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Review Article

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Placenta: A Massive Biological Resource for Clinical applications in Regenerative Medicine

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Abstract

The placenta, with the amniotic fluid, umbilical cord, and the cord blood can be often classified as pregnancy specific biological substances with enormous applications in regenerative medicine. The human placenta is chorioallantoic because it can form both the chorion and the allantois. It remains connected to the growing fetus via the umbilical cord. The blood-placental barrier allows the selective exchange of nutrients, gas, helps in maintaining the thermoregulation of the fetus. It also helps in removing the waste from the fetus's blood. Grossly, the placenta is made up of the amnion, the innermost layer surrounding the fetus, the allantois in the middle and chorion, the outermost fetal layer. Since, 1999, Bhattacharya et al., has been successfully using the application of freshly collected, serologically tested negative, amniotic membrane from the placenta as a biological wound dressing model in patients with burns and non-healing ulcers.

Introduction

In Latin, the word Placenta means "Cake". The importance of placenta as an important barrier between the fetus and the mother was first realized around 5 decade's back [1]. Broadly the placenta can be divided into two parts, the maternal part known as the decidua basal is which develops from the maternal uterine tissues and the fetal part known as the chorion frondosum developing from the blastocyst [2]. One of the most important functions of the placenta is to provide a micro-environment to the fetus required for its nutrition, growth, and development, and a physical and functional barrier against pathogens and maternal immune system. It also plays a role in helping the secretion of different hormones, cytokines and growth factors required for the fetus [3].

Development of the Placenta

The initial development of the placenta starts with the process of outstripping of the embryo. Invagination of the surrounding deciduas occurs by the syncytiotrophoblasts. This process continues till the blastocyst remains surrounded by the circulating maternal blood. Roughly around 3 weeks time, the cytotrophoblast or the primitive extra-embryonic layer starts developing as cellular columns along with the syncytiotrophoblast layer and together they extend into the maternal blood lacunae resembling the primary villi [4]. The development of the secondary villi initiates after the mesodermal invasion into the core of the primary villi. The tertiary villi form after the cellular differentiation in the villi mesoderm results in the formation of a network of blood vessels. At this stage of fetal development, the primitive placenta, the chorionic plate, the developing embryo stalk and each of the vascular villus components gets connected with each other [5].

The cytotrophoblast penetrates through the syncytiotrophoblast layer where many villi reach the decidual region and forms the anchoring villa [6,7]. The villi develop into extensive tree-like branches into the lacunar or intervillous spaces, thereby enabling a larger surface area for gaseous exchange. The cytotrophoblast gets reduced and the distance also shortens between the fetal villi and the maternal intervillous space thus marking the maturation of the villous [5]. The rudimentary umbilical vessels or the allantoic gets formed during this stage. The developing embryo remains attached to the chorion by a body stalk which forms the rudimentary umbilical vessels or the allantoic. During the embryonal growth, due to the shifting of the connecting stalk from the ventral side to its initial posterior position, a large open region is created at the ventral end which gets constricted as the development of the body wall grows and closes. This results in the body wall surrounding the yolk stalk, allantois and the developing vessels to form the primitive umbilicus or the umbilical cord. There is a rapid growth of the placenta from the third month of gestation which continues till term when the matured placenta becomes oval and flat in shape. The placenta at this point, on an average normally weighs 500 grams, with a thickness of 23 mm and an average diameter of 18.5 cm [8].

Development of the fetomaternal circulation and neovascularization

Increased flow of maternal blood towards the placenta is accompanied by vasodilation due to decrease in the resistance of the maternal arterial pressure [9-11]. After decidualisation, the spiral arteries remodel themselves. This remodeling results in the fewer convolutions of the arteries thereby to increase the size of the arteries. During this time, post decidualisation, there is also an increased flow of blood from the maternal side to the placental intervillous space and fetal villi [12]. The maternal and the fetal blood comes directly with each other without the exchange of fluids. The deoxygenated blood flows back into the endometrial valves due to a decrease in the pressure**.** The umbilical arteries radiate and form the chorionic arteries at the junction of the placenta and the umbilical cord. These umbilical arteries undergo further division at the junction to form the arterio-capillary venous branches in the villi. They bring the fetal blood in close proximity to the maternal blood without their mixing because of the presence of the syncytiotrophoblast [3].

