Review

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The Placenta as an Organ and a Source of Stem Cells and Extracellular Matrix: A Review

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Key Words
Placenta · Stem cells · Extracellular matrix · Decellularization · Tissue engineering

Abstract
The placenta is a temporal, dynamic and diverse organ with important immunological features that facilitate embryonic and fetal development and survival, notwithstanding the fact that several aspects of its formation and function closely resemble tumor progression. Placentation in mammals is commonly used to characterize the evolution of species, including insights into human evolution. Although most placentas are discarded after birth, they are a high-yield source for the isolation of stem/progenitor cells and are rich in extracellular matrix (ECM), representing an important resource for regenerative medicine purposes. Interactions among cells, ECM and bioactive molecules regulate tissue and organ generation and comprise the foundation of tissue engineering. In the present article, differences among several mammalian species regarding the placental types and classifications, phenotypes and potency of placenta-derived stem/progenitor cells, placental ECM components and current placental ECM applications were reviewed to highlight their potential clinical and biomedical relevance.

Introduction

The placenta is an active and complex-structured organ that contributes to the development and nutrition of the offspring, promotes fetomaternal immunotolerance and has a huge array of morphological variations among mammals [Carter and Mess, 2013; Mess, 2014]. Placenta-

Abbreviations used in this paper

AGM	aorta-gonad-mesonephros
ECM	extracellular matrix
EMP	erythro-myeloid progenitors
HSC	hematopoietic stem cell
PlGF	placenta growth factor
VEGF	vascular endothelial growth factor

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tion has been defined as the 'approximation or combination of an embryo's tissues with those of its natural or surrogate parent for physiological interchange' [Mossman cited in Mess and Carter, 2007], whereas the newly formed tissue, i.e. the placenta, has been defined as the structure that enables exchange between mother and offspring [Starck cited in Mess and Carter, 2007], including nutrients and gases [Mess and Carter, 2007]. Defective implantation and placentation reduce or limit reproductive efficiency across mammalian species [Johnson et al., 2003].

Placentation represents an important step in the evolutionary process [Carter, 2012; Garratt et al., 2013; Mess, 2014]. The growth of an embryo inside a shell, i.e. within an egg (oviparity), allowed vertebrates to live on land and breed independent of water [Ferner and Mess, 2011]. Such embryos (including birds and other terrestrial, non-mammalian species, as well as some mammals) are enclosed in four extraembryonic membranes (amnion, yolk sac, allantois and chorion) from which the mammalian placenta has evolved [Mess and Carter, 2007]. Current knowledge of the placental diversity in various taxa of mammals is consistent with distinct paths of evolutionary differentiation, with particular relevance for placental (eutherian) mammals that have chorioallantoic placentas [Ferner and Mess, 2011].

Placentas, especially the chorioallantoic ones, have important immunoregulatory features, which are well represented by tolerance of the embryo and fetus by the maternal immune system in a process that involves inhibition of excessive inflammation after blastocyst implantation, modulated by uterine natural killer cells [Moffett and Colucci, 2014; Soares et al., 2014] and proliferation of regulatory T cells [Tripathi and Guleria, 2015]. Additionally, placentas are reported to have anti-inflammatory, antibacterial and antiscarring activities. Those properties, plus the propensity to discard placentas after birth, have promoted interest in using placentas for applications in cell therapy and some regenerative medicine strategies [Lopez-Espinosa et al., 2009; Hong et al., 2010; De et al., 2011; Choi et al., 2013].

Placental Classifications

Placenta formation in eutherian mammals starts with the implantation phase (nidation), which is characterized by the apposition and adhesion of the hatched blastocyst to the endometrium. Thereafter, in the vast majority of mammalian species, this phase is followed by varying degrees of invasion (penetration) of the fetal trophoblast into the uterus. The extent of this process varies greatly among eutherian mammals and can be intrusive penetration (e.g. humans, rhesus monkey and guinea-pig, with the first species being more invasive than the others), displacement penetration (rat and mouse) or fusion penetration (rabbit and ruminants) [Bischof and Martelli, 1992]. Penetration of the conceptus trophectoderm into the endometrium, which in humans can also occur with relatively high frequency at ectopic sites, share several properties with invasion by a carcinoma, in the sense that it requires altered expression of adhesion molecules and elevated expression of matrix-degrading proteinases [Bischof and Martelli, 1992].

The placenta, fetal membranes and umbilical cord are commonly referred to as the fetal adnexa [Parolini et al., 2008]. Fetal membranes are comprised of the amnion (amniotic membrane), an innermost layer that surrounds the fetus and encloses the amniotic fluid, and the chorion, an external layer that attaches to the decidua, which forms the maternal part of the placenta [Parolini et al., 2008; Makhoul et al., 2013]. The amnion, a mostly avascular tissue, has been described as either a three- or five-layered structure, comprised of: (1) an inner compact layer, (2) a mesenchymal cell layer and (3) an outer intermediate or spongy layer (for a three-layer membrane), or in the case of a five-layer membrane: (1) an amniotic epithelial layer (a single layer of cuboidal to columnar cells), (2) a basement membrane, (3) a compact or stromal layer, rich in collagen fibers, (4) a fibroblastic layer and (5) a spongy or mucin-rich layer, the closest to the chorion [Parolini et al., 2008; Niknejad et al., 2013]. The chorion is composed of mesenchyme and a region of extravillous proliferating trophoblast cells [Parolini et al., 2008].

In eutherian embryos, the amnion and major components of the yolk sac (endoderm) and allantois are derived from the inner cell mass of the blastocyst, whereas the outer layer of cells gives rise to the trophoblast and contributes to the formation of extraembryonic membranes [Lillegraven, 2003]. Both the yolk sac and allantoic membrane, in conjunction with the chorion, could be involved in fetomaternal exchange [Ferner and Mess, 2011]. In early pregnancy, embryo-maternal exchange may be facilitated by apposition of the yolk sac endoderm to the chorionic trophoblast (termed the bilaminar omphalopleura). However, when the mesoderm (which includes vitelline blood vessels) is interposed between the endoderm and trophoblast, maternal-fetal exchange is stabilized [Carter and Enders, 2004]. This trilaminar structure, comprised of endoderm, vascularized mesoderm

