Send Orders of Reprints at reprints@benthamscience.net

6 *Current Stem Cell Research & Therapy,* **2013**, *8*, 6-14

Soluble Factors of Amnion-Derived Cells in Treatment of Inflammatory and Fibrotic Pathologies

Antonietta Silini[1], Ornella Parolini[1], Berthold Huppertz[2] and Ingrid Lang*[,2]

[1]*Centro di Ricerca E.Menni, Fondazione Poliambulanza, Brescia, Italy;* [2]*Institute of Cell Biology, Histology, and Embryology, Medical University of Graz, Graz, Austria*

Abstract: Inflammation is a complex defense mechanism characterized by leukocyte migration from the vasculature into damaged tissues and subsequent deposition of extracellular matrix resulting in tissue repair. The inflammatory process is generally categorized into an acute, rapid response, and a persistent but slowly evolving chronic condition, which may progress into inflammatory diseases. An excessive deposition of extracellular matrix leads to overgrowth, hardening, and/or scarring of tissues, defined as fibrosis.

The amnion has been used as biomaterial in medicine for over 100 years and has been proven valuable for the treatment of different pathological conditions including wound healing. In light of recent reports, this article will review the effects of the amnion and its cellular components within the inflammatory-fibrotic scenario and the factors described so far that could be involved in these immunomodulatory actions. As proof of principles, we will also discuss pre-clinical and clinical applications of the amnion where advantage has been taken of its anti-inflammatory and anti-fibrotic properties. It is conceivable that the local host environment in which the amnion is placed may have a profound role in influencing the production and function of soluble factors and the shift towards different steps in triggering healing. The healing effect depends on time, dosage, and location of cytokine/growth factor production by the amnion, together with the influence of the host microenvironment. Indeed, determining the specific cascade of events that may define the role of the amnion in a given clinical situation remains a challenge.

Keywords: Amnion, fibrosis, inflammation, placenta, soluble factors, wound healing.

1. INTRODUCTION

The recent interest in placenta-derived cells displaying stem cell-like features is based on the fact that such cells have the potential to differentiate into certain cell types and thus may be involved in regeneration of tissues [1, 2].

Several types of placental cells with variable plasticity have been described [1], including amnion-derived epithelial cells (hAEC), amnion-derived mesenchymal stromal cells (hAMSC) and chorion-derived mesenchymal stromal cells (hCMSC). All three cell types have been shown to differentiate towards the adipogenic, osteogenic, chondrogenic, skeletal myogenic and neurogenic lineage [1], while hAEC and hAMSC also exhibit cardiomyogenic and pancreatic differentiation potential, and hAEC are also able to differentiate into hepatic cells [1].

The endothelial differentiation potential of hAMSC into mature endothelial cells is controversial [3-5]. However, hAMSC induced towards the endothelial lineage show some angiogenic properties like endothelial morphology, LDL uptake and network formation in Matrigel as well as expression/secretion of angiogenic factors with survival-enhancing effects on endothelial cells [4]. This data emphasize their important role in promoting angiogenesis, stabilization of blood vessels and wound healing [4, 5].

Also the umbilical cord harbors cells with multipotent differentiation potential such as: hAEC from the cord-lining amnionic epithelium, mesenchymal stromal cells from the cord-lining sub-amnion (CL-MSC) and mesenchymal stromal cells from the Wharton´s jelly (WJ-MSC) [6, 7]. Besides the minimal criteria for MSC including osteogenic, adipogenic and chondrogenic differentiation capacities, differentiation into neuronal [8, 9], endothelial [10], cardiogenic [11], hepatic [12] and pancreatic [13] lineages have been described for WJ-MSC. To date it is not clear whether there are significant differences between the differentiation potential of CL-MSC and WJ-MSC [6]. The isolation of CL-MSC is a very time-consuming process, while preparation of WJ-MSC could lead to heterogeneous cell populations containing perivascular and vascular cells [6]. Therefore it is important to prove the identity of the cells by absent staining with endothelial markers. Placental MSC, CL-MSC and WJ-MSC do not express the hematopoietic markers CD34 and CD45 or the selective macrophage marker CD163 [14, 15]. As unique feature CL-MSC express CD14 [6, 14], and CD68 expression was detected in WJ-MSC [15]. CD14 and CD68 are commonly known macrophage markers. Their expression on MSC seems to be responsible for immunomodulatory functions. Soluble CD14 can downregulate T cell activation

*Address correspondence to this author at the Institute of Cell Biology, Histology, and Embryology, Medical University of Graz, Graz, Austria;
E-mail: ingrid.lang@medunigraz.at

