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Biotechnological application of human amniotic membrane in skin reconstruction

Dan Navolan[1], Ioana Ciohat[1], Simona Vladareanu[2], Radu Vladareanu[3], Ovidiu Nicodin[4], Bogdan Panaite[4], Dragos Nemescu[5], Nicolae Bacalbasa[6], Diana Badiu[7], Tony Hangan[7]

1. Department of Obstetrics-Gynecology and Neonatology, "Victor Babes" University of Medicine and Pharmacy, Romania; navolan@yahoo.com
2. Department of Neonatology, "Carol Davila" University of Medicine and Pharmacy, Elias University Hospital, Romania; simconst69@gmail.com
3. Department of Obstetrics and Gynecology, "Carol Davila" University of Medicine and Pharmacy, Elias University Hospital, Romania; vladareanu@gmail.com
4. Department of Gynecology, "Carol Davila" University of Medicine and Pharmacy, Central Military University Emergency Hospital "Dr. Carol Davila", Bucharest, Romania; ovnicodin@yahoo.com
5. Department of Obstetrics & Gynecology, "Gr.T.Popa" University of Medicine and Pharmacy Iasi, Romania; dnemescu@yahoo.com; dragos.nemescu@umfiasi.ro
6. „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania; nicolae_bacalbasa@yahoo.ro
7. Faculty of Medicine, "Ovidius" University of Constanta; tony@medcon.ro; tonyhangan@yahoo.com

All authors have equally contributed to this work.

Abstract

The human amniotic membrane (HAM) has a long history of use in surgical diagnostic. Therefore, the discoveries of cell populations from HAM, which are capable to differentiate into different skin types have been stimulating further research in characterizing this cells and their biotechnological application. Since the discovery of HAM-derived cell characteristics, the interest of big pharmaceutical industry has become more visible in application to skin grafts. In this context, we provide an overview of the recent progress and future perspectives of HAM-derived cells in skin tissue reconstruction and further biotechnological application.
Keywords: *amniotic membrane, amniotic-derived cells, skin types, reconstruction, biotechnology*

1. Human Amniotic Membrane (HAM) as a Dressing Biomaterial

The inner layer of the fetal membrane is HAM which was already used for different investigation as biomaterial in various purposes in transplants although until present, has been proven to facilitate also bone growth and both soft tissue and cartilage healing[1]. The first method discovery for processing and storage of HAM was started at the end of the 1990s and first studies reported an alternative substrate for treating different corneal epithelial defects[2,3].

In this context, it was seen that the combination of HAM with other antioncogenic agents could lead to an innovative potential material for cancer therapy. Some studies showed that HAM was used together with antioncogenic agents and the results concluded to be an innovative potential biomaterial for cancer treatment. In this regard, Horch and contributors have recently started to work on a new stem-cell technology which may be able to change the main role of tumor growth as well as tumor-related angiogenese[4].

Although different various dressing biomaterials were applied to cover parts of skin and becoming skin grafts, HAM have been used as a start for many clinicians to treat infected wounds, ulcers, burns and different trauma.

Based on the clinicians criticism, HAM still represents a good covering, accounting the angiogenic factors and could cover the unexplained success in surgical practice[5].

In another study, the authors showed the application of placental membrane in the case of burns and the results were that HAM appears to surprisingly reduce the bacterial surface contamination[6].

Nowadays, regenerative medicine uses already a combination of living cells to replace the normal function of different damaged tissues by identifying the molecular pathways that lead to disease, as well as targets for therapy[7]. Whatsoever, the source of these cells is becoming to be more searched and developed for the next therapy generation. Although embryonic stem cells were highly used in the different future therapy, still many of them presented memory of the first source cells, and further showed a clear risk of tumor differentiation[8].

Surprisingly, the amniotic fluid stem cells represents the multipotent cells part from the inner layer of the fetal membrane and can be maintained under undifferentiated state for long-time, representing all three germ layers[9]. In comparison with other source of stem cells, the amniotic-derived cells have been showed not to form tumors in the cases when were transplanted *in vitro* and may be a safer alternative to any other embryonic stem cells[10].

Therefore, the development of HAM-derived cells as a dressing biomaterial for different skin-grafts has not been yet fully investigated. Most commonly used dressing techniques still presented some disadvantages, as the accumulation of blood at the base of the wound which make the wound healing process very difficult, being also an expensive material.