Table 1. Duration of gestation, size and weight of placentas in various species

Species	Gestation, days	Breed	Size of the placenta	Weight	Study
Human	270		~22 cm (diameter)	470 g	Benirschke et al., 2012
Horse	338	Thoroughbred	~12.9×10^3 cm^2	3.8 kg	Allen et al., 2002
	338	Pony	~8.3×10^3 cm^2	1.7 kg	Allen et al., 2002
	340	Warmblood mares	–	5.3 kg	Klewitz et al., 2015
Dog	43	Boxer	~1.8 cm (width)		Almeida et al., 2003
	63	Maltese	~4.4 cm (diameter)		Son et al., 2001
	48	Beagle	~4.8 cm (diameter)		Yeager et al., 1992
Cat	60	Mongrel	~4.6 cm (width)	21 g	Illanes et al., 2007
	58	Mongrel	–	20 g	Malassiné and Ferré, 1979
Cattle	294	*Bos indicus*	–	8.03 kg	Ribeiro et al., 2008

Note the huge range in length of gestation (weeks to months) and placenta weight (grams to kilograms).

and trophoblast, forms the choriovitelline or yolk sac placenta (also called the trilaminar omphalopleura), which persists until term in some species (e.g. rodents and lagomorphs), but not in others (e.g. humans) [Carter and Enders, 2004; Mess and Carter, 2007]. In these species, growth of the allantois leads to partial or full displacement of the yolk sac from the chorionic trophoblast, so that a chorioallantoic placentation is established and vascularized by allantoic or umbilical vessels [Mess and Carter, 2007].

In chorioallantoic placentas, three fetal and three maternal layers are juxtaposed just before placenta formation: (1) the fetal endothelium from the allantoic capillaries, (2) the fetal connective tissue from the chorioallantoic mesoderm, (3) the chorionic epithelium formed by the trophoblast, (4) the uterine epithelium, (5) the uterine stroma or connective tissue of the endometrium (decidua) and (6) the maternal capillary endothelium from the endometrial blood vessels [Mess and Carter, 2007; Mess, 2014]. The decidua corresponds to the outermost part of the maternal side of the maternal-fetal interface, whereas on the fetal side, this is represented by the placental trophoblast [Johnson et al., 2003].

Several aspects of placentas are highly variable, including their size and weight (table 1). Therefore, they are commonly classified according to their macroscopic appearance, fetal-maternal interface (the placental barrier) and fetal-maternal interdigitation or internal structure.

Regarding their macroscopic features, which include the distribution of contact sites between fetal membranes and the endometrium, placentas are termed discoid (a single area of contact that gives rise to the placenta is formed, e.g. in humans, primates and rodents), cotyledonary (multiple areas of chorioallantoic attachments to the endometrium, e.g. in ruminants), diffuse (the vast majority of the chorioallantoic surface is involved in placenta formation, e.g. in horses, pigs and whales) and zonary (the placenta forms a band surrounding the fetus, e.g. in elephants and in carnivores, including dogs, cats and bears). In cotyledonary placentas, the fetal portion of a contact site is termed the cotyledon, the maternal portion is the caruncle and the cotyledon-caruncle complex is termed the placentome [Carter and Enders, 2004; Mess, 2014] (fig. 1).

According to the fetal-maternal interface (i.e. relation between the fetal trophoblast and maternal endometrial surfaces), placentas are classified as epitheliochorial, where the trophoblast is juxtaposed through simple microvillar interdigitation to the uterine epithelium (horses, pigs and ruminants, including cattle, sheep, goats and deer), synepitheliochorial, where there is apposition of the trophoblast to the maternal connective tissue but persistence of the uterine epithelium that is modified by migration of trophoblastic binucleate/giant cells (this term is currently used in lieu of syndesmochorial), endotheliochorial, in which the trophoblast is in contact with endothelia of maternal blood vessels (e.g. carnivores), and hemochorial, where the trophoblast is in direct contact with maternal blood (humans, apes, monkeys, rodents and lagomorphs). However, a combined pattern of these in-

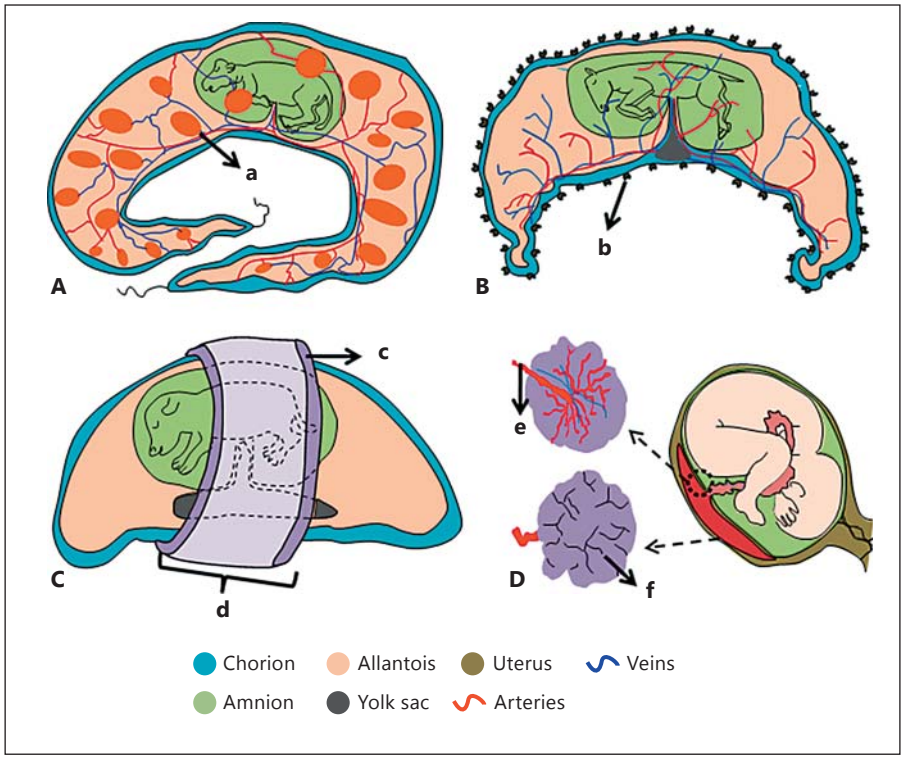

Fig. 1. Types of placentas according to gross anatomy. **A** Cotyledonary placenta, in cattle and others ruminants; cotyledons (**a**). **B** Diffuse placenta, in horses, pigs and whales; microcotyledons (**b**). **C** Zonary placenta, in carnivores; marginal hematoma (**c**), zonary placenta (**d**). **D** Discoid placenta, in humans, primates and rodents; umbilical cord (**e**), cotyledonary furrows (**f**). Modified from Steven and Morriss [1975], Benirschke et al. [2012] and Vejlsted [2012].

terfaces occurs in various species, such as ruminants. In addition, the hemochorial type can be subdivided into hemomonochorial, hemodichorial and hemotrichorial, depending upon the number of trophoblastic cell layers at the villous surface, as in primates [Carter and Enders, 2004; Peter, 2013; Mess, 2014] (fig. 2).

