Angiogenic Potential of Extracellular Matrix of Human Amniotic Membrane

Siti Nurnasihah Md Hashim¹, Muhammad Fuad Hilmi Yusof¹, Wafa' Zahari¹,
Khairul Bariah Ahmad Amin Noordin¹, Thirumulu Ponnuraj Kannan^{1,2},
Suzina Sheikh Abdul Hamid³, Khairani Idah Mokhtar⁴, Azlina Ahmad^{1*}

¹School of Dental Sciences, Universiti Sains Malaysia, Kelantan, Malaysia

²Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia

³Tissue Bank, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia

⁴Kulliyyah of Dentistry, International Islamic University Malaysia, Pahang, Malaysia

Combination between tissue engineering and other fields has brought an innovation in the area of regenerative medicine which ultimate aims are to repair, improve, and produce a good tissue construct. The availability of many types of scaffold, both synthetically and naturally have developed into many outstanding end products that have achieved the general objective in tissue engineering. Interestingly, most of this scaffold emulates extracellular matrix (ECM) characteristics. Therefore, ECM component sparks an interest to be explored and manipulated. The ECM featured in human amniotic membrane (HAM) provides a suitable niche for the cells to adhere, grow, proliferate, migrate and differentiate, and could possibly contribute to the production of angiogenic micro-environment indirectly. Previously, HAM scaffold has been widely used to accelerate wound healing, treat bone related and ocular diseases, and involved in cardiovascular repair. Also, it has been used in the angiogenicity study, but with a different technical approach. In addition, both side of HAM could be used in cellularised and decellularised conditions depending on the objectives of a particular research. Therefore, it is of paramount importance to investigate the behavior of ECM components especially on the stromal side of HAM and further explore the angiogenic potential exhibited by this scaffold.

Tissue Eng Regen Med 2016;13(3):211-217

Key Words: Angiogenic; Human amniotic membrane; Extracellular matrix; Tissue engineering

BIOLOGICAL SCAFFOLD IN TISSUE ENGINEERING

Tissue engineering is a field that has gone through much advancement since 1990s. It started when the scientist began to expand the cells on a scaffold of the desired tissue region with the help of external inducer to re-engineer the tissue. Currently, the combination between tissue engineering and other fields such as cell biology, biomaterials and cell imaging have brought in a great transition in this field, even though the intention is still on improving and repairing tissue function [1]. Apart from the selection of scaffold, the types of cells and growth factors used need to be chosen correctly to ensure the success in tissue reconstruction [2,3].

The selection of the best scaffold is important and need to be synchronised with the end target of the research. Basically, scaffolds which are also known as matrices, should be able to improve, repair and regenerate tissue function caused by injury, inflammation or disease [1,4,5]. A good scaffold should also provide a better environment for cell adhesion, proliferation and differentiation by allowing enough and efficient transportation of oxygen, nutrients and regulatory factors [1,6]. Besides, it needs to be biodegradable, biocompatible, and should provide structural integrity for both mechanical and physical strength [3,7]. Scaffold that provides a 3D micro-environment must also mimic the real conditions to enable the cell's function [3].

There are two types of scaffold, one made up of synthetic materials and the other, derived from a natural one. Examples of synthetic materials include poly(lactic acid), poly(glycolic acid) and polycaprolactone which are capable to sustain the growth of many types of stem cells due to their degradable fibrous structures [7]. Synthetic polymer has been shown to have a low possibility of contamination and readily to be engineered [8].

Received: July 13, 2015

Revised: August 14, 2015

Accepted: August 24, 2015

***Corresponding author:** Azlina Ahmad, School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia. Tel: 609-767 5827, Fax: 609-767 5505, E-mail: azlinakb@usm.my

However, modification into highly porous scaffold in synthetic polymer is needed. Porosity of scaffold ensures the cells to interact efficiently with the environment [1]. Furthermore, scaffold porosity is important to support cell's growth, and important for cell seeding *in vitro* as well as for blood vessel invasion *in vivo* [5].