Certainly, much research is still needed to better understand the full intimal mechanism and the role that HAM-derived cells plays as a dressing biomaterial in skin grafts.

However, the reports reviewed until present regarding the use of HAM as wound covering, showed that in some particularly cases, pig-skin xenografts might be also considered[11]. The aim of the present review is to evaluate the usefulness of HAM-derived cells as an alternative dressing biomaterial with further biotechnological applications.

2. The Implication of HAM in Skin-Tissue Regeneration

HAM is the innermost fetal layer from amniotic cavity. In contrast, the outer layer, which is the chorionic membrane, separates the fetus from maternal tissue. Until present, many studies have been showed that HAM not only provides the main support for the fetus, but also a metabolically active carrier through a direct transport of different materials, including growth factors, and cytokines[12]. In accordance with these studies, it was showed that the umbilical cord represents also the channel for gas exchange, nutrient supply, and metabolites excretion[13].

These translucent, avascular and wound healing properties in particular of HAM allow this biomaterial function beyond its role *in vivo* assuming in the same time a wide range of application in reconstruction medicine and biotechnology industry[14].

Until present, the shelf life of HAM has been extended by different methods like irradiation and cryo-preservation which will be expected to grow and extend in the future therapy for the use in scars and defects by reconstruction of skin and other surfaces. Moreover, in different clinical applications, the use of HAM was achieved by the presence of different growth factors like epidermal growth factor, which may uncover in the future the proper mechanism of action[15].

Interestingly, it was showed that epithelial cells can be rapidly identified as a single layer to the amniotic fluid[16]. Having in the view that mesenchymal stromal cells come from the outer layer, both cell types have been further investigated for their characteristics. In this respect, amniotic membrane has been showed to be a promoter of epithelialization, and was further applied in ocular reconstruction, genito-urinary tract, and skin, including head and neck[17].

When the human amnion-derived fibroblast-like cells were evaluated to differentiate into neural cells, it has been showed that pluripotency markers were present following the induction of differentiation, like neuron specific enolase, neurofilament-medium, beta-tubulin isotype III, and glial fibrillary acidic protein[18].

Nonetheless, it was accepted that multiple cells can be triggered by culturing amniotic mesenchymal stromal cells under the same conditions[19]. Based on methods like immunohistochemical and genetic analysis, amniotic epithelial cells have the potential to differentiate to all three germ layers, being capable to produce different cells like liver, pancreas, cardiomyocyte, and neural cells[20].

Human amniotic epithelial cells showed to be also clonogenic, and primary cultures could be induced to differentiate into cardiomyocytic, myocytic, osteocytic, adipocytic (i.e. mesodermal), pancreatic, hepatic (i.e. endodermal), and neural (i.e. neuroectodermal) cells *in vitro*, as defined by phenotypic and mRNA expression[21].

The development of biological substitute's grafts to replace damaged tissue may involve either the replacement parts in transplantation either the direct administration of these cells to the damages tissue[22].

Mesenchymal-epidermal interactions are known to be responsible for organogenesis. On the basis of this mechanism, Li and contributors constructed methods of traditional tissue engineering skin (i.e. tissue engineering skin with human fibroblasts and keratinocytes) and then established a new bilayered skin based on amniotic cells. They showed that the constructed skin could successfully repair full thickness skin defects on experimental mice[23], which could also be used in different skin lesions which appear during pregnancy period[24].

Some studies described the differentiation potential and beneficial use of these cells and analysed their role for in vitro differentiation into mesodermal cell lineages until present[25]. These studies established the role of HAM in biodelivery from many compounds. In an attempt to discover a suitable material which can be used as scaffold, HAM showed in time to offer a great potential owing to its many desirable properties. HAM is inert, biocompatible, biodegradable being a compatible surgical patch[26]. Since its surgical use, HAM has been showed to be excellent tissue transplantation with many clinical applications, including ocular surface reconstruction[27] and scaffold for host tissue presenting mechanical property and low immunogenicity[28].

3. Biotechnology Application of HAM-Derived Cells in Skin Reconstruction

Through different histological, biochemical and biological techniques it was tried to isolate and determinate the use of HAM-derived cells in different biotechnological industry. Cells from amniotic membrane are just started to be investigated and the industrial market is therefore premature. About 1 million people affected by burns and other wounds in the US have started to benefit from these cell products[29].

