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# Successful Anatomic Repair of Fetoscopic Access Sites in the Mid-gestational Rabbit Model Using Amnion Cell Engineering

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**Abstract.** *Background/Aim: Our aim was to evaluate the impact of in vitro cultured amnion cells, injected and/or seeded in different scaffolds, on in vivo fetal membrane repair. Materials and Methods: Amnion cells, isolated from allogeneic fetal membranes, were cultured on three different scaffolds for 14 to 21 days. In 33 mid-gestational rabbits, fetoscopic access sites were randomly allocated to four closure study groups: conventional collagen plug, as well as collagen plug, collagen foil, and fibrin glue as scaffolds for the cultured amnion cells. All membrane access sites were sealed with fibrin glue, and the myometrium closed with sutures. Fetal survival, amnion membrane integrity, and the presence of amniotic fluid were evaluated one week later. Results: Cultures showed good survival in the collagen scaffolds. The use of collagen plug as a scaffold for the in vitro cultured amnion cells improved the integrity of fetal membranes to 80%, better than that of any other study group. Conclusion: Despite the need for additional studies, the present data suggest that amnion cells can be a practical and important source of cells for the engineering of constructs for sealing of the fetal membrane.*

In the 1990s, the first animal model for the evaluation of closure techniques for fetal membranes after iatrogenic trauma, as well as their healing, was proposed (1). In this mid-gestational pregnant rabbit model, after the evaluation of feasibility of fetoscopy and the creation of fetal membrane defects, different closure techniques have been proposed (2-4), and were shown to demonstrate good functional and anatomic membrane sealing rates, and achieve multilayer closure of the fetoscopic access sites (5). Therefore, this animal model appears to be one of the most important experimental models toward a better *in vivo* understanding of fetal membrane repair and for evaluation of surgical closure techniques after iatrogenic trauma, since iatrogenic preterm rupture of fetal membranes still remains a significant drawback after intrauterine surgery, even by the endoscopic approach, although it appears to be minor compared with the open technique (6). Thus, further investigation of effective membrane closure techniques is mandatory for the further development of intrauterine surgery, as well as for the better treatment of spontaneous rupture of the fetal membranes, very well known in clinical practice.

With the progress of tissue engineering in different fields of medicine, new opportunities have been created for intrauterine surgeons towards better tissue healing and reconstruction (7). Concerning fetal membrane healing, two directions have been consolidated: Several workgroups have been active in developing different possible matrix scaffolds (8). On the other hand, we have proposed the use of the *in vitro* culture of amnion cells in order to improve the healing of the fetal membranes after fetoscopy in an *in vivo* model: the mid-gestational rabbit (9). In that study, we demonstrated the feasibility of this animal culture system, the good survival of the cultured amnion cells and their proliferation in the matrix scaffold.

In the present study, we suggest a further development of membrane closure techniques for fetoscopic access sites, evaluating and comparing this new model, where the collagen plug for fetal membrane defect has been used as the matrix

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Table I. *Fetal outcome parameters at 30 days of gestational age.*

| Treatment group | Survival | | Amniotic integrity | | Amniotic fluid presence | |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % |
| Negative control (n=78) | 78/78 | 100† | 78/78 | 100† | 75/78 | 96.2† |
| Positive control (n=18) | 14/18 | 77.8 | 0/14 | 0 | 0/14 | 0 |
| Collagen plug (group I) (n=19) | 19/19 | 100† | 6/19 | 31.6* | 5/19 | 26.3* |
| Collagen plug with amnion cells (group II) (n=20) | 20/20 | 100† | 16/20 | 80*† | 13/20 | 65*† |
| Collagen foil with amnion cells (group III) (n=20) | 19/20 | 95† | 11/19 | 57.9*† | 10/19 | 52.6*† |
| Fibrin glue with amnion cells (group IV) (n=10) | 5/10 | 50 | 0/5 | 0 | 0/5 | 0 |

**Versus* negative controls: $p<0.001$, †*versus* positive controls: $p<0.05$ (Fisher exact two-tailed test).

scaffold for *in vitro* cultured amnion cells, with other possible matrices known from our previous series, including fibrin glue and collagen foil, and the best surgical closure technique from our first series the conventional collagen plug.