With regard to the type of fetal-maternal interdigitation (i.e. internal structure of the villi), placentas are described as trabecular, where the fetal blood vessels form branches of globular folds (resembling leaves) which are surrounded by maternal blood in an intertrabecular space (some neotropical primates), folded (pigs) or lamellar (some carnivores), which is partly similar to the trabecular placenta, labyrinthine, where the fetal capillaries are parallel to maternal capillaries or blood channels (rodents, lagomorphs, such as rabbits, and insectivores), and villous (humans), where a branching villi pattern forms a free-floating villous tree surrounded by maternal blood in an intervillous space [Carter and Mess, 2013; Mess, 2014; Gundling and Wildman, 2015] (fig. 3).

Several characteristics are used to describe a placenta. For instance, a human placenta can be appropriately classified as discoid, chorioallantoic, hemochorial and villous. Furthermore, not all combinations of placental classifications have been described.

The Placenta and Hematopoiesis

Hematopoiesis has been commonly described as a two-phase process (a primitive or first wave, followed by a definitive or second wave), although an additional transient stage has been described, particularly in the early embryonic development of vertebrates. The initial onset of hematopoiesis occurs at the ventral mesoderm of the embryo, which gives rise to hemangioblasts, a process initially mediated by the *aggf1* gene, as reported in zebrafish [Li et al., 2014]. Hemangioblasts are multipotent progenitors that express *etsrp*, *fli1*, *scl* and *lmo2*, and can either initiate primitive hematopoiesis or produce angioblasts. In primitive hematopoiesis, erythroid (*gata 1* is a key regulator) or myeloid progenitors (*pu.1* marker) are generated. Angioblast differentiation (*etsrp* is a key marker) will give rise to endothelial cells *(kdrl, cdh5)*, hemogenic endothelium and hematopoietic stem cells (HSC; *runx1, c-myb*), which in turn will give rise to erythroid cells, granulocytes, monocytes/macrophages and lymphoid cells in the definitive hematopoiesis phase, persisting throughout life [Choi, 1998; Lacaud et al., 2001; Dzierzak and Speck, 2008; Tavian et al., 2010; Antas et al., 2013; Li et al., 2014].

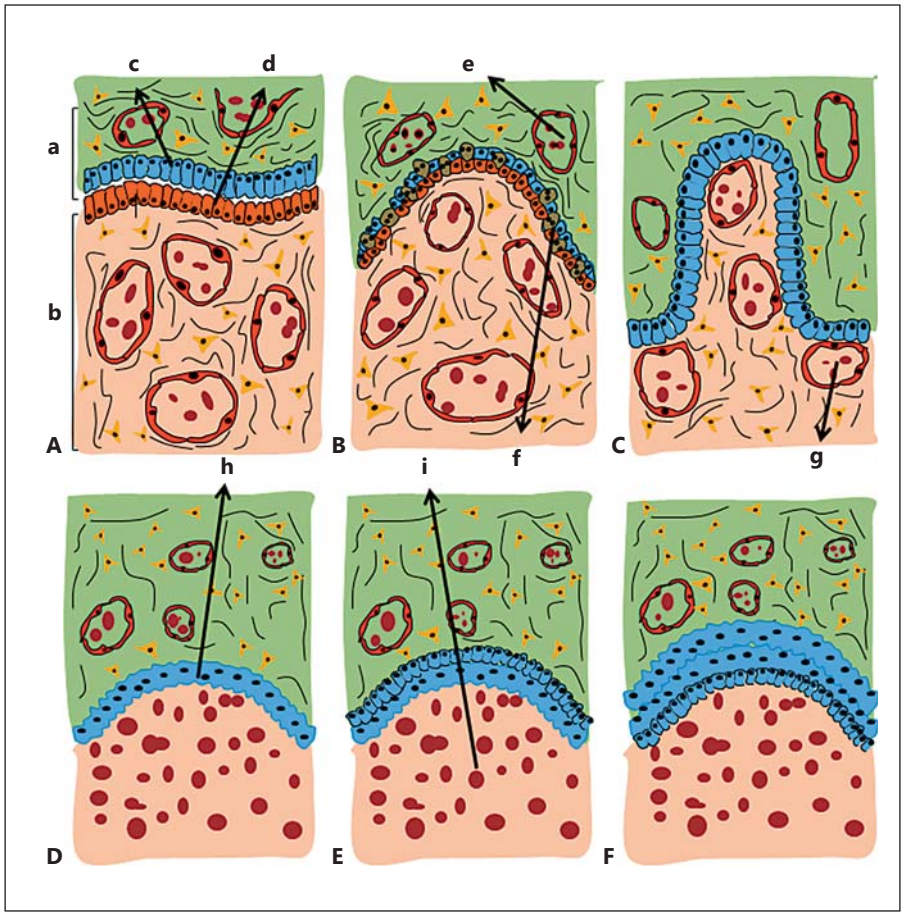

Fig. 2. Representative schemes of the fetal-maternal interface (barrier). **A** Epitheliochorial, in ruminants, horses and pigs; placental fetal components (**a**), placental maternal components (**b**), cytotrophoblasts (**c**), endometrial epithelium (**d**). **B** Synepitheliochorial (also called syndesmochorial), in some ruminants; fetal vessels (**e**), binucleate cells (**f**). **C** Endotheliochorial, in carnivores; maternal vessels (**g**). **D** Hemomonochorial, with one layer of trophoblast, in guinea pig and human placenta at term; syncytiotrophoblasts (**h**). **E** Hemodichorial, with two layers of trophoblastic cells, in rabbits and first-trimester humans; red blood cells (**i**). **F** Hemotrichorial, with three layers of trophoblasts, in mice and hamsters. Modified from Vejlsted [2012] and Furukawa et al. [2014].

