

Original Article

Evaluation of lymphangiogenesis in acellular dermal matrix

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ABSTRACT

Introduction: Much attention has been directed towards understanding the phenomena of angiogenesis and lymphangiogenesis in wound healing. Thanks to the manifold dermal substitute available nowadays, wound treatment has improved greatly. Many studies have been published about angiogenesis and cell invasion in INTEGRA®. On the other hand, the development of the lymphatic network in acellular dermal matrix (ADM) is a more obscure matter. In this article, we aim to characterize the different phases of host cell invasion in ADM. Special attention was given to lymphangiogenic aspects. **Materials and Methods:** Among 57 rats selected to analyse the role of ADM in lymphangiogenesis, we created four groups. We performed an excision procedure on both thighs of these rats: On the left one we did not perform any action except repairing the borders of the wound; while on the right one we used INTEGRA® implant. The excision biopsy was performed at four different times: First group after 7 days, second after 14 days, third after 21 days and fourth after 28 days. For our microscopic evaluation, we used the classical staining technique of haematoxylin and eosin and a semi-quantitative method in order to evaluate cellularity counts. To assess angiogenesis and lymphangiogenesis development we employed PROX-1 Ab and CD31/PECAM for immunohistochemical analysis. **Results:** We found remarkable wound contraction in defects that healed by secondary intention while minor wound contraction was observed in defects treated with ADM. At day 7, optical microscopy revealed a more plentiful cellularity in the granulation tissue compared with the dermal regeneration matrix. The immunohistochemical process highlighted vascular and lymphatic cells in both groups. After 14 days a high grade of fibrosis was noticeable in the non-treated group. At day 21, both lymphatic and vascular endothelial cells were better developed in the group with a dermal matrix application. At day 28, lymphatic endothelial cells had organized themselves, engineering the pseudocylindrical structure better disposed in the ADM group than in the control group, and the lymphatic cells were detectable inside the vessels' lumen in this group. **Conclusion:** This study has made it possible to demonstrate the absolute importance of an ADM in proper wound healing and has shown better definition of both the qualitative and quantitative aspects of lymphangiogenesis compared to the second intention healing. A major grade of organization of the extracellular matrix and a minor grade of fibrosclerosis in ADM allowed a well-structured morphologic and functional development of the endothelial and lymphatic vascular structures. This study hopes

to represent a clinical basis for a wider use of ADM in lesions where lymphatic complications are common.

KEY WORDS

Acellular dermal matrix; INTEGRA®; lymphangiogenesis

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INTRODUCTION

Acellular dermal matrices (ADMs) such as INTEGRA® are usually employed for abdominal, breast,^[1-4] head^[5,6] and neck reconstruction, genital^[7] and in post burn injuries,^[4,8,9] ulcers^[10], cancer^[5,11,12] and post trauma^[13] surgery. The clinical importance of these skin substitutes derives from the fact that they are resistant and pliable and can be rapidly integrated into the surrounding tissues.^[14-18] The biological benefits of INTEGRA®; INTEGRA® are manifold. Their incorporation in tissue is thought to improve revascularization of the matrix, invasion of adjacent host-derived cells and their transition into specific tissue based on the local microenvironment.^[14]

Despite the fact that others have studied^[19,20] the histological aspects of INTEGRA® explants in various settings and the characterization of host cell invasion into ADM, the development of the lymphatic network has not been described in the literature. A broader understanding of lymphangiogenesis into INTEGRA® may better define the current use in reconstructive surgery.^[16,17,21] Given the potential usefulness of ADM, we wish to characterize lymphatic invasion into the matrix at defined time intervals. We have used a model in which INTEGRA® was implanted in the lateral rat thighs. The tissue was harvested at 7, 14, 21 and 28 days for subsequent histologic analysis. We analysed the wound contraction in ADM compared with secondary intention wound healing and characterized host cell invasion using cell type specific immunohistochemistry: Anti-PECAM for vascular angiogenetic characterization and anti-PROX1 for lymphangiogenetic definement.

Based on observations made from our initial histological analysis, we hypothesized that INTEGRA® is capable of supporting lymphangiogenesis.