## Materials and Methods

*The in vitro model: amnion cell isolation and culture.* Allogeneic preterm amnion cells were isolated under sterile conditions, 2 to 3 weeks prior to surgery after amnion membrane biopsies from a mid-gestational pregnant rabbit. Fetal amnion membrane was harvested under aseptic conditions and was minced into small pieces after animal euthanasia. The details of our isolation technique have been reported elsewhere (9).

Seven to ten days prior to surgery the isolated amnion cells were 90% confluent and were removed. Cells were counted and vitality was determined by trypan-blue staining. Afterwards, the cells were re-suspended, and $1\times10^6$ cells in 1 ml keratinocyte-medium were injected into each collagen matrix scaffold (TissuFleece®, TissuFoil E® or Tissucol Duo S 2 ml Immuno®; Baxter Deutschland GmbH, Unterschleissheim, Germany), which was used for plugging the fetoscopic access site. Cells were infiltratingly injected using different injection sites to guarantee a regular spread of cells. Drained-off cell suspension was seeded on the top of both collagen matrix scaffolds. After 20 minutes of incubation, as has been reported elsewhere in detail (9), the dish was filled with 3-4 ml keratinocyte-medium as far as the top of the sponge or/and the foil was covered with medium. Then the collagen matrices, loaded with the cells, were incubated at 37˚C and 5% $CO_2$, up to the time of implantation.

*The in vivo model: operative procedure and matrix application.* Thirty-three timed pregnant New Zealand rabbits were housed in a quiet area and at normal room temperature two days prior to surgery. At approximately 23 days gestational age, the first operation was performed as described previously elsewhere (2, 5, 9).

Fetoscopy was performed with a short, 1.2 mm, 10,000-pixel, 0˚ fibre endoscope (Karl Storz, Tüttlingen, Germany) housed within a 14-gauge needle. Up to a maximum of one in two amniotic sacs were randomly assigned to four study groups, according to the closure technique and/or the matrix scaffold that was to be tested (n=87), excluding the gestational sacs above the cervix. The others served as positive (n=18), as well as negative controls (n=78; Table I).

After the withdrawal of the fetoscope, the access site was closed according to the assigned study group: In study group I (n=19) the fetoscopic access site was sealed with a conventional collagen plug (TissuFleece®; Baxter Deutschland GmbH, Unterschleissheim, Germany) and 0.3 ml fibrin glue (Tissucol Duo S 2 ml Immuno®; Baxter Deutschland GmbH) (5). In group II (n=20) the collagen plug was loaded with *in vitro* cultured allogeneic amnion cells prior to surgery, and positioned into the amnion and chorion membrane defect, then covered with fibrin glue in the same way as in group I (9). In group III (n=20), the amnion and chorion membrane defect was covered with a collagen foil (TissuFoil E®; Baxter Deutschland GmbH), which was seeded with the *in vitro* cultured allogeneic amnion cells, and sealed also with fibrin glue as the other groups. Finally in group IV (n=10), fibrin glue (Tissucol Duo S 2 ml Immuno®) was used not only as a sealant but also as matrix for the cultured amnion cells.

In all study groups, the myometrial layer was closed micro-surgically with a nylon 6/0 suture (Prolene®; Ethicon GmbH, Norderstedt, Germany) (Figure 1) (5, 9). Eighteen sacs were left unclosed (positive controls), and the 78 non-operated sacs served as negative controls. After repositioning of the uterus, the abdomen was closed in layers using polygalactin 3/0 (Vicryl®; Ethicon GmbH) for the fascia and intracutaneous nylon 2/0 suture (Ethilon®; Ethicon GmbH) for the skin. Postoperative uterine relaxation was achieved administering 4.5 mg/kg medroxyprogesterone-acetate *i.m.* (150 mg Depo-Clinovir®; Pharmacia & Upjohn GmbH, Erlangen, Germany). The animals were housed for the next 8 days under the same conditions as those prior to surgery.