The first phase of hematopoiesis (primitive hematopoiesis) emerges in the mesodermal intermediate cell mass as well as in the yolk sac of mice at E7.25 (also described as E7.0–7.5). It produces a limited range of transient blood cell types, including large and nucleated (primitive) red blood cells, granulocytes and macrophages. Erythroid burst-forming units, BFU-E, have been described in the yolk sac of mice shortly after this time point (E8.25) and just prior to the onset of circulation. This population is part of the erythro-myeloid progenitors (EMP), which are HSC-independent definitive progenitors. However, EMP do not subsequently colonize the zebrafish thymus; therefore, it has been suggested that EMP-definitive progenitors should be considered a transient hematopoietic wave and classified as part of the definitive phase [Gekas et al., 2005; Ivanovs et al., 2011; Frame et al., 2013].

The definitive wave (also termed second phase or 'adult-like' hematopoiesis, which will give rise to HSC that will persist throughout life) starts at the aorta-gonad-mesonephros (AGM) region and is associated with multiple highly vascularized anatomical sites in the conceptus (the products of fertilization that includes the embryo or fetus, placenta and extraembryonic membranes) at different times (E10.5 and 5 weeks for murine and human embryos, respectively) [Choi, 1998; Lacaud et al., 2001; Hartenstein, 2006; Dzierzak and Speck, 2008; Ivanovs et al., 2011; Frame et al., 2013; Li et al., 2014]. Definitive hematopoiesis occurs in sequential activities that involve the AGM (where de novo HSC are generated; they correspond to the first cells with lympho-myeloid potential that develop during definitive hematopoiesis), fetal liver (main site for hematopoietic expansion and differentiation, but not HSC generation, during fetal life), thymus (which presents progenitors with myeloid and T potential cells), spleen (hematopoietic organ developed and colonized by HSC after the thymus and before bone marrow) and bone marrow (the last hematopoietic microenvironment developed in the fetus and the most important niche in the adult; it also supports the end-stage differentiation

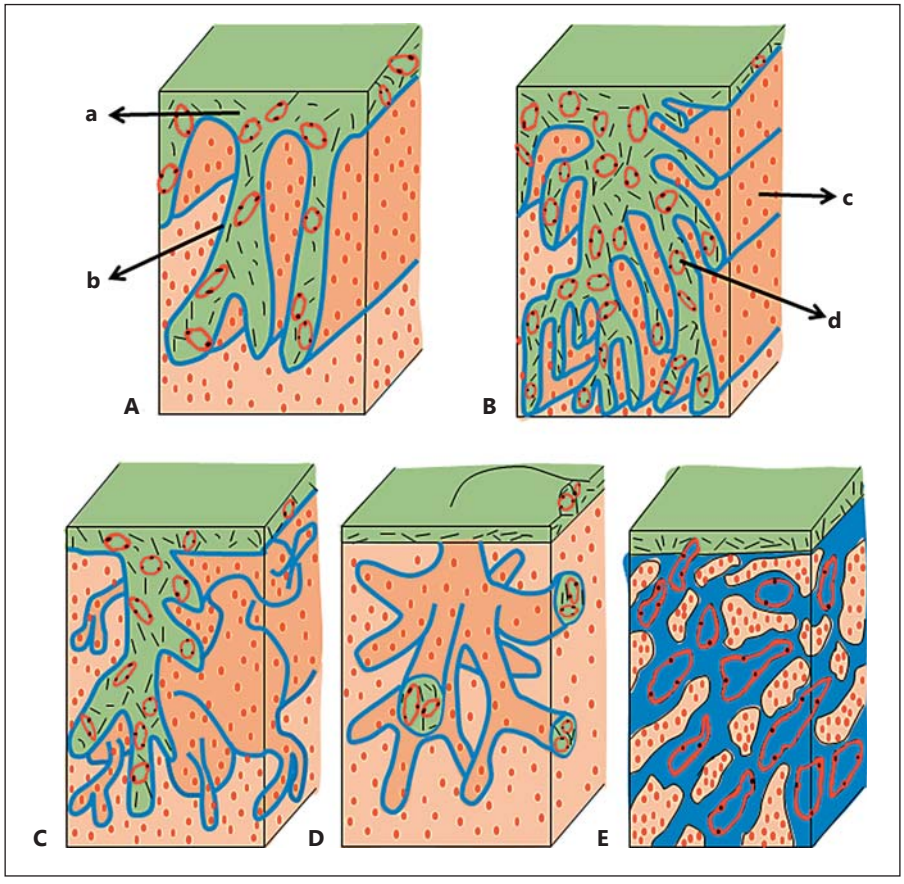

Fig. 3. Placental classification according to the fetal-maternal interdigitation. **A** Folded placenta, in pigs and other species with diffuse placentas; fetal tissues (**a**), trophoblasts (**b**). **B** Lamellar placenta, in some carnivores; maternal tissues (**c**), fetal vessels (**d**). **C** Trabecular placenta, in some primates. **D** Villous placenta, in ruminants, higher primates and humans. **E** Labyrinthine type of placenta, in rodents, some lower monkeys, lagomorphs and insectivores. Modified from Benirschke et al. [2012].

of lymphoid, myeloid and erythroid cell lineages in adult mammals) [Tavian et al., 1996, 2001, 2010; Orkin, 2000; Palis and Yoder, 2001; Kumaravelu et al., 2002; Mikkola et al., 2005; Ottersbach and Dzierzak, 2005; Tavian and Péault, 2005; Cumano and Godin, 2007; Bell and Bhandoola, 2008; Frame et al., 2013; Golub and Cumano, 2013]. Hematopoietic progenitors, located on the ventral side of the aortic endothelium, have been phenotypically described in humans as $CD45^+$ $CD34^+$ $CD31^+$ GATA-2^+ GATA-3^+ c-myb^+ SCL/$TAL1^+$ c-kit^+ flk/KDR^+ $CD38^-$ [Labastie et al., 1998; Tavian and Péault, 2005; Tavian et al., 2010].

In the mid-gestation of mice (approximately E9.5–E11.5), the placenta also functions as an important niche for HSC development without promoting concomitant myeloid and erythroid differentiation [Alvarez-Silva et al., 2003; Gekas et al., 2005; Mikkola et al., 2005; Parolini et al., 2008; Golub and Cumano, 2013]. It is noteworthy that placental hematopoietic activity starts before HSC are in circulation, occurring when the fetal liver HSC reservoir is in its initial growth, and has been observed concomitantly with hematopoiesis at the AGM site [Mikkola et al., 2005]. The placenta seems to represent a pathway for the distribution of HSC, from the AGM towards the fetal liver, which will then be colonized [Gekas et al., 2005]. Vascular connections of the yolk sac and the placenta with the fetal liver may allow hematopoiesis in the fetal liver rudiment. In that regard, definitive red cells are derived from yolk sac-derived progenitors, whereas the placenta may represent a prehepatic HSC niche that provides initial maturation and expansion of HSC [Gekas et al., 2005; Mikkola et al., 2005].