MATERIALS AND METHODS

After the consent for the execution of this study by Local and National Animal Ethical Committee we selected 57 Sprague-Dawley murine models. We removed two 1.5 cm² full thickness tissue lozenges on the lateral side of both rat thighs. As previously described in other studies,^[22,23] we opted for thigh model in order to have symmetric results and to easily show the changes during the period of evaluation. The left thigh defect healed by second

intention. The right one was covered by INTEGRA®, an ADM comprised of cross-linked bovine tendon collagen and glycosaminoglycan (GAG) broadly used as skin replacement in chronic and traumatic, pressure, diabetic, chronic wounds, and vascular ulcers.^[12,16,24-29]

Among the 60 rats, we formed four excision biopsy groups so divided:

- 15 rats: Biopsy after 7 days
- 15 rats: Biopsy after 14 days
- 15 rats: Biopsy after 21 days
- 15 rats: Biopsy after 28 days.

After general anaesthesia with (ketamine cloridate [80-100 mg/kg] + Xilazine cloridrate [10 mg/kg]), bilateral trichotomy of lateral side of the thigh, disinfection with chlorhexidine 0.5%, we proceeded with a bilateral incision until the fascial plane; we made curettage of the fascial plane until obtaining only a light and homogeneous bleeding. Accordingly, an artificial dermal matrix sheet of 1.5 cm was laid down on the right side and fixed with metallic clips. Both wounds were treated with non-adherent dressing and Elizabethan collar was positioned. During the post-operative period, we checked the wounds, and we cleansed the areas with saline every 72 h.

We performed excisional biopsy, after chlorhexidine 0.5% disinfection, and we took off the metallic clips and the silicon layer; a skin and subcutaneous tissue incision with 2 mm of healthy tissue was performed by removing a square of tissue including neodermis on the right-hand side and granulation tissue on the left. At the end of the procedure, the laboratory animals were killed by intravenous injection of the lethal dose of KCl.

In order to attest INTEGRA®'s capability of supporting lymphangiogenesis, we have performed histological staining with haematoxylin and eosine and immunohistochemical analysis with PROX-1 Ab^[30-35] for endothelial lymphatic cells and CD31/PECAM^[36-38] for endothelial hematic cells.

In order to evaluate the amount of cellularity in the tissue implanted with ADM and a semi-quantitative analysis was used.

To maintain an objective evaluation, and to measure wound areas and contraction, we completed a semi-quantitative analysis at days 7, 14, 21 and 28.

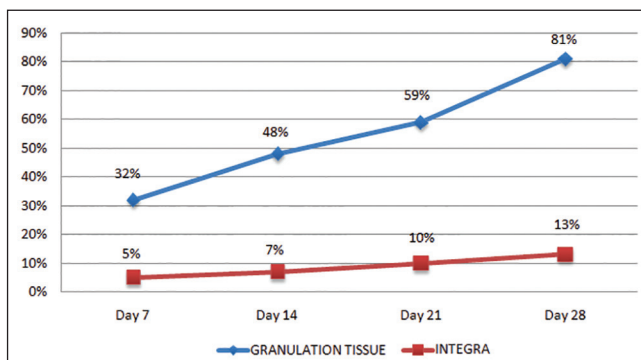
RESULTS

Macroscopically a considerable contraction in wounds healed by second intention (30% at day 7 and 50% at day 14; 60% at day 21 and 80% at day 28) was observed; however the contraction of defects covered with dermal matrix (approximately 5% at day 7, 7% at day 14; 10% at day 14, 13% at day 28) was more limited Graphic 1 and Figure 1.

Microscopic results and lymphangiogenesis

Results at 7 days

Optical microscopy showed a more plentiful cellularity in granulation tissue compared to the regeneration dermal matrix as demonstrated with a semi-quantitative cell counting of 100× zoom images. We divided the sections into different zones, and we discovered a higher quantity of cells in or in the area of the muscular fascia to the superficial skin. Examination of the tissue sections at 7 and 14 days post-implantation revealed a directly proportional correlation of the cellular density with implantation time. Our semi-quantitative analysis of average cell quantity showed a 4-fold increase in granulation tissue at 14 days. Also, within the INTEGRA® there was a 1.5-fold increase at 14 days. It had been