Fetomaternal exchanges of carbon dioxide, water, urea starts normally from the first trimester. They enter into the uterine circulation from the fetal circulatory system accompanied by the exchange of carbohydrates, lipids, amino acids, proteins and vitamins from the maternal side to the fetal end [3]. The deoxygenated blood is carried by the umbilical artery from the fetus to the placenta. During this time, any disruption to the fetomaternal circulation will result in fetal hypoxia [13,14]. During this stage, the hormones control the vascular function of the placenta. This helps in maintaining a balance between the vasoconstrictors and the vasodilators [15,16]. Angiogenesis initiates in the placenta after 4 weeks of gestation and continues till the 25th week. During the 15th week of gestation, regression of the peripheral capillaries occurs. Presence of the vascular endothelial growth factors or VEGF, Placental growth factor or PGF remains high post 25 days of gestation and continues till the second trimester [17,18]. The remaining capillaries develop into the primitive veins and the arteries. The flooding of the placental intervillous space starts by the mid of the first trimester and increases till the end of the first trimester [19,20]. With increase in the gestational week, the levels of the angiogenic factors also increases resulting in the increased sprouting of new blood vessels form the pre-existing ones for transporting blood [21-23]. These further develop into well defined veins and arterioles [23]. Through the placenta, there is exchange of nutrients, gases, oxygenated and deoxygenated blood continuously in a stable manner [24,25]. During the second trimester, the sprouting of multiple villi branches takes place and are continuously replaced by the immediate villi till the term [21,26].

Fetomaternal exchange of the Placenta and its mechanism

The placental exchange between the mother and the fetus normally follows Fick's law. It is defined as : $Q/T = K \times A(Cm-Ct)/D$ where Q/T is the rate of diffusion, K is the diffusion coefficient, A is the area of the membrane, D is the thickness of the membrane and (Cm-Ct) is the concentration gradient [26]. Simple diffusion involving gaseous exchange, active transport for facilitating the transfer of iron, calcium and iodine, and facilitated diffusion for the passage of glucose are some of the different types of mechanism by which feto-maternal exchange occurs in the placenta. Amino acids can pass through secondary active transport system whereas water and other important electrolytes are normally exchanged via the bulk transporter system. IgG, low density lipoprotein enters the placenta by specialized processes like endocytosis and exocytose and can be transported by the vesicles so as to negate the effect of any phagolysosomal degradation before it enters the fetus [27]. Also, lipid-insoluble molecules can facilitate the process of transfer of these molecules via the extracellular pores across the placenta [28,29]. Para-cellular channels and pores allow the diffusion of chloride, phosphate and sulphate ions which remain at a higher concentration in the maternal plasma. Stereo-specificity another important physical property plays an important role in the transfer of the amino acids. During the time of placental development, at different stages of the fetal development, transporters are also expressed as they play a major role in efflux of harmful toxic metabolites and selective feto-maternal exchange. Presence of P-gp a well known efflux transporter and a member of the active binding cassette (ABC), present on the human and mouse syncytiotrophoblast is expressed during the first trimester. It is an important component of the blood-placental barrier as it protects the developing fetus from the harmful toxic effects of drugs and metabolites [30-32]. Pgp, although expressed on both sides of the maternal and fetal villi, its expression on the maternal villi is found to be more [33]. In the second trimester, with the

down-regulation in the expression of the Pgp, multidrug resistance or MRP-2 is synthesized more [34,35]. OATP2B1 another anionic transporter along with the breast cancer resistance protein or BRCP is also expressed on the placenta during the first and second trimester [36]. Multidrug resistance transporters, MDR 1 & 2 along with MRP-5 has shown to be highly expressed during the first trimester [37]. The fetal trophoblast also plays an important role in helping the survival of the fetus during the pregnancy period. As the fetus is an allograft in nature, due to the presence of paternal antigens, there is always a possibility of fetal rejection by the maternal immune system. However, this is not the case, during the time of embryo development in the first trimester; over-expression of HLA-G is found on the trophoblast. HLA-A, B remains absent and there is a weak expression of HLA-C during the first trimester [38]. HLA-G expressed on the surface of the trophoblast, binds to killer cell-like Ig like receptors of NK cells or KIR's which reduces the NK cells activity by blocking it [39]. Leukemia Inhibiting factor or LIF is synthesized by the maternal decidua on the placenta in the first trimester and acts as an immune barrier [40].