2212-3946/13 $58.00+.00

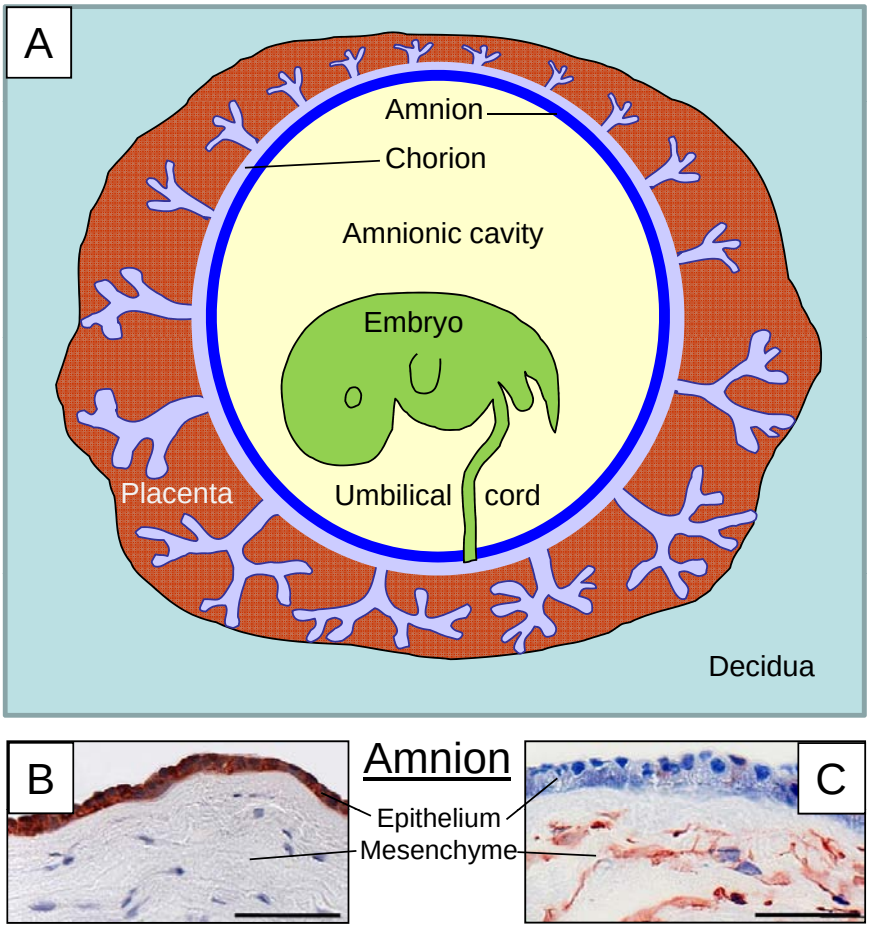

Fig. (1). Overview of the placenta and fetal membranes (A); modified from [5]. The amnion consists of an epithelial layer expressing cytokeratin-7 (B), and a mesenchymal layer with cells stained by anti-vimentin (C). Scale bar 50µm.

[16] and thus modulate immune cells. Also CD68 is supposed to contribute to the hypoimmunogenic and tolerogenic nature of MSC. Additionally, WJ-MSC constitutively express CD200, a molecule known to initiate immunosuppressive and anti-inflammatory effects, in higher amounts than in MSC from bone marrow and adipose tissue. Thus, CD200 could be exploited as a novel WJ-MSC marker [17], but a possible expression in placental MSC has to be determined.

Compared to the umbilical cord and the placental chorionic plate, the placental amnion has some advantages. It is an avascular tissue which can be easily detached from the chorionic plate. Consequently, a homogeneous quality of hAEC and hAMSC can be obtained in large amounts.

To date, the differentiation capacities of placenta- and umbilical cord-derived MSC remain a debated topic, because diverse protocols for isolation and culture conditions may result in heterogeneity of cells and discordant data [4, 6, 7]. However, at the same time an increasing body of evidence suggests that the regenerative effects originating from amnion membrane or amnion-derived cells is due to factors released from these cells rather than cell differentiation [18, 19]. The soluble factors exert healing effects by trophic actions on cells close to the area where an injury or a defect had occurred and modulatory functions on inflammatory cells recruited to the injured site.

The amnion lining the fetal side of the placenta and umbilical cord (Fig. **1** modified from [20]) seems to be a very special tissue, since diverse properties have been attributed to it, including anti-inflammatory and anti-fibrotic properties [21-25]. The mechanisms by which the amnion acts to exert these effects are largely unknown, but could be attributed to the multiple soluble factors released from it. Hence, the amnion can be considered a reservoir of cytokines and growth factors which have been documented to have a role in the stepwise progression of the normal tissue healing process [26]. Amnion-derived cellular cytokine solution promotes macrophage activity [27], accelerates epithelialization of experimental partial-thickness burns [28] and improves the healing of acute and chronic wounds [29, 30].

In this review, we describe the effects of the amnion as a whole and of its cellular components within the inflammatory-fibrotic scenario and the factors described so far that could be involved in these immunomodulatory actions. As proof of principles, we will also discuss pre-clinical and clinical applications of the amnion where advantage has been taken of its anti-inflammatory and anti-fibrotic properties.

2. INFLAMMATION

Inflammation is a complex defense mechanism characterized by leukocyte migration from the vasculature into damaged tissues to control tissue damage induced by pathogenic (bacterial or viral), traumatic, or toxic injury [31]. Inflammation is generally categorized into an acute, rapid response, and a persistent but slowly evolving chronic condition, which may progress into inflammatory diseases. At sites of injury, phagocytic cells, namely macrophages and neutrophils, provide innate cell-mediated immunity and initiate the inflammatory response. Macrophages secrete cytokines that attract neutrophils to leave the blood stream and enter the injured area. The arrival of neutrophils initiates the inflammatory response, by which cells and molecules of innate immunity are recruited into sites of wounding or infection.

At sites of bacterial infections, prominent cytokines secreted by activated macrophages include interleukins (IL-1, IL-6, IL-8, and IL-12) and tumor necrosis factor-α (TNF-α) [32, 33] as well as plasminogen activator, phospholipase C and other enzymes. At the same time, prostaglandins, oxygen radicals, peroxides, nitric oxides, and platelet-activating factor (PAF) further contribute to inflammation and tissue damage [34]. In the course of complement activation, the soluble complement fragments c3a and c5a recruit neutrophils from the blood stream into infected tissues and stimulate mast cells to degranulate, releasing inflammatory molecules such as histamine and TNFα.

When human cells become infected by viruses, they respond with the production and secretion of interferon-α and -ß (IFN-α and -β) which block the spread of viruses to uninfected cells. This in turn activates natural killer cells (NK) to develop cytotoxic effector functions. Stimulation of NK cells with IL-12 favors production of cytokines such as IFNγ, which in turn activates macrophages. The secretion of IL-12 by macrophages in combination with the secretion of IFNγ by NK cells creates a system of positive feedback that increases the activation of both cell types within the site of infection.

Damaged epithelial and/or endothelial cells release inflammatory mediators initiating an anti-fibrinolytic cascade which triggers blood clot formation. Platelets exposed to extracellular matrix components triggers aggregation, clot formation and haemostasis. Next, epithelial and endothelial cells secrete growth factors and chemokines that stimulate the proliferation and recruitment of macrophages and neutrophils which eliminate tissue debris, dead cells and extraneous organisms/materials. They also produce cytokines and chemokines that: a) act as mitogenic and chemotactic factors for endothelial cells which begin to surround the injured site and also to migrate towards the center of the wound to form new blood vessels; and b) recruit and activate T cells which produce pro-fibrotic factors such as IL-13, transforming growth factor-ß (TGF-ß) and platelet-derived growth factor (PDGF) which further activate macrophages and fibroblasts [31, 35, 36].