Graphic 1: Graphic resuming the difference in contraction between INTEGRA (approximately 5% at day 7, 7% at day 14; 10% at day 14, 13% at day 28) and control group (32% at day 7 and 48% at day 14; 59% at day 21 and 81% at day 28)

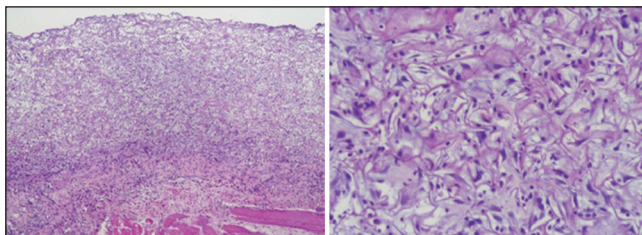


Figure 2: Microscopic results with ×200 (left) immunohistochemical process with PECAM and ×1100 (right) with PROX-1 in INTEGRA® dermal matrix at day 21. Notice, marked with red circle, a vascular outline formed by lymphatic vascular cells

invaded by phagocytes (neutrophils and macrophages), which had immigrated through the three-dimensional scaffold. The cellular component followed a gradient rising from the wound's bed to matrix the surface.

This immunohistochemical process [Figures 2a, b and 3] highlighted a vascular endothelial cellular proliferation (PECAM +) located, above all, at the borders and at the bed of the lesion and uncommon lymphatic vascular cells (PROX-1 +) both in the granulation tissue and dermal matrix (INTEGRA®) [Figures 4-6].

Results at 14 days

Fourteen days after implantation, it was possible to observe the degradation of dermal matrix collagen by phagocytes. The granulation tissue showed a notable fibrosis. Confluent endothelial lymphatic cells (PROX-1 +) were visible both in granulation tissue and dermal matrix [Figures 7 and 8].

Results at 21 days

The number of vascular (PECAM +) and lymphatic (PROX-1 +) endothelial cells grew in both tissues but in INTEGRA® dermal matrix, they formed better structured lymphatic and vascular outlines; in granulation tissue there was an evident and remarkable fibrosclerotic reaction. Semi-quantitative analysis of PROX-1 + cells

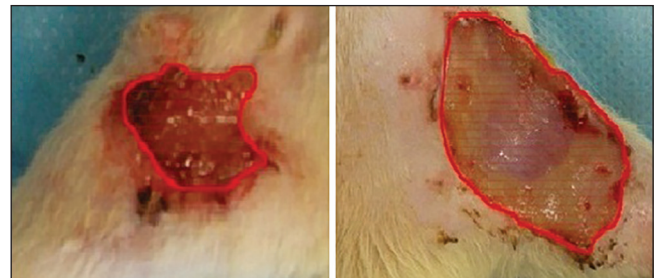


Figure 1: Left side: Macroscopic check in second intention healing of thigh wound at day 28. Notice the remarkable contraction of the granulation tissue and the initial reepithelisation. Right side: Macroscopic check in defect after application of artificial dermal matrix (INTEGRA®) at day 28. Notice the lack of wound contraction and the more acceptable appearance of the wound

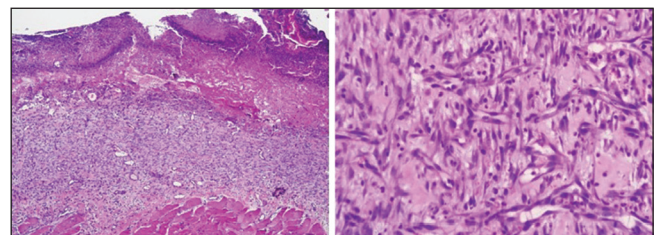


Figure 3: Microscopic results in ×200 (left) immunohistochemical process with PECAM and ×1100 (right) with PROX-1 in non-treated tissue at day 21. Notice the lower plenty and loss of organization of lymphatic vascular cells and the abundant fibrosclerosis of tissue

suggested a quantitative improvement in lymphatic cells in INTEGRA® implanted group [Figures 2 and 3].

Results at 28 days

Blood and lymphatic vessels appeared better structured and organised in the regeneration dermal matrix than the vessels in granulation tissue. Lymphatic vascular structures (PROX-1 +) with lymphocytes were detectable inside the lumen of vessels [Figures 9 and 10].