The Placenta as an immunological barrier

Presence of secretory immune system (SIS) on the maternal side of the placenta also helps in imparting a barrier between the mother and the fetus [41-45]. During the fetal development in the first trimester, the presence of secretory chains (SC) also functions as a barrier against the entry of foreign pathogens inside the fetal compartment [3]. The development of the placenta resembles tumorogenesis in many ways. A balance between the tumorigenesis and normal placental formation is however maintained by the up-regulation of DNA methylation, his tone modifications of tumor suppressor genes such as Maspin, RASSFIA, and APC in the placenta [46]. Genetic imprintation and its control also affect the formation and development of the placenta [47]. DNA methylation normally remains up regulated in the embryo formation period compared to the placental development stage. However, in the trophectoderm, there remains a lack of DNA methylation [48]. Mutation of the polycomb family, an absence of Asc12, Phlda2 and Peg10 genes fails to form the placenta [49,50].

Applications of amniotic membrane in Regenerative Medicine

The placenta consists of the amniotic membrane and the umbilical cord and together with the amniotic fluid and the fetal umbilical cord blood can be considered as pregnancy specific biological substances as they support the process of pregnancy. The use of placental membranes as biological dressing models for healing of woods and burn injuries have been practiced since long including the corneal dressings [51]. After its proper collection and screening for infections, these biological materials can be widely used for the isolation of stem cells, progenitor cells and applied in the field of regenerative medicine [52]. One of the most important applications of the placenta has been its amniotic membrane and amniotic fluid since the last century or so [52]. Grossly, the amniotic membrane of the placenta reveals three important layers. The innermost thin transparent layer is known as the amnion covering the embryo, the middle layer consisting of a collagen-rich connective tissue layer which remains connected with the third and an outer collagen-rich reticular chorionic layer [53]. Both the amnion and the chorion consist of the basement membrane and a stromal layer [54]. The amnion part is rich in mesenchymal stem cells, amniotic epithelial stem/cells, embryonal like cells and progenitor cells [55]. Some of the important properties of amniotic membrane are good water retention capacity, ability to cover large wound areas due to its large size, can be easily and ethically available post birth from the placenta, hypo-antigenic due to poor expression of HLA-A,B,C & DR [56,57]. The amniotic membrane has a similar structure like that of the skin and has anti-microbial properties due to the presence of Beta-defensins, elafin, lysozymes and can prevent the loss of proteins, electrolytes, water by forming a moist environment essentially required for healing [58]. The epithelial stem cells from the amniotic membrane help in re-epithelialization and closure of large wounds [55]. Fibroblasts and other extracellular matrix substances like fibronectin, proteoglycans, laminins, collagen present in the amniotic membrane helps in providing strength to the tissues and act as a scaffold. These cells also have the capability to home to the site of injury. Both amniotic membrane and amniotic fluid have a cocktail of cytokines and growth factors like FGF, PDGF, EGF, TGF-β [55]. Presence of MMP's and their inhibitors or TIMP's are present to counterbalance the excessive growth. The amniotic fluid is rich in amniocytes which are a large pool of self-renewing cells fetal in nature are also present in the amniotic fluid. These amniocytes also have shown to contain pluripotent markers such as TRA 1-60, SSEA-1 – 3, TRA 1-81 [59,60]. Amniocytes have a proliferative capacity, non-tumorigenic in nature, and can grow without feeder layer with a faster doubling rate [61].