3. WOUND HEALING AND FIBROSIS

The recruitment of inflammatory cells and the subsequent deposition of extracellular matrix during wound repair is a normal response to persistent infections, autoimmune reactions, allergic responses, chemical insults, radiation, and tissue injury. During wound healing, fibroblasts differentiate into myofibroblasts, which restored homeostasis in damaged tissue by migration, proliferation, contraction and synthesis of extracellular matrix [37]. However, when the synthesis and deposition of new collagen exceeds its rate of degradation, this causes fibrosis [35, 38]. Fibrosis is defined by the overgrowth, hardening, and/or scarring of tissues and is attributed to excess deposition of extracellular matrix components. The leading cause for fibrosis is the accumulation and persistence of myofibroblasts during tissue repair and healing [39]. Normally, myofibroblasts get lost after re-epithelialization of the wound by removal or apoptosis (Fig. **2**, modified from [40]).

Myofibroblasts derive from a variety of cells including mesenchymal, endothelial and epithelial cells undergoing epithelial-mesenchymal transition, as well as from circulating fibroblast-like cells called fibrocytes that are derived from bone-marrow stem cells [41]. Myofibroblasts are activated by a variety of mechanisms, including paracrine signals from lymphocytes and macrophages, autocrine factors secreted by myofibroblasts, and pathogen-associated molecular patterns (PAMPS) produced by pathogenic organisms that interact with pattern recognition receptors (i.e. TLR) on fibroblasts. Myofibroblast recruitment to the site of tissue damage is controlled by chemokines. Specifically, CCL3 (macrophage inflammatory protein 1α) and CCL2 (monocyte chemoattractant protein-1) are chemotactic for mononuclear phagocytes and were identified as profibrotic mediators [42]. Macrophages and epithelial cells are supposed to be the key sources of CCL3 and CCL2 [43, 44].

CD4+ T cells have also been shown to play an important role in fibrosis. Studies conducted with cytokine-deficient mice have shown that liver fibrosis is strongly correlated with the development of a CD4+ Th2 cell response and involves IL-4, IL-5, IL-13 and IL-21 [45, 46]. Importantly, TGFβ has been linked with the development of fibrosis in a number of diseases [47-49].

4. IMMUNOMODULATORY PROPERTIES OF THE HUMAN AMNION AND ITS PRECLINICAL AND CLINICAL APPLICATIONS

The amnion has been used as biomaterial in medicine for over 100 years. Its first successful implementation was reported in skin transplantation in 1910 [50]. Since then, fetal membranes of human placenta have proven valuable for the treatment of different pathological conditions, including wounds of various origins (*e.g.* burn injuries, post-traumatic skin wounds and leg ulcers) [51-53] and in reconstructive surgery (*e.g.* urogenital and orofacial reconstructive surgery [54, 55] and in a variety of ocular disorders [56-58]. Contraindications, failures, or intolerance to standard medical treatments have paved the way for the use of the human amnion.

The underlining mechanisms of the effects of the amnion and /or amnion-derived cells in disease treatment are still largely unknown, but constitute clear evidence of the anti-inflammatory and anti scarring capacity the amnion. Studies

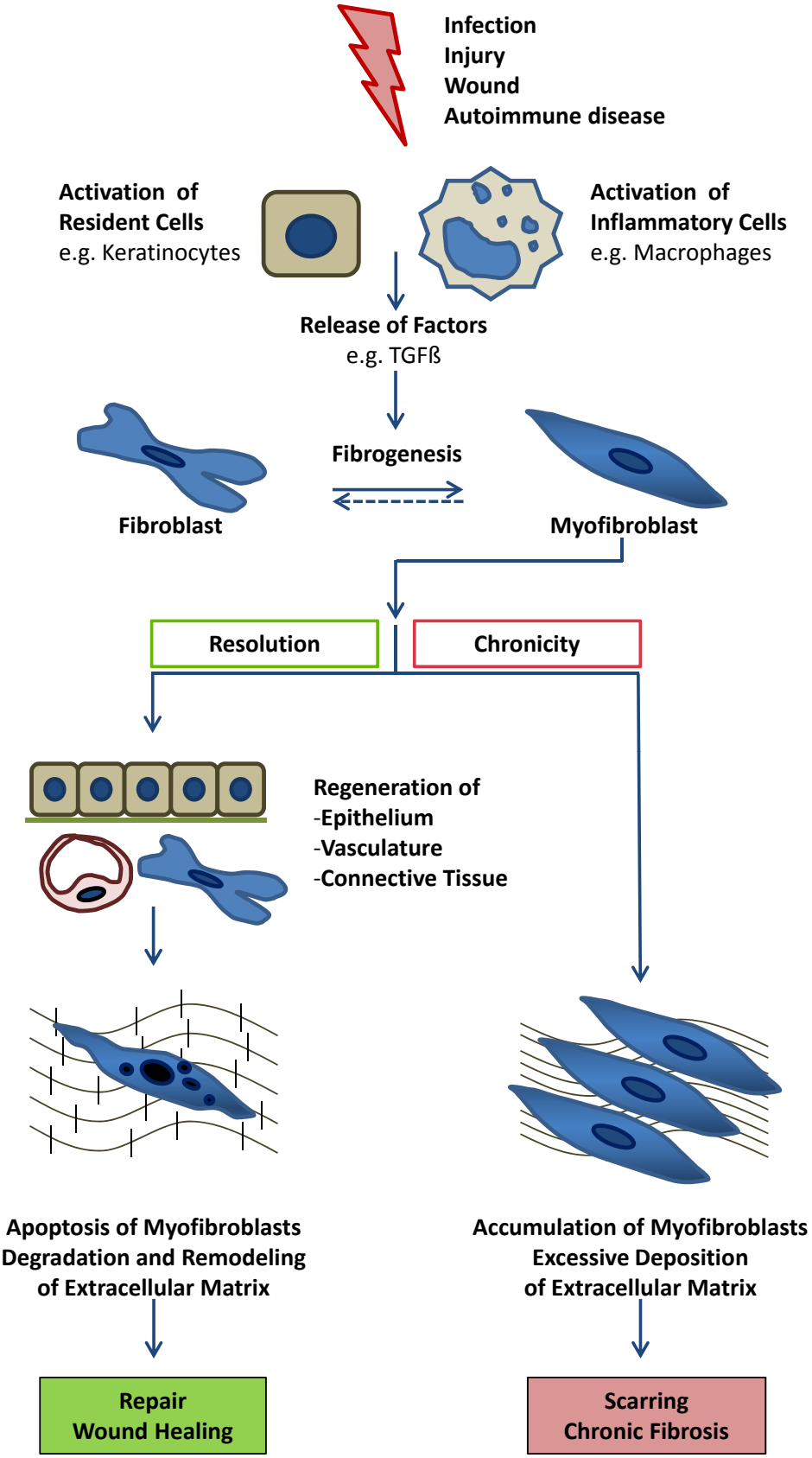

Fig. (2). Model of wound healing and fibrosis, modified from [40].

have documented the role of the amnion in the stepwise progression of the normal tissue healing process including: (1) proliferation, migration and chemotaxis of various cell types, such as fibroblasts, inflammatory and endothelial cells, (2) production and remodeling of extracellular matrix, (3) inhibition of apoptosis, and (4) synthesis of cytokines and growth factors [26].

