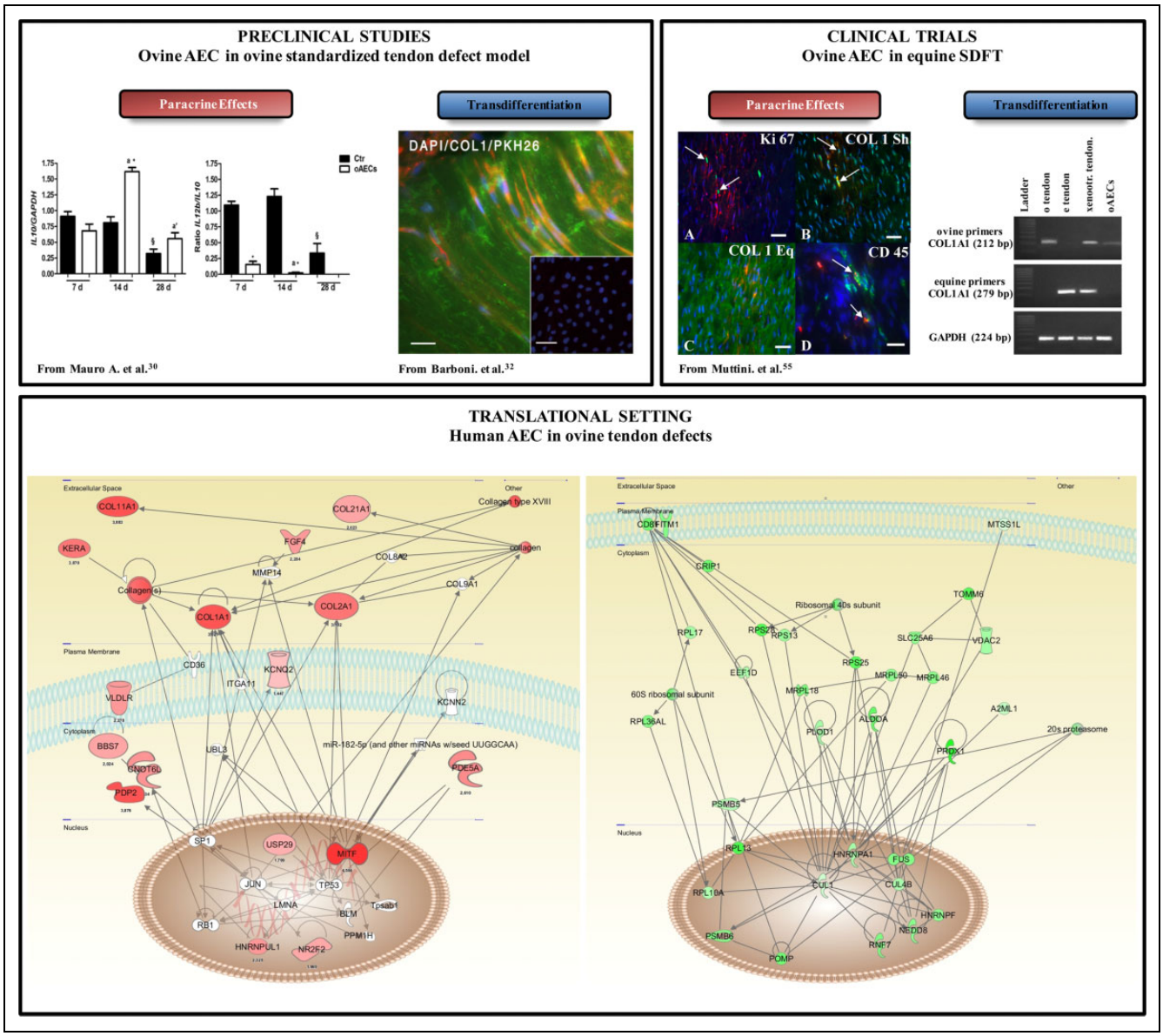

Figure 2. Regenerative mechanisms involved in tendon healing after amniotic epithelial cell (AEC) transplantation. The 3 boxes summarized the major scientific data clarifying the mechanism promoted by AECs for tendon regeneration in preclinical (ovine amniotic epithelial stem cells [oAECs] into ovine damaged tendon: left top box), clinical settings (oAECs into equine spontaneous tendinopathies: right top box), and translational (human amniotic epithelial stem cells [hAECs] into ovine tendon: bottom box). In all of the experimental settings, both paracrine and in situ differentiation data have been documented. (Left top box) The preclinical studies had documented the immunomodulatory influence of AECs through the higher expression of anti-inflammatory cytokines recorded in host tissue 28 d after transplantation (see histograms). The AEC in situ transdifferentiation (right image) was confirmed by immunohistochemistry. In particular, the image shows some PKH26-positive oAECs recorded in the experimental injured calcaneal tendons. Some of the AECs showed a fusiform shape and started to synthetize collagen type I (COLI). The latter event was demonstrated by the colocalization of the green (anti-COLI; Chemicon Int., Billrerica, MA, USA) and red (PKH 26; Sigma-Aldrich, St. Louis, MO, USA) fluorescence. The inserted box shows a group of freshly isolated AECs before transplantation that are negative for COLI. The cells were identified by the DAPI(Sigma-Aldrich, St. Louis, MO, USA) counterstained nuclei (blue fluorescence in small insert). In both the images, the scale bars is 50 μm. (Right top box) The clinical trials were performed using ovine AECs to cure superficial digital flexor tendons (SDFT) spontaneous tendinopathies diagnosed in sportive horses. The effect of oAEC treatments had been mainly documented on the basis of the positive clinical outcomes and of the athletic performances follow-up carried out for 18 mo after cell transplantation. However, after 