The majority of colonies of HSC derived from collagen-treated placentas of mice are described as c-kit^{hi} $CD34^+$, similar to most fetal liver HSC. However placentas also have a small population of c-kit^{hi} $CD34^-$ cells. Serial transplantations of placental HSC demonstrated their capacity to self-renew, but not to allow differentiation of the myeloerythroid lineage, as previously mentioned. Conversely, fetal liver HSC can reconstitute the entire adult hematopoietic system after transplantation into irradiated adult recipients. It is noteworthy that apparent

differences between microenvironmental cues of both fetal liver and placenta remain obscure. Furthermore, as the end of gestation approaches, placental HSC activity significantly declines in mice [Gekas et al., 2005; Mikkola et al., 2005; Ottersbach and Dzierzak, 2005].

Hematopoiesis and vasculogenesis also occur in human placentas, mostly in the first trimester of gestation [Challier et al., 2005; Aplin et al., 2015]. However, erythropoiesis in full-term placentas was recently reported [Kuchma et al., 2015], a process which seems to occur throughout human fetal development. Additionally, placentas host hematopoietic progenitors, although full-term placentas have a lower percentage of lineage-committed cells than the fetal liver but a greater proportion than the cord blood [Kuchma et al., 2015].

Erythroblasts derived from both the human placenta and yolk sac, during primitive hematopoiesis, express different immunophenotypical markers [Challier et al., 2005]. Thus, hematopoiesis in the placenta may represent a strategy for oxygen support, mostly during early gestation.

Cryopreservation of full-term placental fragments has enabled the isolation of viable hematopoietic progenitor cells [Kuchma et al., 2015], representing a potential strategy for tissue banking. For this purpose and all other potential clinical applications of placental stem cells and extracellular matrix (ECM), placentas obtained after Caesarean section may be preferred over those from vaginal delivery, as the former may minimize placental contamination.

Placental Stem Cells

Placentas are considered an important source of stem cells, particularly due to the high cell yield and noninvasive harvesting methods [Mihu et al., 2008]. Placenta-derived stem cells are multipotent [Zhang et al., 2004; Fierabracci et al., 2015; Shafiee et al., 2015] and can be isolated from distinct components, e.g. the fetal and maternal portions of the placenta, amniotic membrane and fluid, chorion, umbilical cord and cord blood [In't Anker et al., 2004; Mihu et al., 2008; Parolini et al., 2008; Corradetti et al., 2011; Vellasamy et al., 2012; Makhoul et al., 2013; Patel et al., 2014; Proudfit et al., 2014; Fierabracci et al., 2015; Kmiecik et al., 2015; Shafiee et al., 2015]. Furthermore, stem cells are widely accepted to reside in a perivascular niche in virtually all organs, including the placenta [Caplan, 2008; Crisan et al., 2008; da Silva Meirelles et al., 2008; Castrechini et al., 2010; Corselli et al., 2010; Park et al., 2011; Asatrian et al., 2015].

The expression of biomarkers is among the criteria commonly used to define adult mesenchymal stem cells, along with the ability to adhere to untreated plastic culture ware, clonogenicity, and the capacity to differentiate into osteogenic, chondrogenic and adipogenic lineages [Dominici et al., 2006; Castrechini et al., 2010]. It is generally accepted that stem cells should present the following antigen profile: $CD73^+$ $CD90^+$ $CD105^+$ $CD14^-$ $CD11b^-$ $CD34^-$ $CD45^-$ $CD19^-$ $CD79\alpha^-$ [Dominici et al., 2006; Castrechini et al., 2010]. However, perivascular stem cells, particularly those isolated from adipose tissue, have been widely characterized and correspond to a combination of pericytes ($CD146^+$ $CD34^-$ $CD45^-$) and adventitial cells ($CD34^+$ $CD146^-$ $CD45^-$); consequently, they have been successfully isolated via FACS (fluorescence-activated cell sorting) using $CD146^+$ $CD34^+$ $CD45^-$ expressions as a gating strategy [Corselli et al., 2013; Asatrian et al., 2015]. Identification of stem cells using immunophenotypical biomarkers remains controversial and varies among tissues. For instance, adherent cells derived from placentas have been described as $CD29^+$ $CD44^+$ $CD73^+$ $CD166^+$ [Zhang et al., 2004; Luan et al., 2013]. Stromal cells isolated from amniotic membrane and chorionic mesenchyme are $CD90^+$ $CD73^+$ $CD105^+$ $CD45^-$ $CD34^-$ $CD14^-$ [Parolini et al., 2008]. Stem cells derived from the fetal portion of the placenta express slightly higher levels of CD146 and nestin compared to bone marrow-derived stem cells, and are negative for CD271, which is very low in bone marrow cells [Roson-Burgo et al., 2014]. Differences in the expression of antigens have been described, not only among stem cells isolated from various parts of the placenta [Roson-Burgo et al., 2014; Kmiecik et al., 2015; Kuchma et al., 2015], but also among placental stem cells isolated at various gestational ages [Jones et al., 2012; Proudfit et al., 2014; Lankford et al., 2015] and among placentas derived from mothers of different ages [Alrefaei et al., 2015].

High expectations regarding applications of mesenchymal stem cells in regenerative medicine and cell therapies are primarily based on their capacity to produce and release several bioactive molecules, which confers the ability to modulate cell responses in distinct microenvironments, including modulation of the immune response [Caplan and Correa, 2011; Caplan and Sorrell, 2015]. This knowledge has prompted investigations on the clinical utilization of allogeneic stem cells, although it remains a very debatable subject [Aldoss et al., 2015; Huang et al., 2015; Mori et al., 2015; Tanaka et al., 2015].

Placenta-derived stem cells also synthesize several cytokines, including stem cell factor, Flt3 ligand, interleu-

kin-6 and macrophage colony-stimulating factor. Therefore, they can be used as a coadjuvant for the expansion of $CD34^+$ cells [Zhang et al., 2004; Parolini et al., 2008] and have been tested for multiple applications, including neuroprotection in hypoxic-ischemic brain damage in a murine model [Ding et al., 2015], as a gene delivery vehicle [Chen et al., 2012] and for myocardial therapy [Makhoul et al., 2013]. Similar to bone marrow mesenchymal stem cells, placenta-derived stem cells inhibit T-cell proliferation and secretion of IFN-γ; the immunosuppressive activity of placenta-derived stem cells against T cells involves PD-L1 (programmed death-ligand 1, also known as CD274) [Luan et al., 2013; Tripathi and Guleria, 2015]. The placenta is also considered an important source of natural killer cells for potential applications in cellular immunotherapy [Kang et al., 2013].