At 30 days gestational age the pregnant rabbits were euthanized with 60 mg/kg pentobarbital (Narcoren®; Rhone-Merieux, Laupheim, Germany) injected intravenously, to undergo a second-look laparotomy, as has been described elsewhere (2, 5, 9). Main outcome measures were fetal survival, integrity of the amniotic membranes, and the presence of amniotic fluid. The living fetuses, which died shortly after the mother due to pentobarbital, were weighed (fetal body weight, FBW) and dissected to assess the wet fetal lung weight (FLW) and to calculate the fetal lung-to-body weight ratio (FLBWR). Macerated stillborn fetuses were noted as non-surviving and not further included in the statistical processing. Furthermore, because of the obvious adverse effects of the fibrin glue as a matrix scaffold on fetal survival and amnion integrity, the performance of this group was interrupted after the 10th amnion sac was used, and not included further in the study.

Table II. *Comparison of fetal lung to body weight ratio according to integrity of amnion.*

| | Negative control group with intact membranes (n=78) | Treatment groups, sacs with intact membranes (n=33) | Treatment groups, sacs with ruptured membranes (n=39) |
|---|---|---|---|
| Body weight (g)† | 33.1±8.7 | 32.5±7.0 | 30.1±7.8 |
| Lung weight (g)† | 1.1±0.3 | 1.1±0.3 | 1.0±0.3 |
| Fetal lung to body weight ratio | 0.034±0.005 | 0.034±0.005 | 0.033±0.006 |

Values are means±SD. No significant differences between any groups were found by ANOVA (LSD). †Stillborn fetuses not included.

All animals were treated in accordance to the current guidelines on animal welfare and the experiments were approved by the Ethical Committee for Animal Experimentation of the District Government of Upper Bavaria (AZ: 209.1/211-2531-73/04). Statistics were carried out using the Chi-square Fisher exact two-tailed test for nominal variables and ANOVA for continuous variables using SPSS 12.0 for Windows software package (SPSS Inc., Chicago, IL, USA).

## Results

Good survival of the cultured amnion cell was confirmed in the collagen matrix scaffold, as has been demonstrated previously elsewhere (9), as well as increased cellularity, survival and proliferation (Figure 2). No adverse effects on animal pregnancy and the fetuses was documented, after the utilization of the *in vitro* cultured amnion cells in collagen matrix scaffolds; fetal survival was similarly high in the collagen groups, compared to the positive control group ($p<0.05$) (Table I).

Complete integrity of the amniotic membranes of non-operated sacs near term was demonstrated (100%), statistically better than in the other study groups ($p<0.05$ and $p<0.001$) (Table I). In contrast, in the amniotic sacs after fetoscopy and without any attempt to close the membranes (*i.e.* the positive control group) 7 days later, no healing of the amniotic membranes was observed. All used techniques, as well as matrix scaffolds, improved amniotic integrity rate; a statistical improvement was shown when study groups II and III were compared to the positive control group at second-look operation ($p<0.05$) (Table I). Finally, the use of collagen plug as a matrix scaffold for the *in vitro* cultured amnion cells improved the integrity of fetal membranes to 80% (study group II), better than any other study group.

The FLBWR of non-manipulated sacs with intact membranes and treated gestational sacs of surviving fetuses (positive control and study groups: n=72) with intact and ruptured membranes were also compared (Table II). FBW tended to be higher in the negative control group (33.1±8.7 g) and study groups (32.5±7.0 g) with membrane integrity compared to the study groups with ruptured membranes (30.1±7.8 g), but no statistical significance was demonstrated. FLW, as well as FLBWR, showed no statistically significant differences between the different groups, however they were similarly higher in the negative controls and the study groups with intact membranes than in fetuses from treated gestational sacs without membrane integrity.