60 d, 1 patient died for causes unrelated to the treatment and allowed us to collect more detailed information. Left images (A, B, C, and D) are examples of PKH26-labeled AEC (red fluorescence) paracrine effects obtained with immunohistochemical analyses. (A) The proliferative marker Ki-67 (Dako Cytomation, Denmark) was observed either in oAECs (PKH26-positive cells: cells indicated with arrows) or in several neighboring endogenous proliferating cells. (B) Flattened ovine AECs (PKH26-positive cells) parallel to the longitudinal axis of the horse tendon fibers were observed. Some of them colocalized within the cytoplasm species-specific ovine COLI (oCOLI; Chemicon Int., Billrerica, MA, USA) antibody (cells indicated with arrows). (C) PKH26-positive oAECs were also identified among the equine COLI (eCOLI; Abcam, Cambridge, UK) fibers (green fluorescence). (D) CD45; AbD Serotec, Oxford, UK marker (green fluorescence) was used to record the leukocyte infiltration and to identified ovine

ovine models have demonstrated that 3-dimensional scaffolds engineered with PCs are able to repair different types of bone defects. Indeed, Jang et al.[54] have demonstrated that the orthotopic implantation of canine UCBMSCs mixed with beta-tricalcium phosphate (β-TCP) was able to enhance osteogenesis in a dog diaphyseal radius defect model. Additionally, UCBMSCs were applied to a dog with nonunion fracture. Histomorphometric analysis revealed a significant increase in new bone formation at 12 wk after implantation, indicating that a mixture of UCBMSCs and β-TCP is a promising osteogenic material for repairing bone defects. Moreover, Kang et al.[32] carried out another in vivo orthotopic implantation assay on radial diaphysis of Beagle dogs by demonstrating that MSCs derived from AT, BM, UCB, and UCM have similar osteogenic capacities even higher than cell-free implants. However, clinical application is more feasible for the MSC source that can be most easily and noninvasively collected such as UCB and UCM.