Unfortunately, there is a paucity of literature characterizing placenta-derived stem cells with regards to phenotype, intrinsic properties, potential therapeutic applications, hematopoiesis and vasculogenesis among various eutherian mammals, as well as potential differences in the same parameters among mammals with different placental features, including macroscopic aspect, type of fetal-maternal interface or fetal-maternal interdigitation.

Placental ECM and Its Tissue Engineering Applications

Remodeling of endometrial and chorionic ECM is fundamental for placentation and implantation [Korhonen and Virtanen, 1997; Guillomot et al., 2014]. Equally important is placental remodeling, which occurs throughout pregnancy, resulting in differences in placental ECM composition at distinct gestational ages [Korhonen and Virtanen, 1997; Johnson et al., 2003; Guillomot et al., 2014].

ECM is synthesized by cells and provides functional and structural support for tissues and organs [Bornstein and Sage, 2002; Lutolf et al., 2009; Schultz and Wysocki, 2009]. Additionally, ECM is a key component of the tissue microenvironment (niche) that comprises biochemical and biophysical signaling provided to the cell by the ECM, other neighboring cells, the immune system and various soluble factors, including cytokines, hormones and growth factors [Xu et al., 2009]. Synthesis and remodeling of ECM (the degradation/synthesis cycle) is crucial in morphogenesis, wound healing and tissue maintenance [Schultz and Wysocki, 2009; Xu et al., 2009]. Thus, combined with the biochemical signaling, ECM provides biophysical properties such as mechanical and architectural/topographical cues, which provide valuable insights regarding cell behavior [Bornstein and Sage, 2002; Lutolf et al., 2009].

Cell-ECM interactions regulate cell fate through a process called dynamic reciprocity, in which the ECM and cellular nucleus are constantly regulating one another [Bissel et al., 1982; Xu et al., 2009]. These dynamic and reciprocal interactions rely on integrin bindings which prompt the reorganization of actin, other cytoskeleton components (e.g. microfilaments, intermediate filaments and microtubules) and lamins, which are structural proteins of the nuclear envelope and connect to cytoskeletal actin through nesprin, ultimately influencing global and specific loci of chromatin [Xu et al., 2009]. Conversely, cells can actively remodel ECM through cytoskeletal tension, which may, for instance, modulate fibronectin assembly and regulate the transcription of matrix metalloproteinases. Therefore, biochemical and physical cues (mediated by mechanotransduction pathways), nuclear localization, movement, gene expression and tissue homeostasis are all directly regulated [Xu et al., 2009].

ECM proteins are classified into four major groups: (1) structural proteins (e.g. collagens and elastin), (2) multidomain adhesive glycoproteins (fibronectin, laminin and vitronectin), (3) glycosaminoglycans (hyaluronan) and proteoglycans (versican, syndecans, glypicans and perlecan, also known as heparin sulfate proteoglycan core protein – HSPG – or heparin sulfate proteoglycan 2 – HSPG2) and (4) matricellular proteins (i.e. proteins with an unusual diversity of functions that are nonstructural but that interact with structural proteins as well as with cell receptors, proteases, hormones and other bioactive molecules; an example is SPARC – secreted protein acidic and rich in cysteine – which includes osteonectin, thrombospondin 1 and 2, tenascin C and X, osteopontin, CCNs and periostin) [Bornstein and Sage, 2002; Schultz and Wysocki, 2009; Morris and Kyriakides, 2014]. Matricellular proteins (previously described as pericellular matrix) are localized in a subcompartment of the ECM adjacent to the cells and classified as distinct, as they are present in low levels in adult tissues. Their expression is increased in development, certain pathologies and after injuries, they can be soluble and insoluble, they function contextually as modulators of cell-matrix interactions, they often induce the deadhesion of cells as opposed to the adhesion properties of most matrix proteins, and they have a grossly normal or subtle altered phenotype in knockout mice [Bornstein and Sage, 2002; Bornstein, 2009; Morris and Kyriakides, 2014].