Amnion-derived mesenchymal cells (hAMSC) in the presence of differentiating dendritic cell cultures abolished the production of pro-inflammatory cytokines TNF-α, C-X-C motif chemokine ligand 10 (CXCL10), CXCL9 and chemokine C-C motif ligand (CCL5) [59]. Further, hAMSC exerted anti-allergic effects on mast cells and eosinophils by suppressing 1) the proliferation of activated fibroblasts and 2) the production of TGFß1, IL-8 and granulocyte-macrophage colony-stimulating factor (GM-CSF) by activated fibroblasts [60].

4.1. Preclinical and Clinical Applications

Examples of the successful use of the amnion in preclinical and clinical setting are available for a variety of pathologies, including lung, liver and ocular pathologies and others. Fibrotic diseases represent a significant and growing burden of disease for which there is no specific intervention today. Only recently treatment of fibrotic diseases in the lung and liver using amnion-derived cells has been started to be under investigation.

4.1.1. Applications in Lung Pathologies

Amnion-derived cells, a mix of the epithelial (hAEC) and mesenchymal cell (hAMSC) populations are able to significantly reduce the severity of lung fibrosis along with a concomitant decrease in neutrophil infiltration when transplanted intraperitoneally or intratracheally into bleomycin-challenged immunocompetent mice [61]. Since the beneficial effects were observed in the presence of only a small amount of donor cells in the host lungs, it has been hypothesized that these effects were mostly due to the paracrine actions exerted by soluble molecules secreted by the transplanted cells rather than their engraftment and differentiation. Moreover, the sole injection of media conditioned from amnion-derived cells resulted in reduced fibrosis progression [18]. On the other hand, hAEC exert beneficial effects in lung fibrosis also by their differentiation into type II pneumocytes *in vivo* [62].

hAEC injection reduced levels of pro-inflammatory cytokines such as IL-1, IL-6, and TNFα, and of pro-fibrogenic cytokines such as TGFß in the lungs. TGFß plays a prominent role in fibrosis. Therefore, a decrease in TGFß signaling by human amnion cells and thus the inhibition of fibroblast activation could be the most plausible mechanism in reducing fibrosis progression [63]. Importantly, hAEC transplantation reduced fibrosis and collagen deposition and induced a collagen-degrading environment by altered proteases levels in the injured lungs [62, 64].

4.1.2. Applications in Liver Pathologies

The engraftment of hAEC in the liver and their differentiation towards the hepatogenic lineage *in vitro* and *in vivo* supports the use of hAEC in restoring hepatic tissues [1, 65].

Furthermore, the amnion is useful for treating biliary fibrosis. Fragments of the human amnion applied as a patch onto the rat liver surface significantly reduced the severity and slowed down the progression of liver fibrosis in a rat model of bile duct ligation [66]. The exact mechanism about the beneficial effects of the amnion on bile duct ligation-induced liver fibrosis is still unknown.

4.1.3. Applications in Ocular Pathologies

Examples of the successful use of the amnion in clinical settings are especially available for ocular pathologies (ocular diseases and injuries) including persistent corneal epithelial defects, corneal ulcers, glaucoma, pterygium, conjunctival surface reconstruction, bullous keratopathy, chemical or thermal burns, limbal stem cell deficiency, and patients with Stevens-Johnson syndrome [24, 67-70].

Ophthalmology studies have also reported on beneficial effects of hAEC-conditioned medium for corneal ulcers resulting in fewer HLA class II antigen presenting cells at the site of injury and a generally reduced inflammatory process [71]. This implies that hAEC can inhibit the chemotactic migration of neutrophils and macrophages to the site of injury, similar to studies of hAECs in lung pathologies [61, 62, 64].

Human corneal and limbal fibroblasts grown on the matrix surface of the amnion down-regulated TGFß signaling, which prevented fibroblast activation into myofibroblasts [22]. Moreover, the de-differentiation of already differentiated myofibroblasts back to fibroblasts has also been attributed to the amnion, which may help to attenuate already an established fibrotic disease [72].

4.1.4. Applications in Adhesion Related Pathologies

Adhesion often arises as a complication after surgery. It is generally prevented or minimized by targeting various steps of the physiopathological process such as the use of anti-inflammatory agents and fibrinolytics [73]. The anti-fibrotic properties of the amnion are of great use for the prevention of adhesion formation. The amnion reduced adhesions in experimental intraperitoneal onlay mesh repair [74] and served as efficient anti-adhesive in rats when used in cecal abrasion and ligature of the adjacent parietal peritoneum, resulting in reduced neovascularization, inflammation and fibrosis. The constant presence of the amnion on the damaged surface without physically changing location played an important role in the positive effect [73]. The adhesion scores increased again in animals in which the amnion slipped off the damaged surface [75].

4.1.5. Applications in Chronic Ulcers of Different Origins

Ulcerations secondary to e.g. diabetes or radiation therapy are often chronic non-healing ulcers. The application of cryopreserved [53], lyophilized [76], freeze-dried irradiated [77] or decellularized amnion membranes [78] as a biological wound dressing helped decreasing the healing time and ulcer surface. Amnion membranes promoted proper epithelialization while suppressing excessive fibrosis with release from pain [53, 77].

5. SOLUBLE FACTORS RELEASED FROM AMNION *IN VITRO* AND THEIR PUTATIVE IMMUNO-MODULATORY ACTIONS

The human amnion expresses various anti-inflammatory and anti-fibrotic factors:

5.1. Hyaluronic acid

Hyaluronic acid is present at high levels in the amnion. Hyaluronic acid mediates the entrapment of inflammatory cells, including lymphocytes, through the binding to CD44 expressed on inflammatory cells [79]. Additionally, HC•HA, a covalently linked complex formed by hyaluronan (HA) and a heavy chain (HC) of inter-α-inhibitor, has been biochemically purified from extracts of amnion and was found to exert potent anti-inflammatory actions [80].