Similar successful results on bone regeneration were obtained by implanting ovine AECs into a sheep tibia defect and into a maxillary sinus lift model[220,221]. The labeled AECs survived in the experimental tibia lesions for 45 d and supported consistent bone neoformation and reduced the infiltration of inflammatory cells, thus showing the potential applications in osteogenic regenerative medicine for this type of PCs. Notable mechanistic advantages have been gained in oral bone regeneration settings. All of these preclinical studies were carried out in a sheep model by mimicking the sinus augmentation lift human maxillofacial procedure. The surgical experimental protocol for extraoral maxillary sinus augmentation has been previously validated and tested for its translational value[269] by comparing size, structural, and functional parameters with humans[270,271]. Furthermore, ovine AECs were able to enhance bone regeneration after maxillary sinus augmentation when implanted for 45 and 90 d with synthetic bone substitutes[221]. AEC allotransplantation provided prompt scaffold integration in host tissue. Moreover, sinus explants displayed a reduced fibrotic reaction, a limited inflammatory response, and an accelerated process of angiogenesis when sinus lift was made with engineered AEC scaffolds. The prompt recovery of homeostasis in cell-treated sinuses may contribute to the increased bone deposition and the widespread presence of bone nucleation foci. Additionally, using the maxillary sinus lift model, ovine AECs exerted a relevant paracrine role to modulate VEGF expression and pro- and anti-inflammatory cytokine expression, thus successfully guiding tissue regeneration. AECs directly participated in bone deposition, as suggested by the presence of ovine AECs entrapped within the newly deposited osteoid matrix and by their ability to switch-on the expression of bone-related genes when transplanted into host tissues. Analogously, AFMSCs have demonstrated a similar efficacy role in supporting bone regeneration in maxillary sinuses. Berardinelli et al.[222] engineered a commercial magnesium-enriched hydroxyapatite/collagen scaffold for orthopedic purposes and demonstrated that ovine AFMSCs may improve bone regeneration by persisting for 90 d postimplantation.

Joint/Cartilage Injuries

Sporadic research linked to the use of PCs for experimental joint defects are available to date. Of interest, the preclinical study aimed to verify in horses the safety of allogeneic UCB intra-articular transplantation[121]. It has been demonstrated that there were minimal local responses, such as joint swelling or lameness after UCM and UCBMSCs intrasynovial injection, in healthy horses thus offering the first biological paradigm for the allogeneic use of these cells. Recently, amniotic membrane (AM) samples have also been tested in vivo for their regenerative role in full-thickness femoral cartilage defects[223].

Soft Tissue Preclinical Studies

Neurogenic Disorders

Spinal cord injury (SCI) produces progressive cell death, axonal degeneration, and functional loss in multiple motor, sensory, and autonomic system neurons[272]. All preclinical research on PC-based SCI regenerative medicine has been carried out in dogs using UCBMSCs. The first study performed allogenic UCBMSC transplantation for SCI induced by balloon compression at the first lumbar vertebra. In this research, Lim et al.[45] found that transplantation of the UCBMSCs resulted in recovery of nerve function with a significant improvement in the tissue conduction velocity based on the somatosensory evoked potentials. In addition,

Figure 2. *(continued)* phagocytated PKH26-positive AECs (merged green and red fluorescence and indicated by the arrows). Scale bar = 25 μm. Right box (transdifferentiation). The *oCOLI* expression performed with species-specific primers was used to verify the differentiation of oAECs after xenotrasplantation into the equine SDFT. Reverse transcription-polymerase chain reaction (RT-PCR) analysis, performed 60 d posttransplantation, confirmed the presence of *oCOLI* gene expression in the equine host tissue thus documenting the in situ specialization of the ovine transplanted AECs. (Bottom box) A translational setting was designed by transplanting human AECs into an ovine calcaneal tendon defect for 28 d. Taking advantage of genomic chimerism (human vs. ovine), an active in situ specialization and a paracrine role of hAECs were substantiated by the microarray analysis. Ingenuity Pathway Analysis (IPA)-inferred top network for modulated gene data set analysis was generated for upregulated (red network) and downregulated (green network) transcript data set to disclose functional networks based on their connectivity and enrichment statistics. Color legend spans from dark to light, which reflect more or less downexpression, respectively. Genes labeled in white are not modulated. The network is constructed following the subcellular localization of the genes. (Left image) The majority of the upregulated transcripts support human AEC specialization after transplantation. (Right image) By contrast, a more generic biological role may be associated with the function of downregulated genes.