Although the results of these studies raise higher expectation, many pharmaceutical companies are still in debate regarding the entering on the market. The most remarkable strategies were the signing of agreements between big and small biotechnology companies whose activity is based direct on cell therapies. Therefore, many HAM-derived cell bioproducts are developed first at many developed research institutions. Therapy based on HAM should be considered drugs or products for skin grafts reconstruction, and should follow the same regulation, including a strict control of manipulation. A regulatory framework will be required to ensure patient accessibility to bioproducts and governmental assistance for their regulation and control. In this respect, HAM-derived cells bioproducts should also taking apart to the Current Good Manufacturing Practices, like quality assurance program, which basically establish minimum quality requirements[30].

The key points of the Food and Drug Administration (FDA) regulation for cell therapy include: preclinical safety, no other risk for donor of transmission of in-

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fections, specific and detailed examination of the type of cell, and the efficacy of the product *in vivo*[31]. According to FDA all human cells are considered xenogeneic cells[32]. It should be mentioned whether these cells have been manipulated with biomaterials, which are more common used in wounds application. Moreover, biomaterials for cell therapy should be biocompatible to prevent immune rejection and further biodegradable[30].

Whether natural or artificial, biomaterial type and its use should be related to the route of administration in different tissue regeneration. Biomaterials can be found mainly in a hydrogel state, forming a hydrophilic network, as occurs in collagen. To control the structure of the biomaterials will becomes very important in increasing the efficacy in biotechnology industry application. Therefore, control of development, and quality by using stability tests should also be taken into account. Clinical aspects such as special dose characteristics, stratification and administration should also be considered. Since new HAM-derived cells therapies develop very fast, the regulatory framework should also adapt and the legislation should be changed more rapidly at this level[32].

The future aim is to identify the factors and cytokines expressed during reconstruction and incorporate them to create a smart dressing model for use in a skin equivalent. Recent advances in the use of microarray and proteomic technology are likely to become more studied[32]. HAM-derived cells, coupled with recent advances in non-viral gene delivery and stem cell technologies, may also contribute to novel approaches that would generate a skin replacement on which biomaterials technology will be based[32].

4. Conclusions

In the view of the tissues and cell transplantation, HAM may represent a new era in skin therapy purpose. In this regard, in the near future, we will be able to see the development of new therapies using these cell types isolated from HAM. Before starting to use it in biotechnological application, more preclinical studies are needed for the patient safety in designing scaffolds or matrices for skin reconstruction. ■