In contrast, natural scaffold has a great biocompatibility and bioactivity [7]. Examples of natural scaffolds are gelatin, dextran and fibronectin, and the most commonly used, collagen [9]. However, some cells grown on collagen caused collagen contraction, leading to tight morphology on the surface and generating tension on matrices [9,10]. Apart from its complex structure and lack of mechanical properties, natural polymer needs complicated technical approach to construct a good scaffold matrix [8]. Natural scaffold usually mimics extracellular matrix (ECM) in its structure [11].

BIOLOGICAL SCAFFOLD IN ANGIOGENESIS

Angiogenesis becomes a subject of interest due to its importance in restoring the blood supply to the tissue. Angiogenesis is formation of new blood capillaries from pre-existing vessel caused by hypoxia, a phenomenon that occurs due to a limitation in the effective diffusion of oxygen as a result of the dysregulation of blood vessel by tissue injury, inflammation or disease [4,5,12]. Maintenance and complete restoration of oxygen and nutrition supply is important to increase the density of capillaries sprouting that would stimulate blood vessel formation. Pathogenesis might happen if there is insufficient and abnormal blood vessel formation [13]. For example, impairment in the blood supply of the tissue caused by restriction in a blood vessel leads to ischemia [14]. High degree of vascularity is needed to provide good tissue vitality and construct [15]. Therefore, it is necessary to select a good scaffold for angiogenesis study to allow optimum conditions for cell to maintain its high metabolic activity in a tissue [16].

Jabbarzadeh et al. [17] used poly(lactic-co-glycolic acid) acid porous scaffold to promote blood vessel formation for bone repair by means of cell transfection. The other synthetic material commonly used is bioactive glass [18,19]. Arkudas et al. [18] showed a newly and immature vascular network formation within bioglass with arteriovenous loop scaffold after 3 weeks of implantation *in vivo*. In another study, bioactive glass was coated with vascular endothelial growth factor (VEGF) and shown to increase blood vessel density, contribute in bone healing and indirectly improve angiogenesis as well as bone maturation [19]. VEGF is a well known growth factor which could stimulate endothelial proliferation, migration, permeabil-

ity and adhesion which is vital in angiogenesis [20]. Another material, fabricated micro-channel of poly(D,L-lactic acid) scaffold, uses sacrificial sugar to allow oxygen and nutrients transportation throughout the structure, and directly contributed to endothelial cell adhesion, penetration and proliferation [21].

Natural scaffold has also been used as a carrier to allow slow release of angiogenic growth factor by means of passive diffusion [5]. In one study, injectable scaffold of gelatin/tyramine/heparin-modified hydrogels provided a sustained released of VEGF and retained its bioactivity over a long period of time, thus contributing to angiogenesis [22]. The same strategy had also been implemented by using other types of natural scaffold. VEGF released from fibrin matrix was reported to induce angiogenesis both *in vitro* and *in vivo* by up-regulating VEGFR2 expression at day 5–13 after transplantation and enhancing ischemic flap survival [23]. Even without the addition of growth factor, this natural scaffold, especially heparin, could still promote neovascularization [24]. Interestingly, a natural scaffold of human amniotic membrane (HAM) has been used to construct vascular graft *in vivo* [25]. Thus, this is an indicator of HAM potential in creating angiogenic micro-environment, but with slight modifications of the technical approaches.

HUMAN AMNIOTIC MEMBRANE AND ITS PROPERTIES

HAM, a thin natural scaffold consists of outer chorion and inner part consisting of basement membrane and avascular stroma [6] (Fig. 1). It has a thickness of 0.02 to 0.5 mm [26] without nerves and muscles [27]. The basement side is covered by epithelial cells on its surface while the stromal side is connect-

Figure 1. Schematic diagram of HAM. HAM consists of basement and stromal side. The basement side consists of epithelium, basement membrane and compact layer, and the stromal side consists of fibroblast and spongy layers. HAM: human amniotic membrane.

ed to a spongy layer [28]. ECM of HAM is rich in collagen types I, III, IV, V, and VII, laminin, fibronectin [6,26,28], and components, including growth factors such as keratinocyte growth factor, basic-fibroblast growth factor (b-FGF), transforming growth factor-beta, nidogen growth factor and epidermal growth factor (EGF) [6,29]. These components would provide an optimal surrounding for any cells to grow on HAM. HAM also gives no immunological reaction, and suitable to be used to treat patients [30]. Other than that, it has anti-microbial properties which help to protect the wound from infection [6,27].