Park et al.[46] compared the effects of UCBMSCs at different transplantation time points after SCI induction. These authors identified the best interval between SCI and cell injection, demonstrating that transplantation of UCBMSCs 1 wk after injury induction was more effective in improving clinical signs and neuronal regeneration by reducing fibrosis. The analyzed tissues showed an increased expression of neuronal markers. More recently, Ryu et al.[33] performed a comparative study by using MSCs derived from AT, BM, Wharton's jelly, and UCB. All sources of MSCs survived for 8 wk and reduced interleukin 6 (IL-6) and cyclooxygenase-2 (COX-2) levels, which may have promoted neuronal regeneration in the spinal cord. Although there was no significant difference in functional recovery among the different MSC groups, interestingly, UCBMSCs induced higher nerve regeneration, neuroprotection, and anti-inflammatory activity.

Myocardial Infarction (MI)

MI causes tissue death, and the important goal in this field of regenerative medicine is to replace lost tissue[273]. Only 2 preclinical studies on MI using domestic animal models have been conducted, both in porcine models. One study carried out by Sartore et al.[274] demonstrated the ability of autotransplanted AFMSCs to improve cardiac functional recovery after acute ischemic myocardium experimental defects in a porcine model. The regenerative role was exerted 30 d after transplantation through the transdifferentiation of the cells toward the vascular tissue lineage whereas, by contrast, evidence of in situ cardiomyocyte differentiation has been observed. The surviving AFMSCs downregulated mesenchymal cell markers with the exception of smooth muscle and endothelial antigens but did not express any major cardiac markers such as troponin.

Recently, Kimura et al.[275] demonstrated the therapeutic potential of porcine GFP-transfected AMSCs on a chronic myocardial ischemia model. The AMSCs survived after allogeneic transplantation performed in an immune-competent animal, by gaining the in situ cardiac phenotype through either transdifferentiation or cell fusion, differently from AFMSCs.

Wound Healing

Cutaneous wound healing requires a well-orchestrated integration of the complex biological and molecular events of cell migration and proliferation and extracellular matrix deposition, angiogenesis and remodeling. However, this orderly progression of the healing process is impaired in many chronic diseases[276]. Preclinical studies carried out to test PC regenerative properties for wound healing have been conducted in goat and sheep models. Azari et al.[277] investigated the effects of allotransplanted UCMMSCs on the cutaneous wound healing process. A histopathological study revealed a complete re-epithelialization after 7 d, whereas in control samples, the wounds still showed an incomplete process. An interesting experiment was carried out by Klein et al.[236] who investigated wound healing in fetal lambs. Fetal wound healing involves minimal inflammation and limited scarring. During this study, fetuses received an intra-amniotic infusion of labeled autologous AMSCs, clarifying their direct role in accelerating wound closure and enhancing the extracellular matrix profile rather than the release of soluble factors. The mechanisms highlighted still need to be fully elucidated and hold valuable clues for wound healing and the development of MSC-based regenerative strategies, both perinatally and later in life.

Prenatal Preclinical Studies

Many rigorous preclinical studies have focused their attention on the in utero PC transplantation to ameliorate prenatal congenital disease. All of these studies have been carried out using a sheep model. Shaw et al.[227] were the first to demonstrate the safety of in utero AFMSC autologous transplantation. In this study, GFP-transduced AFMSCs were injected into the peritoneal cavity of each fetal sheep donor. GFP-positive cells were detected in fetal tissues including liver, heart, placenta, membrane, UCB, adrenal gland, and muscle, demonstrating that autologous AFMSCs have widespread organ homing and can offer an alternative treatment for prenatal congenital diseases. These authors were also able to establish the hematopoietic potential of GFP^- sheep AFMSCs selected for CD34 (GFP^-CD34^+ AFMSCs). After autologous in utero transplantation these cells colonized hematopoietic organs and peripheral blood, confirming their potential for the development of cell-based protocols to treat congenital hematopoietic diseases[228].