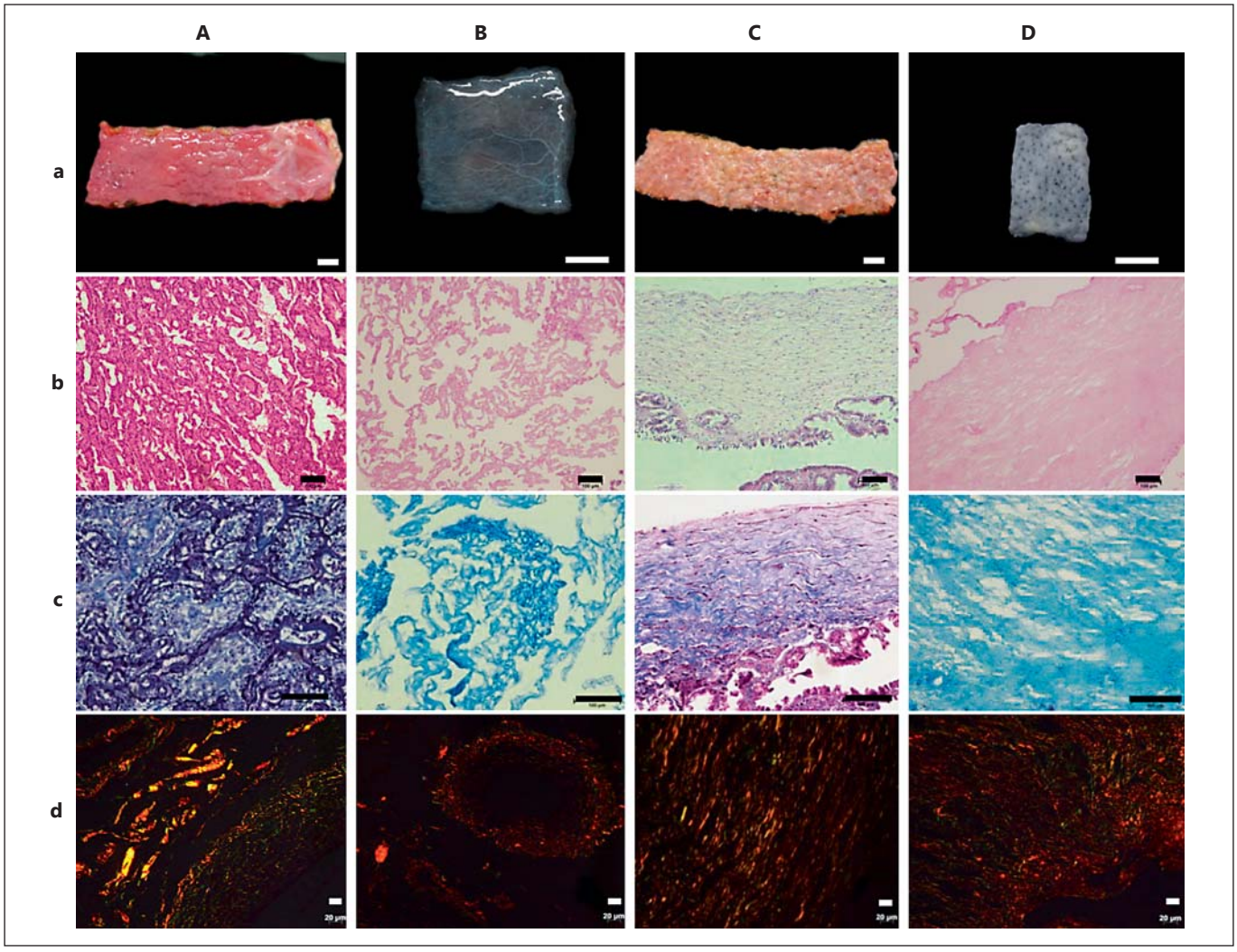

Fig. 4. Gross (**a**) and histological (**b–d**) aspects of fetal (**A**, **B**) and maternal (**C**, **D**) components of a canine placenta, classified as zonary, endotheliochorial, chorioallantoic and with lamellar type of interdigitation, before (**A**, **C**) and after decellularization (**B**, **D**). Decellularization was performed using 1% SDS and 10 mM Tris. **a** Macroscopic aspects. Scale bars = 1 cm. **b** HE staining. Scale bars = 100 μm. **c** Masson's trichrome staining. Scale bars = 100 μm. **d** Picrosirius staining. Objective 16. Scale bars = 20 μm.

During decidualization (i.e. dramatic endometrial remodeling after ovulation in preparation for pregnancy) matricellular proteins (basement membrane) found around individual decidual cells are comprised of laminin α1, α2, β1, β2 and γ1 chains, fibronectin, type IV collagen and heparin sulfate proteoglycan [Wewer et al., 1985; Korhonen and Virtanen, 1997; Gellersen et al., 2007; Oefner et al., 2015]. Decidualizing stromal cells control trophoblast invasion, suppress inflammation and oxidative stresses, and desensitize maternal immune responses [Gellersen et al., 2007].

Osteopontin is another matricellular protein widespread throughout placentation and known to have diverse functions in the uterus. It influences implantation and placental development, with roles from early stages of pregnancy to the end of gestation [Johnson et al., 2003]. Osteopontin has been implicated in adhesion and signal transduction at the uterine-placental interface, is expressed in uterine stroma, has been correlated with the degree of conceptus invasiveness and may regulate immune cell behavior and cytokine production [Johnson et al., 2003].