Other properties of HAM include anti-scarring and anti-inflammatory [6,27]. Besides, HAM can be easily obtained, processed, transported [6], and its function can be maintained through several preservation methods [26]. The great advantage of HAM is, it allows transmission of water vapour and oxygen, which provide moisture to the transplantation surface naturally [30]. This physiologically moist micro-environment contribute directly towards the healing process [31]. Moreover, HAM which has a sponge-like structure, has good cytocompatibility allowing cells to proliferate normally [32]. In addition, HAM is suitable to be used in cell culture because of its optimal pore size, and it has an ability to absorb water directly thus imitates and provides a micro-environment in helping cell growth and proliferation [32]. The basement side of HAM has a tight pore structure, where the diameter size is approximately less than 0.5 μm while its stromal side shows large pore size between 0.5 μm to 2 μm and contains thick collagen fibers [25]. HAM stromal side is made up of larger fibril with loosely packed interstitial collagen matrix as compared to the basement side [33]. Interestingly, its basement mimics other basement part of the body, such as conjunctiva and gingiva [27]. Due to its thin and tough structure, it has been widely used for clinical application such as in assisting wound healing. Figure 2A showed bottled HAM scaffold after being exposed to 25 kGy gamma radiation. Figure 2B showed HAM scaffold incubated in media for *in vitro*

study.

Moreover, the angiogenic factors profile released attribute by HAM is affected by the method of HAM preservation such as b-FGF, VEGF, or platelet-derived growth factor (PDGF) [34]. The ability of HAM in promoting endothelial cell proliferation, migration and differentiation proved that factors released from the ECM of HAM do not directly contribute to the inhibition of angiogenesis [35]. Therefore, this type of scaffold could be utilised in the construction of blood vessel and enhance endothelium function contributing to angiogenesis [36].

APPLICATION OF HAM IN MEDICAL FIELD

Due to the magnificent characteristics of HAM, its usage in accelerating wound healing has begun since 1900s. Its ability in reducing water loss, protein and evaporative heat in wound area has made HAM into a usable temporary dressing, especially for wound healing [37]. Bose [38] used HAM as biological dressing for wound healing due to its availability, and its characteristic that could protect from infection, prevent fluid, protein and energy loss as well as to accelerate fibroblast migration and collagen development. Besides, HAM has been used for the construction of living skin equivalent (LSE) graft [39]. Their results show that LSE graft vascularised well on full-thickness wounds *in vivo*, suggesting that good epidermogenesis occur both *in vivo* and *in vitro*. Since HAM is a reservoir for growth factors and cytokines, HAM has been used together with other bone graft to repair bone fracture and resection [40]. In that particular study, HAM has been proven to generate new bone formation by bridging the bone defect. Another study conducted by Díaz-Prado et al. [41] claimed that cryopreserved HAM helps in human chondrocyte proliferation to repair osteoarthritis cartilage. Interestingly, they compared between the efficacy of using basement side and stromal side of HAM. Their results show that the cells that grew on basement side seemed to compete with the naturally existing epithelial cell, suggesting that the stromal side provides a better niche to promote cell proliferation. These results were concordant with other findings that also clarified the ability of HAM to resist shear force making it suitable for articular cartilage repair [42].

Apart from wound healing, HAM has been manipulated widely in treating ocular diseases. Grueterich et al. [43] in their review stated that limbal stem cell deficiency can be treated by transplanting bio-engineered graft by seeding limbal epithelial stem cells *ex vivo* on intact HAM. They also reported that the basement side of HAM allows stem cell differentiation, thus suggesting the suitability of HAM in promoting cell differentiation. In addition, HAM has an ability to reduce ocular surface

Figure 2. HAM scaffold. (A) Bottled HAM after gamma radiation. (B) HAM scaffold, 5×5 cm sized, incubated in media for *in vitro* experiment. Arrows showed the edges of HAM scaffolds. HAM: human amniotic membrane.

inflammation as its stromal matrix suppressed interleukin gene family expression which are related to inflammation [36,44]. This result proved that HAM has an anti-inflammatory effect and suitable to be used for *ex-vivo* limbal stem cell expansion [43]. HAM has the potential in helping the corneal endothelial cell transplantation, an approachable tool for improving vascular tissue engineering [6,45].