Prenatal studies have also been conducted to treat airway pathologies. Particularly, Gray et al.[229] have shown that AMSC-engineered airways may become an option for perinatal airway repair. Fetal lambs with tracheal defects were implanted with expanded/labeled autologous AMSCs engineered in the de-cellularized leporine tracheal segment. Lambs that survived to term could breathe at birth. Engineered constructs exhibited full epithelialization, displaying a pseudostratified columnar epithelium, a significantly greater degree of increase in elastin levels after implantation than acellular grafts.

Severe congenital tracheal anomalies, namely, long segment stenosis, atresia, and agenesis, represent another typology of unsolved prenatal diseases[267,278]. Engineered cartilaginous grafts with GFP-AFMSCs have been used for fetal tracheal repair by evaluating their effect to term. Respiratory functional tests combined with morphological evidence of the presence of fluorescent protein–positive cells lined with pseudostratified columnar epithelium and remodeled into a predominantly fibrous cartilage pattern were the most relevant results obtained. By contrast,

implants alone did not show any significant changes in glycosaminoglycans, collagen, or elastin content at harvest. Thus, these findings demonstrated that AFMSCs can be a practical cell source for engineered tracheal reconstruction.

The in utero PCs transplantation technique was also used for the treatment of prenatal congenital cardiac malformations. Weber et al.[230] carried out prenatal heart valve interventions aimed at the early and systematic correction of congenital cardiac malformations. In this experiment, fetal implantation was carried out in utero into the pulmonary position of prenatally engineered biodegradable polyglycolic acid- poly-4-hydroxybutyrate (PGA-P4HB) composite heart valves with autologous ovine AFMSCs. Tissue-engineered heart valves showed in vivo functionality with intact valvular integrity and absence of thrombus formation, thus providing evidence that this approach may serve as an experimental basis for future human prenatal cardiac interventions and a promising treatment option in maternal–fetal care.

Clinical Application of PCs in Veterinary Regenerative Medicine

Domestic animal PC clinical application is limited to date and has been used mainly to treat tendinopathies in horses and ocular surface reconstruction both in horses and in dogs.

Musculoskeletal Applications

Tendinopathy

Tendinopathies of the SDFT is a significant cause of lameness and often a career-ending event in Thoroughbred horses because of its high incidence, prolonged recovery period, and high rate of recurrence[97,110,144,145,262,279]. Afflicted horses are prone to distal limb injury due to hyperextension of the metacarpal joint during racing or riding; thus, the SDFT represents the highest frequency of injury in racehorses[280]. After injury, the equine SDFT heals via a process of fibrosis, but the scar tissue that forms is functionally deficient compared to that for the normal tendon and it is the predisposing factor of the high incidence of recurrences[281–283]. However, recently in equine medicine, PCs have been used to treat tendon injuries. The most widely used cells for this purpose are UCB and amniotic-derived cells. The first clinical trial was performed with horse AMSCs to investigate their therapeutic potential and cell tolerance in vivo, when allogeneically injected into spontaneous tendon injuries. The study resulted in a quick reduction in tendon size and ultrasonographic (US) cross-sectional area measurements[146]. The same group also conducted a series of clinical studies that confirmed the efficacy of the amniotic-derived stem cells in curing SDFT tendinopathies demonstrating their better clinical outcome over BMMSCs[284]. In the same year, Lange-Consiglio et al.[141] demonstrated that the conditioned medium obtained from horse AMSCs can also be useful for cell therapy applications in tendon diseases, hypothesizing that these cells may promote tendon repair mainly via paracrine-acting molecules targeting inflammatory processes rather playing a direct regenerative role. This study identified AMSC-conditioned media as a novel therapeutic biological cell-free product for treating horse tendon and ligament diseases.

Another source of amniotic-derived cells has been used with success in the treatment of equine spontaneous tendinopathies. Muttini et al.[142] have demonstrated that ovine AEC xenotransplantation was able to improve the clinical outcome in 15 horses with acute SDFT lesions. In particular, US controls showed infilling of the defect and early good alignment of the fibers, and 12 of the 15 horses resumed their previous activity during the 18 mo after treatment. The clinical data were also substantiated by histological analyses carried out on a treated SDFT of a horse who died for unrelated causes. The results demonstrated that ovine AECs contribute to tendon healing. The recovered transplanted cells were indeed able to deposit ovine COLI in the repaired area, as revealed by using ovine-specific primers and antibodies that did not cross-react with equine COLI. These cells also confirmed their low immunogenicity, as they were able to survive in the healing site for 60 d.