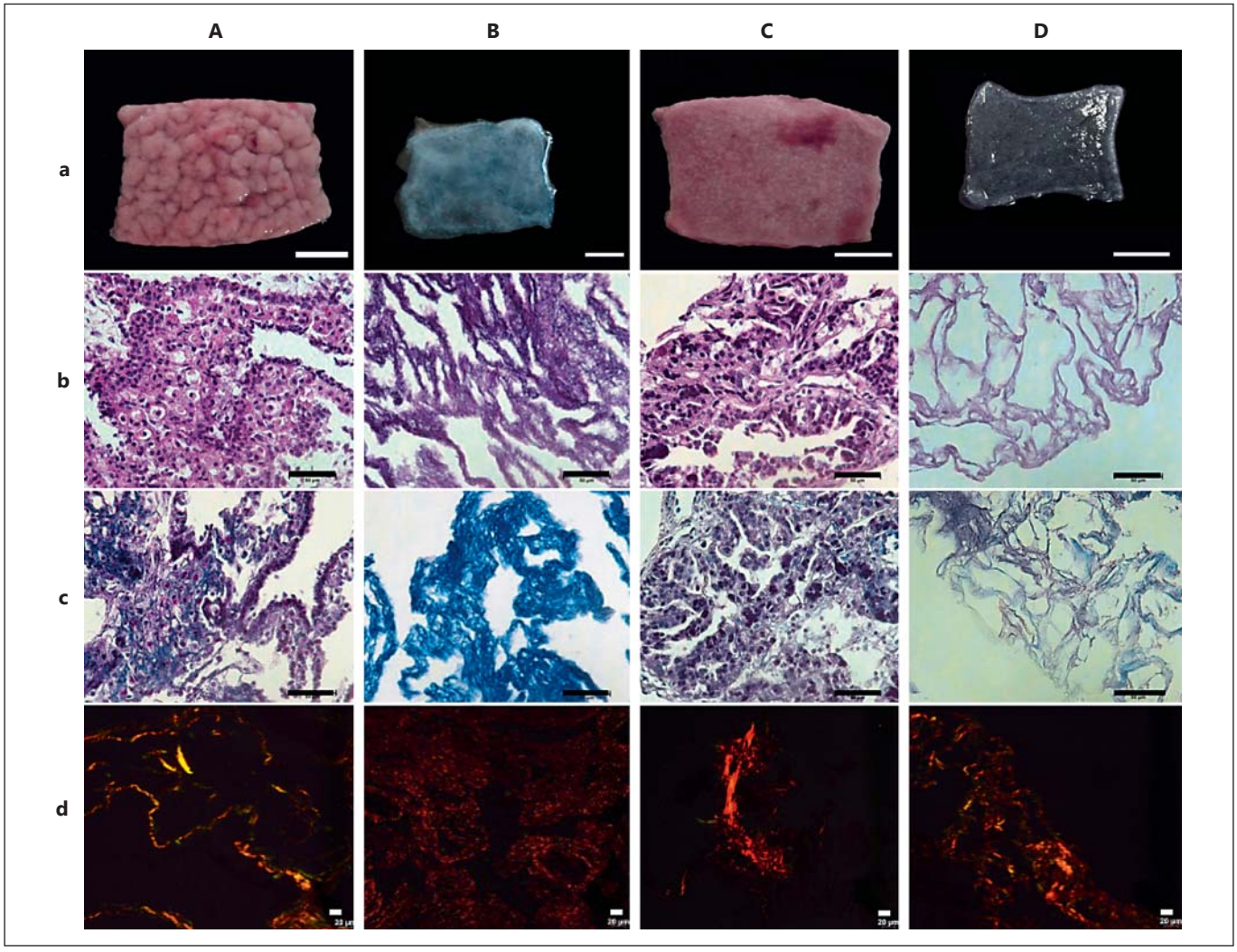

Fig. 5. Gross (**a**) and histological (**b–d**) aspects of fetal (**A**, **B**) and maternal (**C**, **D**) components of a feline placenta, known as zonary, endotheliochorial, chorioallantoic with lamellar fetal-maternal interdigitation, before (**A**, **C**) and after decellularization (**B**, **D**). Solutions of 1% SDS and 10 mM Tris were used for decellularization. **a** Macroscopic aspects. Scale bars = 1 cm. **b** HE staining. Scale bars = 50 μm. **c** Masson's trichrome staining. Scale bars = 50 μm. **d** Picrosirius staining. Objective 16. Scale bars = 20 μm.

Another important matricellular protein is the small leucine-rich proteoglycan decorin. Downregulation of decorin has been associated with the proliferation, remodeling and vascularization of placental tissues; its dysregulation has been associated with placental abnormalities in cattle clones produced by somatic cell nuclear transfer [Guillomot et al., 2014]. Downregulation of decorin gene expression decreases the proliferation of the human microvascular endothelial cell type, probably due to a downstream decrease in *EGFR1*, *IGFR1* and *VEGFR* expression, network formation and thrombin generation [Chui et al., 2014].

ECM has binding sites for growth factors, thus influencing their availability and signaling. The ECM proteins fibronectin, vitronectin, tenascin C, osteopontin, fibrinogen and, to a lower extent, collagen I are particularly important for the binding of growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor, transforming growth factor-β, fibroblast growth factor and neurotrophins [Martino et al., 2014]. VEGFA and placenta growth factor (PlGF) are structurally related proteins. PlGF has two splice variants (PlGF-1 and PlGF-2). The latter contains a heparin-binding se-

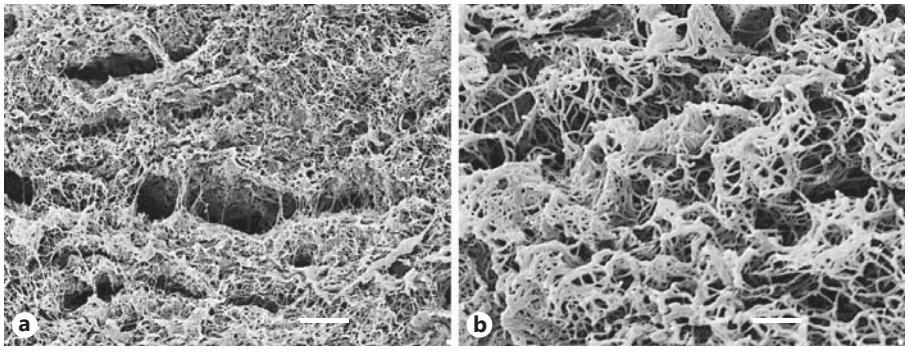

Fig. 6. Scanning electron microscopy of maternal ECM of a canine placenta, treated with 1% SDS and 10 mM Tris solution for 3 days, followed by 1% Triton X-100 solution for 2 days. Scales bars = 10 (**a**) and 3 (**b**) μm.

quence near the C terminus and strongly binds several ECM proteins as opposed to PlGF-1, which lacks ECM binding [Athanassiades and Lala, 1998; Martino et al., 2014]. Furthermore, PlGF is synthesized by human extravillous trophoblast cells and by human placental trophoblast, and interacts only with VEGF receptor Flt-1 (VEGFR-1) but not with KDR (kinase domain-containing region; VEGFR-2) [Athanassiades and Lala, 1998].

Placentas and fetal membranes have been successfully used for tissue engineering applications. For instance, amniotic membrane has been used to prevent adhesions [Kuriu et al., 2009; Petter-Puchner et al., 2011; Ellington and Ferguson, 2014], to repair recto-vaginal fistulas [Roshanravan et al., 2014], to treat skin burns and wounds [Taghiabadi et al., 2015] and chronic leg ulcers [Zelen et al., 2013], and for regeneration of corneal and conjunctival epithelium [Yokogawa et al., 2014; Zeng et al., 2014; Zhou et al., 2015], although it was ineffective for treatment of Mooren's ulcer, a rare but severe ulcerative keratitis [Schallenberg et al., 2013]. In addition, its anticancer properties have been noted, probably due to the down-regulation of HSP90 (heat shock protein 90), cancer cell cycle arrest and inhibition of angiogenesis [Magatti et al., 2012; Niknejad and Yazdanpanah, 2014].

Similar to other tissues and organs, placentas from humans and other species have been decellularized, and the remaining ECM, from both fetal and maternal components, has been used for tissue engineering applications (fig. 4–6). Decellularized ECM may augment endogenous stem cell functions and may be used as carriers for stem/progenitor cells into damaged, diseased or aged tissues and organs [Lutolf et al., 2009], thus fulfilling a broad range of clinical applications. Placental ECM has been applied as a dermal substitute for full-thickness wound healing [Choi et al., 2013], to promote in vitro and in vivo vascularization, and inhibit fibrosis, in lieu of Matrigel [Moore et al., 2015].

Final Considerations

Placentation and placental development represent a vast field of investigation. Current knowledge on placental composition has provided important insights into several aspects of pregnancy disturbances, clone viability and various kinds of pathologies. Immunoregulatory properties, in addition to noninvasive harvesting procedures (at or soon after parturition) make placentas a valuable source of stem/progenitor cells and ECM. Allogeneic stem cells and xenotransplantation of ECM-based biomaterials have gained attention in regenerative medicine. The placenta, being a vast source of ECM, may represent an important resource for both human and veterinary medicine. Finally, the elucidation of various differences regarding stem cell features and properties, as well as ECM composition among placentas derived from various species, is far from complete.

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