The beneficial effects of the amnion in fibrosis are most likely associated with the release of soluble factors which in turn act in a concerted and paracrine manner to support survival and proliferation of host cells. Hyaluronic acid suppressed TGFß and inhibited the differentiation of conjunctival and limbal fibroblasts into myofibroblasts [22], thus ultimately inhibiting collagen synthesis. TGFß protein was decreased in Bleomycin and CCl4 injured lungs and livers of mice receiving hAEC. This was accompanied by an increase in collagen degrading matrix metalloproteases (MMPs) and a decrease in their tissue inhibitors (TIMPs) [62, 81]. Potentially, similar mechanisms may partly account for the reduction in scarring following patching of amnion membranes.

5.2. TIMPs and MMPs

The amnion contains various tissue inhibitors (TIMP-1, -2, -3, -4) of MMPs [82, 83]. They regulate many crucial processes in inflammation and fibrotic processes including chemotactic migration of inflammatory cells, mitosis of fibroblasts, and synthesis and degradation of extracellular components. This can explain, at least in part, the anti-inflammatory and anti-fibrotic action of the amnion [84].

5.3. Interleukins

IL-1 receptor agonist and IL-10 are potent anti-inflammatory cytokines [85]. They are expressed by hAEC and hAMSC on the transcriptional level [82] and significant amounts of IL-10 were also detected in extracts of amnion membranes on the protein level [21]. IL-10 counteracts the action of different pro-inflammatory cytokines, such as IL-6, IL-1, IL-8 and TNF [86-89]. IL-1 receptor agonist as potent inhibitor of IL-1 may suppress IL-1-mediated inflammation [90]. In particular, the stromal matrix of the amnion suppressed the expression of potent pro-inflammatory cytokines IL-1α and IL-1ß at both transcriptional and protein levels in human corneal limbal epithelial cells. Further, the ratio of IL-1 receptor agonist / IL-1α was increased [91].

5.4. Prostaglandins

The amnion produces high levels of prostaglandin E2 (PGE2) [92]. The constitutive secretion of PGE2 by hAMSC and WJ-MSC have been shown to be responsible for the immunosuppressive effects on lymphocytes [93-95]. PGE2 regulates the maturation and antigen presentation of dendritic cells and inhibits T cell proliferation and pro-inflammatory cytokine production [96]. PGE2 was significantly increased in amnion-derived mesenchymal cells co-cultured with peripheral blood mononuclear cells [95]. The anti-proliferative effect of amnion-derived cells on peripheral blood mononuclear cells [95] was reversed by blocking prostaglandin production with indomethacin (Rossi 2012). These results suggest that the immunomodulatory effects of amnion-derived cells are associated with PGE2 production which, together with other soluble factors (such as TGF-β, HGF, and IDO), could be involved in the suppression of the lymphocyte-mediated immunological response.

5.5. Migration-Inhibitory Factor (MIF)

hAEC secrete the macrophage migration-inhibitory factor (MIF) [97], which inhibits migration of macrophages and NK cell-mediated lytic activity [98].

6. CONCLUSION

The manner in which the amnion manifests the properties exerted through the release of its bioactive, and likely paracrine-acting, factors remains to be determined. This is confounded by the release of molecules, which theoretically, have contradictory roles, i.e. pro-inflammatory cytokines (e.g. IL-6 and IL-8) versus anti-inflammatory cytokines (e.g. IL-10 and IL-1 receptor antagonist), MMPs versus their natural inhibitors TIMPs, and anti-angiogenic versus pro-angiogenic factors. It is thus conceivable that the local host environment in which the amnion is sited may have a profound role in influencing the production and function of these molecules and the shift towards different steps in triggering healing. The nature of steps that are triggered thus depends on time, dosage, and location of cytokine/growth factor production by the amnion, together with the influence of the host microenvironment. Indeed, determining the specific cascade of events that may define the role of the amnion in a given clinical situation remains a challenge.

SOURCES OF FUNDING

Fondazione Cariplo and MIUR Progetto Firb, Italy.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Parolini O, Alviano F, Bagnara GP, *et al.* Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells. Stem Cells 2008; 26(2): 300-11.

[2] Parolini O, Caruso M. Review: Preclinical studies on placenta-derived cells and amniotic membrane: an update. Placenta 2011; 32 Suppl 2: S186-95.

[3] Alviano F, Fossati V, Marchionni C, *et al*. Term Amniotic membrane is a high throughput source for multipotent Mesenchymal Stem Cells with the ability to differentiate into endothelial cells *in vitro*. BMC Dev Biol 2007; 7: 11.

[4] König J, Huppertz B, Desoye G, *et al.* Amnion-derived mesenchymal stromal cells show angiogenic properties but resist differentiation into mature endothelial cells. Stem Cells Dev 2012; 21(8): 1309-20.

[5] Warrier S, Haridas N, Bhonde R. Inherent propensity of amnion-derived mesenchymal stem cells towards endothelial lineage: vascularization from an avascular tissue. Placenta 2012; 33(10): 850-8.

[6] Jeschke MG GG, Phan TT, Herndon DN, Kita K. Umbilical Cord Lining Membrane and Wharton's Jelly-Derived Mesenchymal Stem Cells: the Similarities and Differences. Open Tiss Engin Regen Med 2011; 4: 21-7.

[7] Huang YC, Parolini O, La Rocca G, Deng L. Umbilical cord versus bone marrow-derived mesenchymal stromal cells. Stem Cells Dev 2012; 21(15): 2900-3.

[8] Lu LL, Liu YJ, Yang SG, *et al*. Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials. Haematologica 2006; 91(8): 1017-26.

[9] Karahuseyinoglu S, Cinar O, Kilic E, *et al*. Biology of stem cells in human umbilical cord stroma: in situ and *in vitro* surveys. Stem Cells 2007; 25(2): 319-31.

[10] Wu KH, Zhou B, Lu SH, *et al*. *In vitro* and *in vivo* differentiation of human umbilical cord derived stem cells into endothelial cells. J Cell Biochem 2007; 100(3): 608-16.

[11] Hollweck T, Hartmann I, Eblenkamp M, *et al*. Cardiac differentiation of human Wharton's Jelly stem cells-experimental comparison of protocols. Open Tiss Engin Regen Med 2011; 4: 95-102.