Since HAM has high biocompatibility and unique composition of the ECM, it has been a reliable source of exploitation for vascular grafting in coronary artery bypass graft surgery [25]. Its tissue-engineered vascular graft using HAM was found to be cost-effective for vascular application. Besides, the integration between endothelial cells and ECM formed hemidesmosome-like structure which indicate firm basal adhesion due to the increment of integrin expression on basement HAM [36].

HAM: CELLULARISED AND DECELLULARISED

3D scaffold of HAM is very unique because it can be used in both cellularised and decellularised conditions due to its own strength in tissue engineering application. The cellularised HAM, with intact amniotic epithelial cell, preserves its basic structure and serves as adhesion ligands and regulators, which are important in signal transduction in repairing damaged tissue area [46]. However, some of the researchers need to exclude external factor that might bring side effect to the damaged tissue area, termed as decellularisation. Decellularisation is important to block initiation factor involved in immunogenic response such as antigens, cellular components and soluble proteins, that contribute to low immunological response [1,32]. The crucial part is to ascertain that the removal of this cellular material should not give any adverse effect towards scaffold mechanical strength, composition as well as biological activity [1]. There are several methods for decellularisation of HAM and each method varies and produces different outcomes between each other out of which some gives effect to the HAM basement side and its ECM structure [32,47]. Hopkinson et al. [47] examined the efficacy of three different techniques in removing epithelial cells and look into the integrity of ECM. The results are impressive since the amniotic epithelial cells on the basement side are completely removed and give minimal damage towards ECM when using EDTA and thermolysin, both with gentle scraping, but not with dispase [47]. While another transplantation study using EDTA also shows concordant results with the one that applied the same technique using EDTA to decellularise HAM, in treating ocular disease [45]. EDTA has also been used to decellularise HAM for vascular grafting [25]. From this study, the vascular wall cells are able to attach

and proliferate, thus, proves the biocompatibility characteristic of decellularised HAM [25]. Due to that factor, HAM had been used as permanent graft in replacing damage tissue area and as temporary patch for wound healing [48].

ECM OF HAM

Burgos [49] found out that HAM has the angiogenic growth factor that facilitates in promoting angiogenesis by increasing neovascularization. The presence of angiogenic growth factor could be the main reason for use in biological dressing. In early 1980s, a study had been conducted to see the properties of ECM on HAM. Basement membrane contains the collagen type IV and V as well as laminin [33]. Collagen type IV together with laminin help in the rapid tubular formation which are very crucial in the angiogenesis process [11]. Interestingly, cells grown on the stromal side of HAM showed a different behaviour. It seems that the cells proliferate and migrate into the inner part of stromal and stop before reaching the basement side [33]. Other than that, the ECM could control and increase tube formation [11]. This statement is in concordance with the findings by Madri and Williams [33], where the rat capillary endothelial cells, grown on the stromal side of HAM, formed tube-like structure in areas of high cell density if it was cultured for a long-term culture period (approximately about 3–4 weeks) while basement side showed faster tubular formation (approximately about 5 days). Formation of tubular-like structure suggests that there might exist interconnectivity between cells and ECM which could probably be due to the HAM scaffold porosity [1].