DISCUSSION

The biointegration of an ADM follows different phases, similar to the phases we find in normal wound

healing: An inflammatory phase, fibroblast migration, neovascularization, remodelling and maturation.^[39,40] In the inflammatory phase — the matrix is invaded by phagocytes that migrate across the matrix; in the phase of fibroblast migration — macrophages, leucocytes and fibroblasts migrate from wound borders through collagen and GAG network and dispose themselves in the three-dimensional scaffold; the neovascularization phase — demonstrable with anti-PECAM antibodies that show neoangiogenesis with a well-structured organization on hematic vessels. In the remodelling and maturation phase is observable completion of the matrix degradation and a neosynthesis of collagen by the fibroblasts. Neovascularization in INTEGRA® has been proved by several studies;^[41-43] in this study we aim to prove that an ADM as INTEGRA® is capable of supporting

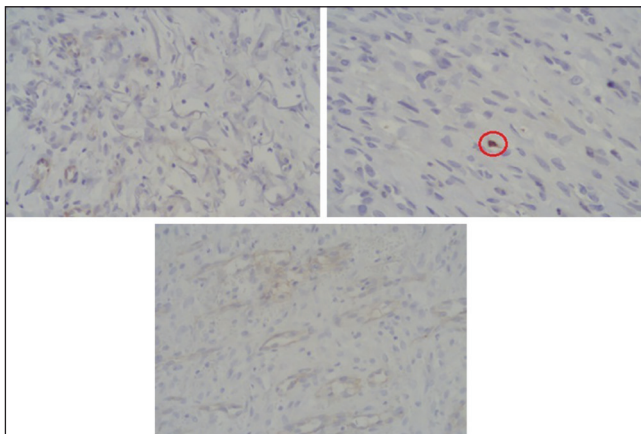


Figure 4: ×40 (left) and ×100 (right) H and E staining in tissue treated with artificial dermal matrix at day 7. Notice the massive infiltration of neutrophils and macrophages in characterization of inflammatory phase

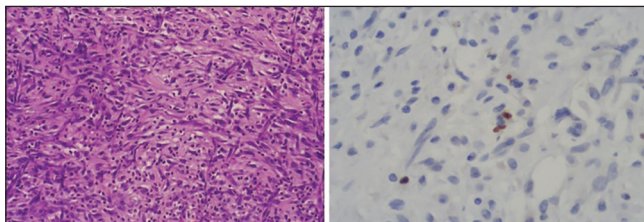


Figure 6: ×100 (left and right) H and E, ×1100 (centre) immunohistochemical process with PECAM and PROX-1 at day 7. Notice (remarked with a red circle) an endothelial lymphatic cell. In Figure 7a and b, that depict artificial dermal matrix, more vascular endothelial cells are detectable

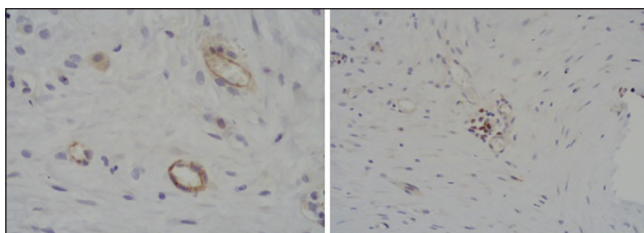


Figure 8: ×100 (left) and H and E, ×1100 (right) immunohistochemical process with PROX-1 in granulation tissue at day 14. Notice the scarce number of PROX-1 + cells in regard to Figure 5 and an initial fibrosis of the tissue

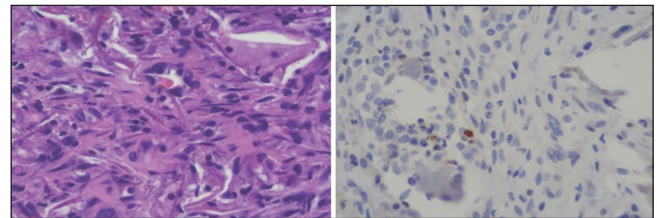


Figure 5: ×40 (left) and ×100 (right) H and E staining in granulation tissue at day 7. Notice the massive infiltration of neutrophils and macrophages in characterization of inflammatory phase and the initial reepithelisation