[12] Anzalone R, Lo Iacono M, Corrao S, *et al*. New emerging potentials for human Wharton's jelly mesenchymal stem cells: immunological features and hepatocyte-like differentiative capacity. Stem Cells Dev 2010; 19(4): 423-38.

[13] Anzalone R, Lo Iacono M, Loria T, *et al*. Wharton's jelly mesenchymal stem cells as candidates for beta cells regeneration: extending the differentiative and immunomodulatory benefits of adult mesenchymal stem cells for the treatment of type 1 diabetes. Stem Cell Rev 2011; 7(2): 342-63.

[14] Kita K, Gauglitz GG, Phan TT, Herndon DN, Jeschke MG. Isolation and characterization of mesenchymal stem cells from the subamniotic human umbilical cord lining membrane. Stem Cells Dev 2010; 19(4): 491-502.

[15] La Rocca G, Anzalone R and Farina F. The expression of CD68 in human umbilical cord mesenchymal stem cells: new evidences of presence in non-myeloid cell types. Scand J Immunol 2009; 70(2): 161-2.

[16] Rey Nores JE, Bensussan A, Vita N, Stelter F, Arias MA, Jones M, Lefort S, Borysiewicz LK, Ferrara P and Labeta MO. Soluble CD14 acts as a negative regulator of human T cell activation and function. Eur J Immunol 1999; 29(1): 265-76.

[17] Najar M, Raicevic G, Jebbawi F, De Bruyn C, Meuleman N, Bron D, Toungouz M and Lagneaux L. Characterization and functionality of the CD200-CD200R system during mesenchymal stromal cell interactions with T-lymphocytes. Immunol Lett 2012; 146(1-2): 50-6.

[18] Cargnoni A, Ressel L, Rossi D, Poli A, Arienti D, Lombardi G and Parolini O. Conditioned medium from amniotic mesenchymal tissue cells reduces progression of bleomycin-induced lung fibrosis. Cytotherapy 2012; 14(2): 153-61.

[19] Parolini O, Alviano F, Bergwerf I, *et al.* Toward cell therapy using placenta-derived cells: disease mechanisms, cell biology, preclinical studies, and regulatory aspects at the round table. Stem Cells Dev. 2010;19(2):143-54.

[20] Huppertz B KJ. The placenta and fetal membranes. In Dewhurst´s Textbook of Obstetrics and Gynaecology 2012.

[21] Tseng SC, Espana EM, Kawakita T, Di Pascuale MA, Li W, He H, Liu TS, Cho TH, Gao YY, Yeh LK and Liu CY. How does amniotic membrane work? Ocul Surf 2004; 2(3): 177-87.

[22] Tseng SC, Li DQ and Ma X. Suppression of transforming growth factor-beta isoforms, TGF-beta receptor type II, and myofibroblast differentiation in cultured human corneal and limbal fibroblasts by amniotic membrane matrix. J Cell Physiol 1999; 179(3): 325-35.

[23] Lee SB, Li DQ, Tan DT, Meller DC, Tseng SC. Suppression of TGF-beta signaling in both normal conjunctival fibroblasts and pterygial body fibroblasts by amniotic membrane. Curr Eye Res 2000; 20(4): 325-34.

[24] Liu J, Sheha H, Fu Y, Liang L, Tseng SC. Update on amniotic membrane transplantation. Expert Rev Ophthalmol 2010; 5(5): 645-61.

[25] Manuelpillai U, Moodley Y, Borlongan CV, Parolini O. Amniotic membrane and amniotic cells: potential therapeutic tools to combat tissue inflammation and fibrosis? Placenta 2011; 32 Suppl 4: S320-5.

[26] Ching YH, Sutton TL, Pierpont YN, Robson MC, Payne WG. The use of growth factors and other humoral agents to accelerate and enhance burn wound healing. Eplasty 2011; 11: e41.

[27] Uberti MG, Lufkin AE, Pierpont YN, *et al.* Amnion-derived cellular cytokine solution promotes macrophage activity. Ann Plast Surg 2010; 66(5): 575-80.

[28] Payne WG, Wachtel TL, Smith CA, Uberti MG, Ko F, Robson MC. Effect of amnion-derived cellular cytokine solution on healing of experimental partial-thickness burns. World J Surg 2010; 34(7): 1663-8.

[29] Franz MG, Payne WG, Xing L, *et al*. The use of amnion-derived cellular cytokine solution to improve healing in acute and chronic wound models. Eplasty 2008; 8: e21.

[30] Steed DL, Trumpower C, Duffy D, *et al.* Amnion-derived cellular cytokine solution: a physiological combination of cytokines for wound healing. Eplasty 2008; 8: e18.

[31] Wynn TA. Fibrotic disease and the T(H)1/T(H)2 paradigm. Nat Rev Immunol 2004; 4(8): 583-94.

[32] Escorcio-Correia M, Hagemann T. Macrophages in the Tumor Microenvironment. Cancer Drug Discovery and Development. 2010;The Tumor Microenvironment: 371-83.

[33] Remick DG. Old friends: pneumonia and interleukin-6. Crit Care Med 2009; 37(5): 1809-10.

[34] Laskin DL, Sunil VR, Gardner CR, Laskin JD. Macrophages and tissue injury: agents of defense or destruction? Annu Rev Pharmacol Toxicol 2011; 51: 267-88.

[35] Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol 2008; 214(2): 199-210.

[36] Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol 2006; 24: 99-146.

[37] Haniffa MA, Collin MP, Buckley CD, Dazzi F. Mesenchymal stem cells: the fibroblasts' new clothes? Haematologica 2009; 94(2): 258-63.

[38] Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. Nat Med 2012; 18(7): 1028-40.

[39] Desmouliere A, Darby IA, Gabbiani G. Normal and pathologic soft tissue remodeling: role of the myofibroblast, with special emphasis on liver and kidney fibrosis. Lab Invest 2003; 83(12): 1689-707.

[40] Darby IA, Hewitson TD. Fibroblast differentiation in wound healing and fibrosis. Int Rev Cytol 2007; 257: 143-79.

[41] Kalluri R, Neilson EG. Epithelial-mesenchymal transition and its implications for fibrosis. J Clin Invest 2003; 112(12): 1776-84.

[42] Gillitzer R, Goebeler M. Chemokines in cutaneous wound healing. J Leukoc Biol 2001; 69(4): 513-21.

[43] Smith RE, Strieter RM, Phan SH, *et al.* Production and function of murine macrophage inflammatory protein-1 alpha in bleomycin-induced lung injury. J Immunol 1994; 153(10): 4704-12.