ADVANTAGES OF ECM

ECM which consists mainly of protein complex, glycoprotein and proteoglycan, had been used and referred to as a main model for developing and designing biomaterial scaffold [50]. The concern arises when cells grown on culture plates could not retain its original function in cell signaling and lose important regulators [32]. In contrast, cell grown on a 3D scaffold could tolerate well in micro-environment condition and sustain the cell function of cells [51]. ECM fulfills the basic requirement needed by cells due to its components. Collagen, one of the components in ECM, is necessary to allow cell proliferation and provides mechanical strength for cell adhesion and flexibility [32]. Integrin, an ECM receptor, is important for cell survival and migration [52]. It initiates the signaling event by binding to the cell surface, and mediates the interaction between the cells and ECM to generate a response that is essential in cell action and regulation [11,32,51,53]. Bayless et al. [54] reported that $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin involved in endothelial cell morpho-

genesis and regulate human endothelial cell vacuolation as well as lumen formation using fibrin matrix system, a system which imitates the wound micro-environment where angiogenesis happened [54]. In another study, the interaction between two molecule of ECM which are integrin and fibronectin give signal to the growth factor such as PDGF, EGF, and b-FGF which are involved in extracellular signal-regulated kinase (ERK) regulation [55]. Previously, fibronectin had been reported involved in vascular morphogenesis since it contained endothelium-line vasculature [56]. According to the PubMed literature search, there is no study conducted in investigating mechanisms of the ECM components of HAM during angiogenesis, but only one which converted the ECM of HAM into microparticles [57]. However, some studies confirmed that several ECM components do play their role in angiogenic mechanism. In a study, matrix metalloproteinase interferes angiogenesis via protein kinase B (AKT) pathway by inhibiting endothelial cell proliferation and reducing the formation of capillary-like structure [58]. In a review by Mavria et al. [59], they mentioned that the down-regulation of Rho-kinase had led to the endothelial proliferation. Both of this study confirmed the involvement of AKT as well as ERK/MAPK pathway in angiogenesis [58,59].

CONCLUSIONS

HAM, a biological scaffold can be developed as a good scaffold to promote angiogenesis. It can be easily obtained, available abundantly and does not require any complicated process before the usage. Besides, HAM has a great biocompatibility due to the unique features shown by its ECM. Although there is no study that specifically indicates the direct involvement of HAM in angiogenic regulation, we believed the ECM components underlying in this scaffold could probably help in angiogenesis. From the previous finding, the ECM components do contribute to the angiogenic regulation. It gives clue to study further on the mechanism and understand the behavior of every molecule on HAM that involved in neovascularization. Hence, we would be able to exploit and use this membrane directly or develop other synthetic materials which have the same exact ECM components as such in HAM. The knowledge of ECM of HAM could be further explored for future prospect in regenerative medicine particularly in treating angiogenic disease. We postulate, HAM is not only helping cells to attach, proliferate and migrate, but it could also enable stem cells to differentiate into other types of cells.

FINAL DIRECTION

In health industry nowadays, people are looking towards pre-

vention rather than medication and this idea is highly related with tissue regeneration. Consensus opinion amongst health operators and biomedical scientists is to find something or some ways to make regenerative medicine successful. Thus, many researchers are working on development of scaffolds for that purposes; synthetic or biological. As some teams are looking at the biological scaffold, they are learning many aspect of making synthetic scaffold mimicking the micro-environment possesses by the biological scaffold. This will eventually lead to the mass production of synthetic scaffold with similar induction towards tissue regeneration as biological scaffold and in this case, HAM. Besides angiogenicity, other tissue formation, such as osteogenicity, could also utilise HAM and its ECM components. In dentistry, the scanty knowledge on regenerative pulp tissue with regards to the suitable scaffold arouses curiosity among researchers. Among the concern is to regenerate adequate tissue vascularisation, which brought in the idea to look into the potential of HAM in promoting angiogenesis. Since the ECM of HAM allows cell attachment and helps the cell to differentiate into other types of cells, the potential of HAM can be well exploited. Moreover, HAM scaffold is available in plenty and affordable. In addition, the angiogenicity characteristic showed by HAM in previous study shows promise.

Acknowledgements

This review was supported by Universiti Sains Malaysia Research University Grant (1001/PPSG/813075).

Conflicts of Interest

The authors have no financial conflicts of interest.

Ethical Statement

There are no animal experiments carried out for this article.