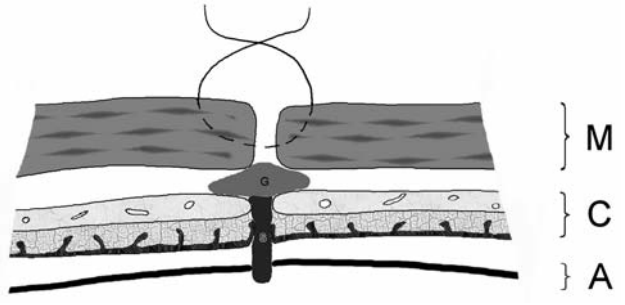


Figure 1. *Schematic presentation of fetal membrane closure technique. M, Myometrium; C, chorion; A, amnion; G, fibrin glue (Tissucol Duo S 2 ml Immuno®); S, depending upon the study group, collagen plug (TissuFleece®) seeded or not, collagen foil (TissuFoil E®) or fibrin glue (Tissucol Duo S 2 ml Immuno®).*

As has been previously reported, histological examination of the access sites confirmed indirectly the anatomic repair of the membranes, since no entrapment of fetal membranes was found in the myometrial wound (9).

## Discussion

Intrauterine surgery is gaining more and more acceptance as a realistic alternative surgical approach for the treatment of life threatening malformations, as well as recently of non life-threatening anomalies such as spina bifida (10). Moreover, recent advances in fetal fetoscopic interventions have allowed a safer intrauterine treatment of the fetus. However, although the iatrogenic uterine and membrane trauma seems to be less than when the open technique is performed, preterm membrane disruption, amniotic fluid leakage, and oligohydramnios remain some of the main drawbacks for extending the indication for of this kind of surgery. Therefore, such interventions are still only performed in a small number

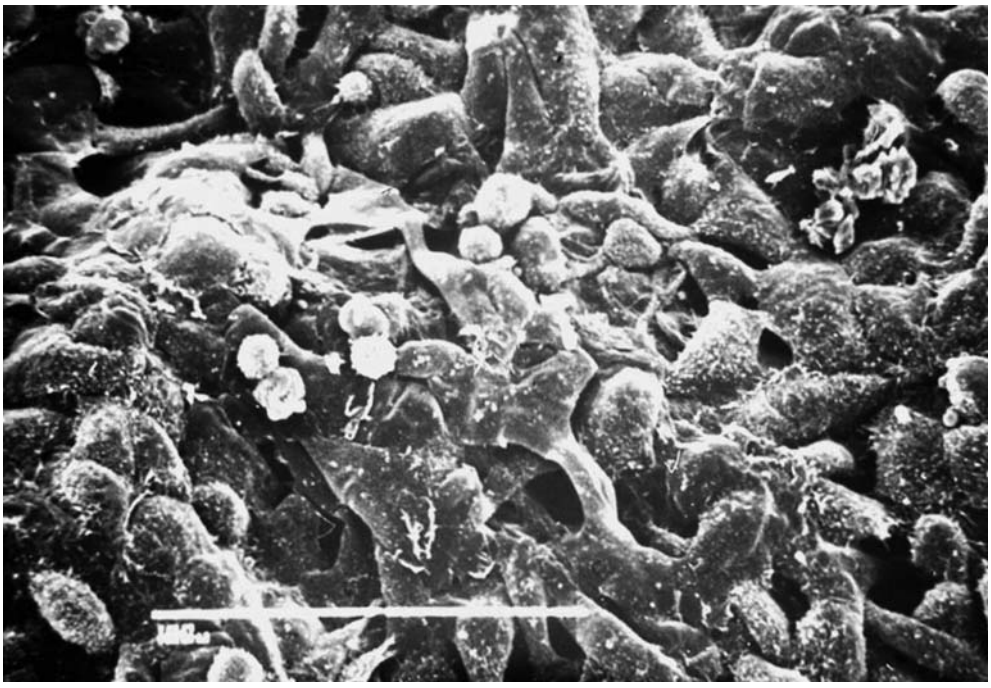

Figure 2. *Increased cellularity and proliferation of the seeded amnion cells on the scaffold can be seen. Scanning electron microscopy; bar=100 μm.*