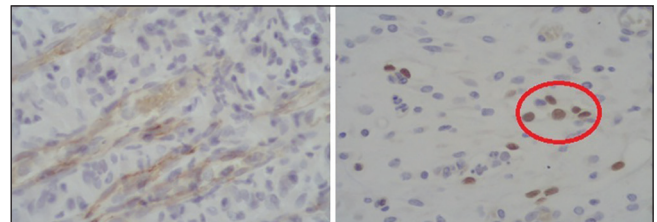


Figure 7: ×200 (left) H and E, ×1100 (right) immunohistochemical process with PROX-1 in tissue treated with artificial dermal matrix at day 14. Notice the abundant number of PROX-1 + cells

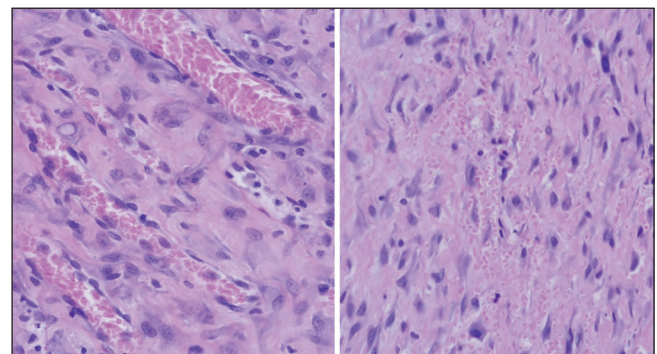


Figure 9: Comparison between artificial dermal matrix (left) and granulation tissue (right) in ×200 microscopic results with H and E staining. Notice the superiority of dermal matrix case in organisation, number and structure of blood and lymphatic vessels

lymphangiogenesis. Only few studies try to connect wound healing and lymphangiogenesis issues.^[44-46] Low lymphatic vessels presence seems to be an important factor in the impairment of diabetic ulcers wound healing.^[47] Moreover, lymphatic vessels are responsible for the maintenance cells equilibrium and normal wound healing. The main role of the lymphatic vessels is the control of the interstitial microcirculation. The lymphatic vessels remove macromolecules and particulate matter too large to reenter the blood capillaries from the extra vascular space. . If these materials are not removed, the osmotic and hydrostatic forces within the tissues change and disease results. Failure of the lymphatic function leads to pollution of the tissues because of the excesses of protein, other macromolecules, and fluid around the cells, resulting in impaired wound healing.^[48]

This study make an effort to demonstrate the role of the artificial dermal matrix (INTEGRA®) in the proper wound healing^[49,50] and underline the better structured pattern of lymphangiogenesis between the two groups considered.^[22] From the 1st week of observation, a minor wound contraction in tissue treated with INTEGRA® implant was evident. A morphological examination of tissue specimen revealed that the response to the ADM was similar during wound healing, involving mostly inflammatory cells in the 1st day, and afterwards, in the mesenchymal cells. The quantitative analysis indicated that the total number of cells within the ADM increased time following a gradient rising from the wound bed to matrix the surface. Our microscopic investigation emphasized the low fibrosclerosis in ADM compared with the granulation tissue in which we observed a

copious fibrosis. We found a major grade of organisation of extracellular matrix and a minor presence of fibrosclerosis in INTEGRA®; furthermore, we observed histological evidence of lymphatic vessels formation within ADM and confirmed this finding by using immunohistochemistry for the lymphatic endothelial cell marker PROX-1. Lymphatic endothelial cells initially diffused inside the thickness of dermal matrix and organized themselves constructing a pseudocylindrical structure better organized both qualitatively and quantitatively compared to granulation tissue. A well-structured morphological and functional development in vascular and lymphatic endothelial structures was observed [Figure 11]. This could give an explication about the low morbidity of the integrate site for seroma formation reported in our experiences as well in the literature. A more structured lymphatic vessels formation could be significant for a more functional lymphatic activity in the new tissue. However, our observation is not enough to understand the functional, so clinical studies still be needed to test the functionality of lymphatic channelling within the ADM.