REFERENCES

1. Dhandayuthapani B, Yoshida Y, Maekawa T, Kumar DS. Polymeric scaffolds in tissue engineering application: a review. *Int J Polym Sci* 2011 [Epub]. DOI:10.1155/2011/290602.
2. Toda A, Okabe M, Yoshida T, Nikaido T. The potential of amniotic membrane/amnion-derived cells for regeneration of various tissues. *J Pharmacol Sci* 2007;105:215-228.
3. Barbara Z, Eriberto B, Stefano S, Giulia B, Chiara G, Ferrarese N, et al. Dental Pulp Stem Cells and Tissue Engineering Strategies for Clinical Application on Odontoiatric Field. In: Pignatello R, editor. *Biomaterials Science and Engineering*. Croatia: InTech; 2011. p.339-348.
4. Nakashima M, Iohara K, Sugiyama M. Human dental pulp stem cells with highly angiogenic and neurogenic potential for possible use in pulp regeneration. *Cytokine Growth Factor Rev* 2009;20:435-440.
5. Zisch AH, Lutolf MP, Hubbell JA. Biopolymeric delivery matrices for angiogenic growth factors. *Cardiovasc Pathol* 2003;12:295-310.
6. Niknejad H, Peirovi H, Jorjani M, Ahmadiani A, Ghanavi J, Seifalian AM. Properties of the amniotic membrane for potential use in tissue engi-