[44] Smith RE, Strieter RM, Zhang K, Phan SH, Standiford TJ, Lukacs NW and Kunkel SL. A role for C-C chemokines in fibrotic lung disease. J Leukoc Biol 1995; 57(5): 782-7.

[45] Pesce J, Kaviratne M, Ramalingam TR, *et al.* The IL-21 receptor augments Th2 effector function and alternative macrophage activation. J Clin Invest 2006; 116(7): 2044-55.

[46] Reiman RM, Thompson RW, Feng CG, *et al*. Interleukin-5 (IL-5) augments the progression of liver fibrosis by regulating IL-13 activity. Infect Immun 2006; 74(3): 1471-9.

[47] Sato M, Muragaki Y, Saika S, Roberts AB, Ooshima A. Targeted disruption of TGF-beta1/Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction. J Clin Invest 2003; 112(10): 1486-94.

[48] Clouthier DE, Comerford SA, Hammer RE. Hepatic fibrosis, glomerulosclerosis, and a lipodystrophy-like syndrome in PEPCK-TGF-beta1 transgenic mice. J Clin Invest 1997; 100(11): 2697-713.

[49] Sime PJ, Xing Z, Graham FL, Csaky KG, Gauldie J. Adenovector-mediated gene transfer of active transforming growth factor-beta1 induces prolonged severe fibrosis in rat lung. J Clin Invest 1997; 100(4): 768-76.

[50] Davis J. Skin Transplantation with a Review of 550 Cases at The Johns Hopkins Hospital. Johns Hopkins Medical Journal 1910; 15: 307.

[51] Branski LK, Herndon DN, Celis MM, Norbury WB, Masters OE, Jeschke MG. Amnion in the treatment of pediatric partial-thickness facial burns. Burns 2008; 34(3): 393-9.

[52] Lo V, Pope E. Amniotic membrane use in dermatology. Int J Dermatol 2009; 48(9): 935-40.

[53] Mermet I, Pottier N, Sainthillier JM, *et al*. Use of amniotic membrane transplantation in the treatment of venous leg ulcers. Wound Repair Regen 2007; 15(4): 459-64.

[54] Arai N, Tsuno H, Okabe M, *et al*. Clinical Application of a Hyperdry Amniotic Membrane on Surgical Defects of the Oral Mucosa. J Oral Maxillofac Surg 2012; 70(9): 2221-8.

[55] Koziak A, Marcheluk A, Dmowski T, Szczesniewski R, Kania P, Dorobek A. Reconstructive surgery of male urethra using human amnion membranes (grafts)--first announcement. Ann Transplant 2004; 9(4): 21-4.

[56] Kruse FE, Joussen AM, Rohrschneider K, You L, Sinn B, Baumann J and Volcker HE. Cryopreserved human amniotic membrane for ocular surface reconstruction. Graefes Arch Clin Exp Ophthalmol 2000; 238(1): 68-75.

[57] Sippel KC, Ma JJ and Foster CS. Amniotic membrane surgery. Curr Opin Ophthalmol 2001; 12(4): 269-81.

[58] Tosi GM, Massaro-Giordano M, Caporossi A, Toti P. Amniotic membrane transplantation in ocular surface disorders. J Cell Physiol 2005; 202(3): 849-51.

[59] Magatti M, De Munari S, Vertua E, *et al*. Amniotic mesenchymal tissue cells inhibit dendritic cell differentiation of peripheral blood and amnion resident monocytes. Cell Transplant 2009; 18(8): 899-914.

[60] Solomon A, Wajngarten M, Alviano F, *et al*. Suppression of inflammatory and fibrotic responses in allergic inflammation by the amniotic membrane stromal matrix. Clin Exp Allergy 2005; 35(7): 941-8.

[61] Cargnoni A, Gibelli L, Tosini A, *et al*. Transplantation of allogeneic and xenogeneic placenta-derived cells reduces bleomycin-induced lung fibrosis. Cell Transplant 2009; 18(4): 405-22.

[62] Moodley Y, Ilancheran S, Samuel C, *et al*. Human amnion epithelial cell transplantation abrogates lung fibrosis and augments repair. Am J Respir Crit Care Med 2010; 182(5): 643-51.

[63] Leask A and Abraham DJ. TGF-beta signaling and the fibrotic response. Faseb J 2004; 18(7): 816-27.

[64] Murphy S, Lim R, Dickinson H, *et al*. Human amnion epithelial cells prevent bleomycin-induced lung injury and preserve lung function. Cell Transplant 2011; 20(6): 909-23.

[65] Miki T, Lehmann T, Cai H, Stolz DB, Strom SC. Stem cell characteristics of amniotic epithelial cells. Stem Cells 2005; 23(10): 1549-59.

[66] Sant'Anna LB, Cargnoni A, Ressel L, Vanosi G, Parolini O. Amniotic membrane application reduces liver fibrosis in a bile duct ligation rat model. Cell Transplant 2011; 20(3): 441-53.

[67] Riau AK, Beuerman RW, Lim LS, Mehta JS. Preservation, sterilization and de-epithelialization of human amniotic membrane for use in ocular surface reconstruction. Biomaterials 2010; 31(2): 216-25.

[68] Baradaran-Rafii A, Aghayan H-R, Arjmand B, Javadi M-A. Amniotic Membrane Transplantation. Journal of Ophthalmic & Vision Research 2007; 2(1): 58-75.

[69] Meller D, Pauklin M, Thomasen H, Westekemper H, Steuhl KP. Amniotic membrane transplantation in the human eye. Dtsch Arztebl Int 2011; 108(14): 243-8.

[70] Parolini O, Solomon A, Evangelista M, Soncini M. Human Term Placenta as a Therapeutic Agent: From the First Clinical Applications to Future Perspectives. Human placenta: Structure and Development, Circulation and Function. 2010: 1-49.

[71] Kamiya K, Wang M, Uchida S, *et al*. Topical application of culture supernatant from human amniotic epithelial cells suppresses inflammatory reactions in cornea. Exp Eye Res 2005; 80(5): 671-9.

[72] Li W, He H, Chen YT, Hayashida Y, Tseng SC. Reversal of myofibroblasts by amniotic membrane stromal extract. J Cell Physiol 2008; 215(3): 657-64.