of centers worldwide, and only for malformations that meet the criteria that International Fetal Medicine and Surgery Society (IFMSS) have established (11).

The prospects for intrauterine treatment of non life-threatening malformations are still unclear and will depend upon if and when the well-known adverse effects of these procedures can be overcome. The animal model presented here was proposed as the first *in vivo* model in order to study fetal membrane healing after iatrogenic trauma of the amniotic sac under experimental conditions, proportional to that of fetoscopy in humans (1). Nowadays, this model appears to be very significant for such studies, and will certainly contribute considerably more towards the further development of intrauterine surgery (1-5, 9, 12-14).

However there are arguments as to whether the rabbit model is appropriate for these kinds of studies. Criticism is focused on the small size of the model and its ability to simulate the environment of the human embryo, and its short gestation period, which provides a shorter postoperative intrauterine time. As alternatives, sheep and monkey models have been proposed. Our opinion is that research should move forward to a larger animal model but only after satisfactory results in the rabbit model are achieved.

It has been postulated that besides the immediate sealing of permeable fetal membranes by scaffolds glued on the leakage side, healing of the fetal membrane defect should be attained by outgrowth of implanted cells and ingrowth of cells of the microenvironment (15). Further, amnion cells are of fetal origin and may hold the potential for regenerative cell therapy (16, 17). Consequently, as an additional option in this model, we introduced the *in vitro* culture of amnion cells and their utilization on a collagen matrix scaffold which could be used as a plug. Despite good survival and localized proliferation of the amnion cells observed in the collagen matrix scaffold, no clear statistically better closure of fetoscopy-induced permanent membrane defects was seen under the described conditions. Therefore, the present study broadens the application of fetal tissue engineering beyond life-threatening anomalies by applying *in vitro* cultured amnion cells in different matrix scaffolds, well-known for their clinical or experimental utilization in the surgical closure of uterine and membrane iatrogenic trauma. However, the use of fibrin glue as a matrix scaffold was not successful, unlike its successful usage with keratinocytes for the reconstitution of epidermis (18), taking advantage at the same time of the sealant effect, as well as its usefulness as a scaffold. Therefore, we intend to revaluate its use in a further series under different and improved conditions. Finally, the plugging of the fetal membranes with collagen matrix scaffold, seeded or injected with *in vitro* cultured amnion cells, and sealed with fibrin glue, as well as myometrial closure with sutures following fetoscopy was found to be reproducible and, as anticipated, still achieved the best results, (9). In our opinion, this method using

materials that can be easily found commercially is still the most promising and has already proven its efficacy in combination with amnion cell engineering *in vivo*. Firstly, like the conventional plug technique (group I), this method is also applicable to percutaneous interventions, and secondly, intensive research is being performed to gain more experience and understanding of the biology of the amnion cells and their healing process, as well as to establish the ideal composition of implantable matrix scaffolds for each desired application. The ideal matrix scaffold would permit maximal cell adherence and growth without compromising tensile elasticity and strength. It is probable that these desired characteristics would necessitate a composite construct consisting of a mixture of different cell types and scaffolds (13, 19-22). Additionally, matrix scaffold composition may affect cell differentiation *in vivo* and *vice versa* (23, 24). Finally, attention needs to be paid to the learning curve of such new techniques and further studies are needed (14).