- neering. *Eur Cell Mater* 2008;15:88-99.
7. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. *J Endod* 2007;33:377-390.
 8. Kanczler JM, Oreffo RO. Osteogenesis and angiogenesis: the potential for engineering bone. *Eur Cell Mater* 2008;15:100-114.
 9. Huang GT, Sonoyama W, Chen J, Park SH. In vitro characterization of human dental pulp cells: various isolation methods and culturing environments. *Cell Tissue Res* 2006;324:225-236.
 10. Plant AL, Bhadriraju K, Spurlin TA, Elliott JT. Cell response to matrix mechanics: focus on collagen. *Biochim Biophys Acta* 2009;1793:893-902.
 11. Sieminski AL, Gooch KJ. Biomaterial-microvasculature interactions. *Biomaterials* 2000;21:2232-2241.
 12. Heil M, Eitenmüller I, Schmitz-Rixen T, Schaper W. Arteriogenesis versus angiogenesis: similarities and differences. *J Cell Mol Med* 2006;10:45-55.
 13. Carmeliet P. Angiogenesis in health and disease. *Nat Med* 2003;9:653-660.
 14. Djonov V, Makanya AN. New insights into intussusceptive angiogenesis. *EXS* 2005;(94):17-33.
 15. Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, et al. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. *J Endod* 2008;34:962-969.
 16. Demarco FF, Conde MC, Cavalcanti BN, Casagrande L, Sakai VT, Nör JE. Dental pulp tissue engineering. *Braz Dent J* 2011;22:3-13.
 17. Jabbarzadeh E, Starnes T, Khan YM, Jiang T, Wirtel AJ, Deng M, et al. Induction of angiogenesis in tissue-engineered scaffolds designed for bone repair: a combined gene therapy-cell transplantation approach. *Proc Natl Acad Sci U S A* 2008;105:11099-11104.
 18. Arkudas A, Balzer A, Buehrer G, Arnold I, Hoppe A, Detsch R, et al. Evaluation of angiogenesis of bioactive glass in the arteriovenous loop model. *Tissue Eng Part C Methods* 2013;19:479-486.
 19. Leach JK, Kaigler D, Wang Z, Krebsbach PH, Mooney DJ. Coating of VEGF-releasing scaffolds with bioactive glass for angiogenesis and bone regeneration. *Biomaterials* 2006;27:3249-3255.
 20. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000;407:249-257.
 21. Sun J, Wang Y, Qian Z, Hu C. An approach to architecture 3D scaffold with interconnective microchannel networks inducing angiogenesis for tissue engineering. *J Mater Sci Mater Med* 2011;22:2565-2571.
 22. Li Z, Qu T, Ding C, Ma C, Sun H, Li S, et al. Injectable gelatin derivative hydrogels with sustained vascular endothelial growth factor release for induced angiogenesis. *Acta Biomater* 2015;13:88-100.
 23. Mittermayr R, Morton T, Hofmann M, Helgersson S, van Griensven M, Redl H. Sustained (rh)VEGF(165) release from a sprayed fibrin biomatrix induces angiogenesis, up-regulation of endogenous VEGF-R2, and reduces ischemic flap necrosis. *Wound Repair Regen* 2008;16:542-550.
 24. Moon JJ, West JL. Vascularization of engineered tissues: approaches to promote angiogenesis in biomaterials. *Curr Top Med Chem* 2008;8:300-310.
 25. Brennan JA, Arrizabalaga JH, Nollert MU. Development of a small diameter vascular graft using the human amniotic membrane. *Cardiovasc Eng Technol* 2014;5:96-109.
 26. Caruso M, Silini A, Parolini O. The human amniotic membrane: A tissue with multifaceted properties and different potential clinical applications. In: Kyle JC, Curtis L, Cetrulo Jr, Rouzbeh R, Taghizadeh, editors. *Perinatal Stem Cells*. 2nd ed. New Jersey: John Wiley & Sons; 2013. p.177-195.
 27. Chopra A, Thomas BS. Amniotic membrane: a novel material for regeneration and repair. *J Biomim Biomater Tissue Eng* 2013;18:106.
 28. 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;31:216-225.
 29. Koizumi NJ, Inatomi TJ, Sotozono CJ, Fullwood NJ, Quantock AJ, Kinoshita S. Growth factor mRNA and protein in preserved human amniotic membrane. *Curr Eye Res* 2000;20:173-177.
 30. Nor Kamalia Z, Suzina SAH, Yusof N. Changes in biophysical properties of human amniotic membranes after different preservation methods and radiation sterilization. *Regen Res* 2014;3:64-70.
 31. Ab Hamid SS, Zahari NK, Yusof N, Hassan A. Scanning electron microscopic assessment on surface morphology of preserved human amniotic membrane after gamma sterilisation. *Cell Tissue Bank* 2014;15:15-24.
 32. Taghiabadi E, Nasri S, Shafieyan S, Jalili Firoozinezhad S, Aghdami N. Fabrication and characterization of spongy denuded amniotic membrane based scaffold for tissue engineering. *Cell J* 2015;16:476-487.
 33. Madri JA, Williams SK. Capillary endothelial cell cultures: phenotypic modulation by matrix components. *J Cell Biol* 1983;97:153-165.
 34. Wolbank S, Hildner F, Redl H, van Griensven M, Gabriel C, Hennerbichler S. Impact of human amniotic membrane preparation on release of angiogenic factors. *J Tissue Eng Regen Med* 2009;3:651-654.
 35. Ma DH, Yao JY, Yeh LK, Liang ST, See LC, Chen HT, et al. In vitro anti-angiogenic activity in ex vivo expanded human limboconal epithelial cells cultivated on human amniotic membrane. *Invest Ophthalmol Vis Sci* 2004;45:2586-2595.
 36. Tsai SH, Liu YW, Tang WC, Zhou ZW, Hwang CY, Hwang GY, et al. Characterization of porcine arterial endothelial cells cultured on amniotic membrane, a potential matrix for vascular tissue engineering. *Biochem Biophys Res Commun* 2007;357:984-990.
 37. Maral T, Borman H, Arslan H, Demirhan B, Akinbingol G, Haberal M. Effectiveness of human amnion preserved long-term in glycerol as a temporary biological dressing. *Burns* 1999;25:625-635.
 38. Bose B. Burn wound dressing with human amniotic membrane. *Ann R Coll Surg Engl* 1979;61:444-447.
 39. Yang L, Shirakata Y, Tokumaru S, Xiuju D, Tohyama M, Hanakawa Y, et al. Living skin equivalents constructed using human amnions as a matrix. *J Dermatol Sci* 2009;56:188-195.
 40. Starecki M, Razzano P, Schwartz JA, Grande DA. Evaluation of amniotic derived membrane biomaterial as an adjunct for repair of critical sized bone defects. *Adv Orthop Surg* 2014 ID 572586.
 41. Díaz-Prado S, Rendal-Vázquez ME, Muñíos-López E, Hermida-Gómez T, Rodríguez-Cabarcos M, Fuentes-Boquete I, et al. Potential use of the human amniotic membrane as a scaffold in human articular cartilage repair. *Cell Tissue Bank* 2010;11:183-195.
 42. Jin CZ, Park SR, Choi BH, Lee KY, Kang CK, Min BH. Human amniotic membrane as a delivery matrix for articular cartilage repair. *Tissue Eng* 2007;13:693-702.
 43. Grueterich M, Espana EM, Tseng SC. Ex vivo expansion of limbal epithelial stem cells: amniotic membrane serving as a stem cell niche. *Surv Ophthalmol* 2003;48:631-646.
 44. 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;85:444-449.
 45. Ishino Y, Sano Y, Nakamura T, Connon CJ, Rigby H, Fullwood NJ, et al. Amniotic membrane as a carrier for cultivated human corneal endothelial cell transplantation. *Invest Ophthalmol Vis Sci* 2004;45:800-806.
 46. Lo V, Pope E. Amniotic membrane use in dermatology. *Int J Dermatol* 2009;48:935-940.
 47. Hopkinson A, Shanmuganathan VA, Gray T, Yeung AM, Lowe J, James DK, et al. Optimization of amniotic membrane (AM) denuding for tissue engineering. *Tissue Eng Part C Methods* 2008;14:371-381.
 48. Liu J, Sheha H, Fu Y, Liang L, Tseng SC. Update on amniotic membrane transplantation. *Expert Rev Ophthalmol* 2010;5:645-661.
 49. Burgos H. Angiogenic factor from human term placenta. Purification and partial characterization. *Eur J Clin Invest* 1986;16:486-493.
 50. Langer R, Tirrell DA. Designing materials for biology and medicine. *Nature* 2004;428:487-492.
 51. Geiger B, Bershadsky A, Pankov R, Yamada KM. Transmembrane cross-