The amniotic membrane of the placenta plays an important role in the process of wound healing. The mechanism of a wound, in brief, involves three general steps which are i. Inflammation, ii. Proliferation and iii. Maturation [58]. Amniotic membrane has a tendency for rapid adherence to the site of wound bed along with the release of different cytokines and growth factors. Further, through unknown and yet to be explored mechanisms, amniotic membrane helps in maintaining a balance between the process of angiogenesis, by controlling the production of matrix metalloproteinases (MMP) and their inhibitors or TIMP's, PMN infiltration, proliferation of mesenchymal stem cells and the secretion of various growth factors from at the wound bed. Together these functions and presence of amniotic epithelial cells are believed to initiate the process of rapid re-epithelialization [62,63]. Application of dry, dehydrated and processed amniotic membrane for treating different disease conditions including burn patients is not new [54]. However, the application of freshly collected and fully screened amniotic membrane was conducted for the first time by Bhattacharya et al., in 1999 in more than 200 patients suffering from different diseases including burns [64].

Applications of amniotic membrane in burn management

Successful treatment of 64 burn patients (age group <10 years to 71 years) with freshly collected amniotic membrane was first reported by Bhattacharya et al., [64] Both male and female patients suffering from chemical and thermal burns were recruited for the study after satisfying the inclusion/exclusion criteria. The process included, application of normal saline water to the infected and wound region to clean and remove the cell debris followed by application of amniotic fluid which was serologically screened for CMV, syphilis, Hepatitis B, C, VDRL, HIV-I & II. In cases where of superficial or partial skin thickness burn injuries the amniotic or fetal side of the amniotic membrane was applied and in case of re-epithelialization and improving angiogenesis the maternal or chorionic side was applied [64,58]. Patients were supplemented with antibiotics intravenously and with improvement were given oral antibiotics. Stabilization, healing were routinely conducted with monthly follow up and physiotherapy. Death was not reported in any of the patients. 6 patients had keloid and hypertrophic scars along with 14 cases of hypo-pigmentation. Further, follow up studies showed no hypo-pigmentation and the rest of the patients reported normal and complete wound healing [64,58].

Amniotic membrane application in Leprosy

Similarly, leprosy patients with gangrene were treated with amniotic membrane. Due to infection, some of the patients were infected with maggots which were removed and treated with normal saline followed by a sprinkling of amniotic fluid as an antiseptic agent after 5 to 10 mins [58]. Freshly collected amniotic membranes were applied in superficial and partial thickness wounds. All patients were on anti-leprosy treatment. 3 months and 6 months follow up study revealed the formation of granulation tissue and re-epithelialization [58]. Freshly collected amniotic fluid was successfully applied in 52 patients suffering from osteoarthritis (age range 39 to 82 years) in the joint spaces. It was a double arm study where one group received intra-articular steroid treatment and the other amniotic fluid [65]. The second group where amniotic membrane was applied showed better prognosis and improved outcome after 4 months of follow up study [65].

Amniotic membrane application in Ophthalmology

In 1940, the first application of amniotic membrane in ocular surgery for managing second-degree chemical burns of the eye was reported [66]. Tusbita et al., reported encouraging results with the amniotic membrane in treating patients with cicatricial pemphigoid and Steven Johnson Syndrome (SJS) [67,68]. Other successful reports where amniotic membrane was successfully applied for treating deep corneal ulceration and their reconstruction has also been reported [69-71]. Positive results using the amniotic membrane in pterygium surgery has also been reported including bullous keratopathy [72-74].

Conclusion

The placenta is an important organ formed during the time of pregnancy. Formation of the placenta is essential for the fetus to survive inside the mother's womb. Through a series of complex developmental stages, the blood-placental barrier is formed which is essential in not only protecting the fetus from harmful toxic drugs, and metabolites but also in the selective diffusion and exchange of different inorganic salts, gases and even fetal cells. The placenta like the cord blood is an untapped resource which can be successfully applied in regenerative medicine and cell-based therapies. The use of properly screened and freshly collected placenta and using its amniotic membranes, amniotic fluid for cell therapy purposes was first attempted in 1999. Since then follow up studies and intense clinical scrutiny has yielded very encouraging and positive results using placental membranes in treating non-healing ulcers of different etiologies and burn wounds with very few reports of graft rejections by the host's immune system due to sloughing or due to Pseudomonas aeruginosin infection [75].

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