For these reasons, further investigation is essential, not only to explore possible postoperative complications using these new surgical methods for the sealing of the iatrogenic rupture of membranes, but also to develop further the possibilities that are given to us nowadays by tissue engineering, always bearing in mind ethical considerations (25), in order to obtain safe and outstanding surgical results (13). Only under these conditions, and with a more thorough understanding of pathophysiology and natural history of fetal malformations, will it eventually be possible to clinically help the human fetus in a wider manner, and therefore in cases of non life-threatening malformations too (10, 26).

## Conclusion

The data presented in this study suggest that amnion cells are a very promising and practical medium for tissue engineering, either injected or seeded on a scaffold. In this effort, the rabbit model seems to be invaluable. In our view, when satisfactory results are obtained on the rabbit model, research should move forward gradually to a larger scale model, such as the sheep and monkey, in order not only to find the ideal material for fetal membrane repair in humans, but also to prove that it is long lasting.

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## Disclosure Statement

No financial conflict of interest to declare.

## References

1 Papadopulos NA, Van Ballaer PP, Ordonez JL, Laermans I, Vandenberghe K, Lerut T and Deprest JA: Fetal membrane closure techniques after hysteroamniotomy in the midgestational rabbit model. Am J Obstet Gynaecol *178*: 938-942, 1998.

2 Papadopulos NA, Dumitrascu I, Ordonez JL, Decaluwe H, Lerut T, Barki G and Deprest JA: Fetoscopy in the pregnant rabbit at mid-gestation. Fet Diagn Ther *14*: 118-121, 1999.

3 Gratacós E, Yamamoto H, Papadopulos NA, Adriaenssens T, Philips T, Lerut TE and Deprest JA: The mid-gestational rabbit as a model for the creation of membrane defects following needle fetoscopy. Am J Obstet Gynecol *180*: 1263-1267, 1999.

4 Deprest JA, Papadopulos NA, Decaluwé H, Yamamoto H, Lerut TE and Gratacós E: Closure techniques for fetoscopic access sites in the rabbit at midgestation. Human Reprod *14*: 1730-1734, 1999.

5 Papadopulos NA, Klotz S, Raith A, Henke J, Kloeppel M, Brill T, Müller D, Kovacs L, Erhardt W and Biemer E: Surgical closure methods for fetoscopic created membrane defects in the midgestational rabbit model. Geburtsh Frauenheilkd *63*: 651-657, 2003 (in German).

6 Papadopulos NA, Deprest JA, Dumitrascu I, Miserez M, Yesildaglar N, Vandenberghe K and Lerut TE: Endoscopic fetal surgery: a new perspective in fetal therapy? Sozialpädiatr Kinder Jugendheilkd *22*: 14-22, 2000 (in German).

7 Fuchs JR, Kaviani A, Oh JT, LaVan D, Udagawa T, Jennings RW, Wilson JM and Fauza DO: Diaphragmatic reconstruction with autologous tendon engineered from mesenchymal amniocytes. J Pediatr Surg *39*: 834-838, 2004.

8 Bilic G, Hall H, Bittermann AG, Zammeretti P, Burkhart T, Ochsenbein- Kölble N and Zimmermann R: Human preterm amnion cells cultured in 3-dimensional collagen I and fibrin matrices for tissue engineering purposes. Am J Obstet Gynecol *193*: 1724-1732, 2005.

9 Papadopulos NA, Klotz S, Raith A, Foehn M, Schillinger U, Henke J, Kovacs L, Horch RE and Biemer E: Amnion cell engineering: A new perspective in fetal membrane healing after intrauterine surgery? Fet Diagn Ther *21*: 494-500, 2006.

10 Fichter A, Dornseifer U, Henke J, Schneider KTM, Kovacs L, Biemer E, Bruner J, Adzick NS, Harrison MR and Papadopulos NA: Fetal spina bifida repair, current trends and prospects of intrauterine neurosurgery. Fet Diagn Ther *23(4)*: 271-286, 2008.