[73] Yetkin G, Uludag M, Citgez B, Karakoc S, Polat N, Kabukcuoglu F. Prevention of peritoneal adhesions by intraperitoneal administration of vitamin E and human amniotic membrane. Int J Surg 2009; 7(6): 561-5.

[74] Petter-Puchner AH, Fortelny RH, Mika K, Hennerbichler S, Redl H, Gabriel C. Human vital amniotic membrane reduces adhesions in experimental intraperitoneal onlay mesh repair. Surg Endosc 2011; 25(7): 2125-31.

[75] Kelekci S, Uygur D, Yilmaz B, Sut N, Yesildaglar N. Comparison of human amniotic membrane and hyaluronate/carboxymethylcellulose membrane for prevention of adhesion formation in rats. Arch Gynecol Obstet 2007; 276(4): 355-9.

[76] Ward DJ, Bennett JP, Burgos H, Fabre J. The healing of chronic venous leg ulcers with prepared human amnion. Br J Plast Surg 1989; 42(4): 463-7.

[77] Lobo Gajiwala A and Sharma V. Use of irradiated amnion as a biological dressing in the treatment of radiation induced ulcers. Cell Tissue Bank 2003; 4(2-4): 147-50.

[78] Letendre S, LaPorta G, O'Donnell E, Dempsey J, Leonard K. Pilot trial of biovance collagen-based wound covering for diabetic ulcers. Adv Skin Wound Care 2009; 22(4): 161-6.

[79] Higa K, Shimmura S, Shimazaki J, Tsubota K. Hyaluronic acid-CD44 interaction mediates the adhesion of lymphocytes by amniotic membrane stroma. Cornea 2005; 24(2): 206-12.

[80] He H, Li W, Tseng DY, *et al*. Biochemical characterization and function of complexes formed by hyaluronan and the heavy chains of inter-alpha-inhibitor (HC*HA) purified from extracts of human amniotic membrane. J Biol Chem 2009; 284(30): 20136-46.

[81] Manuelpillai U, Tchongue J, Lourensz D, *et al*. Transplantation of human amnion epithelial cells reduces hepatic fibrosis in immunocompetent CCl(4)-treated mice. Cell Transplant 2010; 19(9): 1157-68.

[82] Hao Y, Ma DH, Hwang DG, Kim WS, Zhang F. Identification of antiangiogenic and antiinflammatory proteins in human amniotic membrane. Cornea 2000; 19(3): 348-52.

[83] Kim JS, Kim JC, Na BK, Jeong JM, Song CY. Amniotic membrane patching promotes healing and inhibits proteinase activity on wound healing following acute corneal alkali burn. Exp Eye Res 2000; 70(3): 329-37.

[84] Niknejad H, Peirovi H, Jorjani M, Ahmadiani A, Ghanavi J, Seifalian AM. Properties of the amniotic membrane for potential use in tissue engineering. Eur Cell Mater 2008; 15: 88-99.

[85] Dinarello CA. Interleukin-1. Thomson A (Ed), The Cytokine handbook Academic Press, London (1994). 1998:31–56.

[86] Huang S, Xie K, Bucana CD, Ullrich SE, Bar-Eli M. Interleukin 10 suppresses tumor growth and metastasis of human melanoma cells: potential inhibition of angiogenesis. Clin Cancer Res 1996; 2(12): 1969-79.

[87] Hurme M, Lahdenpohja N, Santtila S. Gene polymorphisms of interleukins 1 and 10 in infectious and autoimmune diseases. Ann Med 1998; 30(5): 469-73.

[88] Fortunato SJ, Menon R, Lombardi SJ. Interleukin-10 and transforming growth factor-beta inhibit amniochorion tumor necrosis factor-alpha production by contrasting mechanisms of action: therapeutic implications in prematurity. Am J Obstet Gynecol 1997; 177(4): 803-9.

[89] Fortunato SJ, Menon R, Lombardi SJ. The effect of transforming growth factor and interleukin-10 on interleukin-8 release by human amniochorion may regulate histologic chorioamnionitis. Am J Obstet Gynecol 1998; 179(3 Pt 1): 794-9.

[90] Fidel PL, Jr., Romero R, Ramirez M, *et al*. Interleukin-1 receptor antagonist (IL-1ra) production by human amnion, chorion, and decidua. Am J Reprod Immunol 1994; 32(1): 1-7.

[91] Solomon A, Rosenblatt M, Monroy D, Ji Z, Pflugfelder SC, Tseng SC. Suppression of interleukin 1alpha and interleukin 1beta in human limbal epithelial cells cultured on the amniotic membrane stromal matrix. Br J Ophthalmol 2001; 85(4): 444-9.

[92] Satoh K, Yasumizu T, Kawai Y, *et al*. *In vitro* production of prostaglandins E, F, and 6-keto prostaglandin F1 alpha by human pregnant uterus, decidua and amnion. Prostaglandins Med 1981; 6(4): 359-68.

[93] Rossi D, Pianta S, Magatti M, Sedlmayr P, Parolini O. Characterization of the conditioned medium from amniotic membrane cells:

prostaglandins as key effectors of its immunomodulatory activity. PLoS One 2012; 7(10): e46956.

[94] Najar M, Raicevic G, Boufker HI, *et al*. Mesenchymal stromal cells use PGE2 to modulate activation and proliferation of lymphocyte subsets: Combined comparison of adipose tissue, Wharton's Jelly and bone marrow sources. Cell Immunol 2010; 264(2): 171-9.

[95] Kang JW, Koo HC, Hwang SY, *et al*. Immunomodulatory effects of human amniotic membrane-derived mesenchymal stem cells. J Vet Sci 2012; 13(1): 23-31.

[96] Yanez R, Oviedo A, Aldea M, Bueren JA, Lamana ML. Prostaglandin E2 plays a key role in the immunosuppressive properties of adipose and bone marrow tissue-derived mesenchymal stromal cells. Exp Cell Res 2010; 316(19): 3109-23.

[97] Li H, Niederkorn JY, Neelam S, *et al*. Immunosuppressive factors secreted by human amniotic epithelial cells. Invest Ophthalmol Vis Sci 2005; 46(3): 900-7.

[98] Apte RS, Sinha D, Mayhew E, Wistow GJ, Niederkorn JY. Cutting edge: role of macrophage migration inhibitory factor in inhibiting NK cell activity and preserving immune privilege. J Immunol 1998; 160(12): 5693-6.

Received: July 26, 2012 Revised: November 11, 2012 Accepted: December 22, 2012