- talk between the extracellular matrix--cytoskeleton crosstalk. *Nat Rev Mol Cell Biol* 2001;2:793-805.
52. Bowers SL, Banerjee I, Baudino TA. The extracellular matrix: at the center of it all. *J Mol Cell Cardiol* 2010;48:474-482.
 53. Cukierman E, Pankov R, Yamada KM. Cell interactions with three-dimensional matrices. *Curr Opin Cell Biol* 2002;14:633-639.
 54. Bayless KJ, Salazar R, Davis GE. RGD-dependent vacuolation and lumen formation observed during endothelial cell morphogenesis in three-dimensional fibrin matrices involves the alpha(v)beta(3) and alpha(5)beta(1) integrins. *Am J Pathol* 2000;156:1673-1683.
 55. Miyamoto S, Teramoto H, Gutkind JS, Yamada KM. Integrins can collaborate with growth factors for phosphorylation of receptor tyrosine kinases and MAP kinase activation: roles of integrin aggregation and occupancy of receptors. *J Cell Biol* 1996;135(6 Pt 1):1633-1642.
 56. Astrof S, Hynes RO. Fibronectins in vascular morphogenesis. *Angiogenesis* 2009;12:165-175.
 57. Zhang YQ, Ji SZ, Fang H, Zheng YJ, Luo PF, Wu HB, et al. Use of amniotic microparticles coated with fibroblasts overexpressing SDF-1 α to create an environment conducive to neovascularization for repair of full-thickness skin defects. *Cell Transplant* 2015;25:365-376.
 58. Brauer R, Beck IM, Roderfeld M, Roeb E, Sedlacek R. Matrix metalloproteinase-19 inhibits growth of endothelial cells by generating angiotensin-like fragments from plasminogen. *BMC Biochem* 2011;12:38.
 59. Mavria G, Vercoulen Y, Yeo M, Paterson H, Karasarides M, Marais R, et al. ERK-MAPK signaling opposes Rho-kinase to promote endothelial cell survival and sprouting during angiogenesis. *Cancer Cell* 2006;9:33-44.