11 Harrison MR: Professional considerations in fetal treatment. *In*: The Unborn Patient. 2nd Ed. Harrison MR, Golbus MS and Filly RA (eds.). Philadelphia, PA, USA: WB Saunders, pp. 8-13, 1991.

12 Ochsenbein-Kölble N, Jani J, Lewi L, Verbist G, Vercruysse L, Portmann-Lanz B, Marquardt K, Zimmerman R and Deprest J: Enhancing sealing of fetal membrane defects using tissue-engineered native amniotic scaffolds in the rabbit model. Am J Obstet Gynecol *196(3)*: 264-267, 2007.
13 Mallik AS, Fichter MA, Rieder S, Bilic G, Stergioula S, Henke J, Schneider KTM, Kurmanavicius J and Papadopulos NA: Fetoscopic closure of punctured fetal membranes with acellular human amnion plugs in a rabbit model. Obstet Gynecol *110(5)*: 1121-1129, 2007.
14 Zisch AH and Zimmermann R: Bioengineering of foetal membrane repair. Swiss Med Wkly *138(41-42)*: 596-601, 2008.
15 Ochsenbein-Kölble N, Bilic G, Hall H, Huch R and Zimmermann R: Inducing proliferation of human amnion epithelial and mesenchymal cells for prospective engineering of membrane repair. J Perinat Med *31*: 287-294, 2003.
16 Portmann-Lanz CB, Ochsenbein-Kölble N, Marquardt K, Lüthi U, Zisch A and Zimmermann R: Manufacture of a cell-free amnion matrix scaffold that supports amnion cell outgrowth *in vitro*. Placenta *28*: 6-13, 2007.
17 Kaviani A, Guleserian K, Perry TE, Jennings RW, Ziegler MM and Fauza DO: Fetal tissue engineering from amniotic fluid. J Am Coll Surg *196*: 592-597, 2003.
18 Horch RE, Bannasch H, Kopp J, Andree C and Stark GB: Single-cell suspensions of cultured keratinocytes in fibrin-glue reconstitute the epidermis. Cell Transplant *7*: 309-317, 1998.
19 Niknejad H, Peirovi H, Jorjani M, Ahmadiani A, Ghanavi J and Seifalian AM: Properties of the amniotic membrane for potential use in tissue engineering. Eur Cell Mater *15*: 88-99, 2008.
20 Ilancheran S, Moodley Y and Manuelpillai U: Human fetal membranes: A source of stem cells for tissue regeneration and repair? Placenta *30(1)*: 2-10, 2009.
21 Liekens D, Liesbeth L, Jani J, Heyns L, Poliard E, Verbist G, Ochsenbein-Kölble N, Hoylaerts M and Deprest J: Enrichment of collagen plugs with platelets and amniotic fluid cells increases cell proliferation in sealed iatrogenic membrane defects in the fetal rabbit. Prenat Diagn *28*: 503-507, 2008.
22 Lewi L, Liekens D, Heyns L, Poliard E, Beutels E, Deprest J and Hoylaerts MF: *In vitro* evaluation of the ability of platelet-rich plasma to seal an iatrogenic fetal membrane defect. Prenat Diagn *29*: 620-625, 2009.
23 Langer R and Vacanti JP: Tissue engineering. Science *260*: 920-926, 1993.
24 Freed LE, Martin I and Vunjak-Novakovic G: Frontiers in tissue engineering. *In vitro* modulation of chondrogenesis. Clin Orthop *367(Suppl)*: 46-58, 1999.
25 Horch RE, Pepescu LM, Vacanti C and Maio G: Ethical issues in cellular and molecular medicine and tissue engineering. J Cell Mol Med *12(5B)*: 1785-1793, 2008.
26 Papadopulos NA, Papadopoulos MA, Kovacs L, Zeilhofer HF, Henke J, Boettcher P and Biemer E: Fetal surgery and cleft lip and palate: Current status and new perspectives. Br J Plast Surg *58*: 593-607, 2005.

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