The effective role of ovine AEC treatment has been confirmed in an experimental trial carried out on 2 horses with acute and 1 horse with chronic spontaneous SDFT tendinopathies. Muttini et al.[143] used xenotransplantation with ovine AECs demonstrating that, after 180 d, they were able to induce an almost complete restoration of normal tendon architecture with an optimal alignment of tendon fibers. AECs represent to date the only cell source used for the treatment of tendinopathies where experimental, preclinical, translational, and clinical studies have been combined to demonstrate the efficacy and safety of these stem cell–based protocols.

Studies using UBC-derived stem cells have also demonstrated their efficacy in healing SDFT tendinopathies[147]. Additionally, equine UCBMSCs[148] have also demonstrated a therapeutic effect in clinical cases of desmitis of the suspensory ligament and of the inferior check ligament and tendinitis of the deep digital flexor tendon.

Soft Tissue Applications

Ophthalmology

The positive results in human and veterinary medicine have led to the use of the AM as a clinic dressing protocol to promote, healing of epithelial tissues[248,285]. AM transplantation indeed has an effective clinical role in veterinary ophthalmology for its avascular and strong structure and for the large presence of growth factors (mainly antiangiogenic and anti-inflammatory) that are able to prevent or decrease fibrosis during healing.

Indications for its use are steadily growing from previously human experience and include grafting/patching to replace diseased, missing, or excised tissue. Alternatively, AM has been used as a substrate for the expansion of epithelial cells for transplantation for organs or tissues such as the cornea. In this context, AM transplantation has been demonstrated to preserve the integrity of the globe, optimize the visual outcome, and minimize scarring in severely diseased corneas.

Based on these findings, xenogeneic use of AM was adopted in companion animals as replacement for full-thickness corneal defects (18-treated dogs)[94] and to treat keratomalacia (1 dog)[94], fibrous histiocytoma (1 dog)[94], or symblepharon (1 cat)[95]. In all of these clinical cases, the patients experienced reduced ocular pain by recovery of vision and tissue architecture although some degree of graft rejection was observed[94].

In the same year, AM was also allotransplanted in horses affected by corneal ulceration and severe keratomalacia[205]. The treatment preserved vision and maintained the structural integrity of the globe by maximizing cosmesis in the eyes. The validity of AM transplantation for ocular surface reconstruction in horses was then definitively confirmed by Plummer et al.[206] who conducted a retrospective study on 58 equine clinical cases.

Wound Healing

In domestic animal patients, the use of AM to promote wound healing has been less investigated than in humans to date with the exception of 1 paper by Iacono et al.[193] which compared AFMSCs and platelet rich plasma (PRP) gel treatments in severe decubitus ulcers by demonstrating that the combination of AFMSCs plus PRP promoted a faster healing in aseptic neonatal foal.

Conclusions

Although PCs do not represent the most widespread used progenitors/stem cells in veterinary science and medicine, they probably are the most promising and scientifically solid cell source studied so far[239,249].

Rigorous investigations have begun to clarify the biological properties of PCs in domestic animals by confirming a high degree of conservation among species and reinforcing the idea of that experimental data between species remain robust and can be compared.

One valuable biological characteristic of PCs are their paracrine effects. Several studies using human and animal amniotic-derived cells demonstrated that either the native or induced secretomes contain an array of modulatory molecules and proteins capable of recapitulating most of the regenerative processes involved in the recovery of tissue homeostasis after cell transplantation[286]. A large number of human and animal studies confirm a conserved paracrine effect of amniotic-derived cells in the modulation of inflammatory and antifibrotic mechanisms. More recently, new perspectives on application of cells for in antiaging and antitumor therapies owe to the secretory activities of AECs[287].