References

1. Davis S. Skin transplantation with a review of 550 cases at the John Hopkins Hospital. John Hopkins Hospital Report 1910, 15, 307-10.
2. Lee SH, Tseng SC. Amniotic membrane transplantation for persistent epithelial defects with ulceration. AJO 1997, 123, 3, 303-12.
3. Loeffelbein DJ, Rohleder NH, Eddicks M, Baumann CM, Stoeckhuber M, Wolff KD, Drecoll E, Steinstraesser L, Hennerbichler S, Kesting MR. Evaluation of human amniotic membrane as a wound dressing for split-thickness skin-graft donor sites. BioMed Research International 2014, heep://dx.doi.org/10.1155/2014/572183.
4. Horch RE, Boos AM, Quan Y, Bleiziffer O, Detsch R, Boccacini AR, Alexiou C, Sun J, Beier J, Arkudar A. Cancer research by means of tissue engineering – is there a rationale? Journal of Cellular and Molecular Medicine 2013, 17(10), 1197-206.
5. Faulk WP, Matthews R, Stevens PJ, Bennett JP, Burgos H, His BL. Human amnion as an adjunct in wound healing. Lancet 1980, 31(1), 1156-8.
6. Troensegaard-Hansen E. Amniotic grafts in chronic skin ulceration. Lancet 1950, 6(1), 859-60.
7. Chien KR. Regenerative medicine and human models of human disease. Nature 2008, 453, 7193, 302-5.
8. Ben-David U, Benvenistry N. The tumorigenicity of human embryonic and induced pluripotent stem cells. Nature Reviews Cancer 2011, 11, 4, 268-77.
9. Klemut PA, Vafaizadeh V, Groner B. The potential of amniotic fluid stem cells for cellular therapy and tissue engineering. Expert Opin Biol Ther 2011, 11(10), 1297-314.
10. Miki T, Lehmann T, Cai H, Stolz DB, Strom SC. Stem cell characteristics of amniotic epithelial cells. Stem Cells 2005, 23(10), 1549-59.
11. GRUSS JS, JIRSCH DW, Human amniotic membrane: a versatile wound dressing. Cn Med Assoc J 1978, 20, 118(10), 1237-46.
12. Toda A, Okaba M, Yoshida T, Nnikaido T. The potential of amniotic membrane/amnion-derived cells for regeneration of various tissues. Journal of Pharmacological Sciences 2007, 105, 3, 215-28.
13. Yan J, Zhun Y, Liao H, Su L, Guo Y, Cai J. A case of umbilical cord hemangioma. Rom J Leg Med 2014, 22, 177-80.
14. Nikmejad H, Peirovi H, Jorjani M, Ahmadiani A, Ghanavi J, Seifalian AM. Properties of the amniotic membrane for potential use in tissue engineering. ECM 2008, 15, 88-9.
15. Parolini O, Soucini M, Evangelista M, Schmidt D. Amniotic membrane and amniotic fluid-derived cells: potential toold for regenerative medicine? Regenerative Medicine 2009, 4(2), 275-91.
16. Bourgne G. The foetal membranes. Postgrad Med J 1962, 38(438), 193-201.
17. Mamede AC, Carvalho MJ, Abrantes AM, Laranjo M, Maia CJ, Botehlo MF. Amniotic membrane: from structure and functions to clinical applications. Cell and Tissue Research 2012, 349(2), 447-58.
18. Tamagawa T, Ishiwata I, Ishikawa H, Nakamura Y. Induced in vitro differentiation of neural-like cells from human amniotic-derived fibroblast-like cells. Human Cell 2008, 21, 2, 38-45.
19. Portmann-Lanz CB, Schoeberlein A, Huber A, Sager R, Malek A, Holzgreve W, Surbek DV. Placental mesenchymal stem cells as potential autologous graft for pre- and perinatal neuroregeneration. Am J Obstet Gynecol 2006, 194(3), 664-73.
20. Miki T, Lehmann T, Cai H, Stolz DB, Strom SC. Stem cell characteristics of amniotic epithelial cells. Stem Cells 2005, 23, 10, 1549-59.
21. Ilancheran S, Michalska A, Peh G, Wallance EM, Pera M, Mannelpillai U.Stem cells derived from human fetal membranes display multilineage differentiation potential. Biol Reprod 2007, 77(3), 577-88 (2007).
22. Langer R, Vacanti JP. Tissue-engineering. Science 1993, 260, 5110, 920-6.
23. Li H, Chu Y, Zhang Z, Zhang G, Jiang L, Wu H, Liu S, Yu C, Jun Y. Construction of bilayered tissue-engineered skin with human amniotic mesenchymal cells and human amniotic epithelial cells. Artif Organs 2012, 36(10), 911-9.
24. Diaz-Prado S, Muinos-Lopez E, Hermida-Gomez T, Cicione C, Rendal-Vazquez ME, Fuentes-Boquete I, de Toro FJ, Blanco FJ. Human amniotic membrane as an alternative source of stem cells for regenerative medicine. Differentiation 2011, 81(3),162-71.
25. Fernandes M, Sridhar MS, Sangman VS, Rar GN. Amniotic membrane transplantation for ocular surface reconstruction. Cornea 2005, 24(6), 643-53.
26. Wilshaw SP, Kearney JN, Fisher J, Ingham E. Production of an acellular amniotic membrane matrix for use in tissue engineering. Tissue Eng 2006, 12(8), 2117-29.
27. Ninjnejad 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.
28. Smith D. Commercialization challenges associated with induced pluripotent stem cell-based products. Regen Med 2010, 5, 593-603.
29. Liras A. Future research and therapeutic applications of human stem cells: General, regulatory, and bioethical aspects. J Transl Med 2010, 8, 131-46.
30. Halme DG, Kessler DA. FDA regulation of stem cell-based therapies. N Engl J Med 2006, 355, 1730-5.
31. FDA. Proposed Approach to Regulation of cellular and Tissue-Based Products. Available online. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/UCM062601.pdf
32. Metcalfe AD, Ferguson MW. Tissue engineering of replacement skin: The crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration. J R Soc Interface 2007, 4, 413-37.