Domestic animal PCs, in addition, have offered advanced insights in relevant challenges related to the ex vivo negative effects. Indeed, although most PCs can be maintained ex vivo and expanded, the yield and recovery of them can be quite variable and their biological characteristics appear to be dependent on the genotype, gestational age of the donor, and the collection/amplification methods[225,288–290]. These data introduce some important caveats related to cell culture that may limit the comparison of results among research groups and, as a consequence, the translation of animal and human studies into clinical trials. Data recently obtained on domestic animal AECs offers practical solutions to preserve the native phenotype during in vitro cell amplification, and culture methods have been proposed that may improve the biological regenerative properties of expanded AECs thus increasing the comparability of cell transplantation protocols[291].

The standardization of PC protocols represents, in addition, a prerequisite for developing comparable preclinical treatments. Of utmost importance is that the effectiveness of a treatment designed to replace or regenerate tissues depends on a correct balance between the inherent response of the recipient and the quality of the treatment itself.

The elucidation of the properties of PCs combined with the robust evidence of their safety and regenerative efficacy represents the proof of concept of their therapeutic use[239,292].

Based on the evidence discussed, PC-based therapy is now used in veterinary medicine for muskuloskeletal disorders, mainly in athletic horses[141,146,239].

The clinical translation of the PC-based treatments has been validated by a large amount of data collected in preclinical settings, which demonstrated that PC transplantation in different animal models promoted common mechanisms leading to regeneration. The strategies adopted by PCs to combat pathological phenotypes include the exogenous cell graft persistence, the direct replacement of the dysfunctional cells through the in situ tissue-specific lineage transdifferentiation (e.g., tendon- and bone-derived lineage cells), as well as the improvement of endogenous regenerative milieu realized through the release of pro-angiogenic, pro-neurogenic, anti-inflammatory, and antifibrotic factors[142,216–218,221,274]. However, what is not clear is how long exogenous cells remain viable and active, whether they change significantly with time and/or stimulation in vivo, and how dynamic changes may influence biomolecule production and immunogenicity. The potential extent and impact of PC viability and functionality and the delineation of local and systemic immune responses continue to be vital areas of study.

The biological the biological mechanisms and application of domestic animal PCs to date appears to be more consistent than other sources of progenitor/stem cells that are more commonly used in veterinary medicine such as MSCs

derived from adult tissues. On the basis of the data gathered from preclinical studies and clinical trials, PC-based treatment may have a wide range of applications for treating diseases that affect domestic animals either during adulthood or prenatally.

In light of the robustness of the basic experimental and clinical data obtained using PCs, it is not surprising if the use of these cells moves toward translation to clinical practice in human patients.

Future Perspectives

The demonstration of both safety and efficacy is paramount for translation to clinical applications. However, legislation may mandate delays in translating a method or treatment for veterinary regenerative medicine. Regulating drugs or biologics for veterinary medicine in a manner similar to that in human medicine may not be ideal and in the next future, a legislation in this operative context is desirable in order to be able to certify the safety and efficacy of different cell-based protocols and to standardize these treatments among clinicians.

It has been thoroughly researched and well-supported in preclinical and clinical multicenter studies that PCs possess superior regenerative potential compared to conventional cell-based treatments for horse tendinopathies. In addition, given the high economic value of horses, protocols for cell banking may be highly beneficial for this valuable source of stem cells. This is a practice that vets should consider when preparing for the birth of a foal. PC banking allow for a fast and readily-available supply of stem cells for when an injury occurs, which will give the horse the best chance of a successful recovery. Horses that would have PCs already available would therefore be at a considerable advantage following an SDFT injury. Furthermore, PC cryopreservation is crucial since this would allow for the generation of a quality-controlled stock of cells, transport of cells among investigators, and avoidance of the need for expensive and time-consuming continuous culture.

Furthermore, the consistent availability of data on domestic animal PCs could lead to the use of this cell source and others to treat unexplored animal diseases, which cannot currently be addressed by drug therapy. Detailed characterization of PCs may be also useful insolving emerging and challenging technological issues related to, for example, accuracy and efficacy of cell injection site and doses, cell migration track, and off-target and monitoring long-term cell engraftment. The resolution of these applicative aspects is crucial for standardization of cell-based protocols and for increasing the predictive validity of regenerative medicine applied to human and animal diseases.

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