CONCLUSION

This study made possible the demonstration of the absolute importance of an ADM in proper wound healing^[55] and in the better characterization of the qualitative and quantitative aspects of lymphangiogenesis compared to healing by secondary intention. By using this simple animal model, it was possible to prove that INTEGRA supports the formation of lymphatic structures. Our

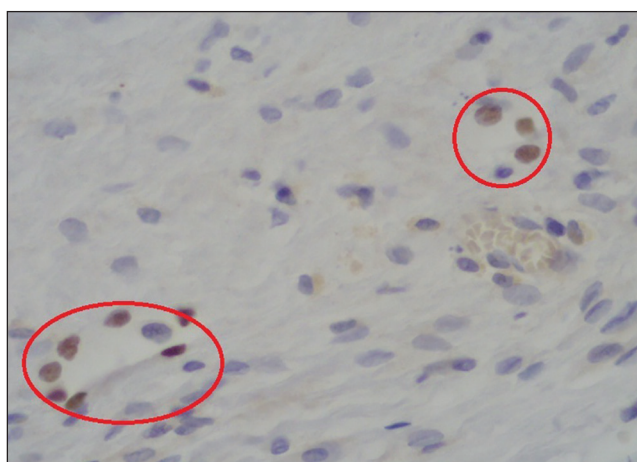


Figure 10: Lymphatic vascular structure with lymphocytes inside vessels lumen highlight by $\times 1400$ immunohistochemical process with PROX-1 at day 28 in tissue treated with INTEGRA®

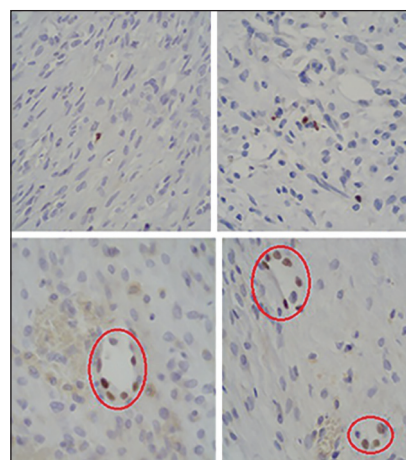


Figure 11: Immunohistochemical process with PROX-1 on tissue treated with dermal matrix (INTEGRA®) underlines an increase of amount of lymphatic endothelial cells and the formation of well-organized cylindric vascular structure (red circles in right-centre and right image). Left (day 7), left-centre (day 14), right-centre (day 21), right (day 28)

observations outline a rational theoretical basis toward which a broader clinical use of skin substitutes in lesions when the risk of lymphedema would compromise both morphological and functional outcomes.^[51-54] Although further studies are necessary in order to better evaluate the development of the lymphatic network in more evolved models, such as porcine or human models, this study represents a rational start in our understanding of the reasons why ADMs have so many useful applications in plastic and reconstructive surgery.

REFERENCES

- Goshtasby PH, Chami RG, Johnson RM. A novel approach to the management of pyoderma gangrenosum complicating reduction mammoplasty. *Aesthet Surg J* 2010;30:186-93.
- Aquilina D, Darmanin FX, Briffa J, Gatt D. Chest wall reconstruction using an omental flap and Integra. *J Plast Reconstr Aesthet Surg* 2009;62:e200-2.
- Palao R, Gómez P, Huguet P. Burned breast reconstructive surgery with Integra dermal regeneration template. *Br J Plast Surg* 2003;56:252-9.
- Tsoutsos D, Stratigos A, Gravvanis A, Zapandioti P, Kakagia D. Burned breast reconstruction by expanded artificial dermal substitute. *J Burn Care Res* 2007;28:530-2.
- Khan MA, Ali SN, Farid M, Pancholi M, Rayatt S, Yap LH. Use of dermal regeneration template (Integra) for reconstruction of full-thickness complex oncologic scalp defects. *J Craniofac Surg* 2010;21:905-9.
- Burd A, Wong PS. One-stage Integra reconstruction in head and neck defects. *J Plast Reconstr Aesthet Surg* 2010;63:404-9.
- Valdatta L, Maggiulli F, Scamoni S, Pellegatta I, Cherubino M. Reconstructive management of degloving trauma of male external genitalia using dermal regeneration template: A case report. *J Plast Reconstr Aesthet Surg* 2014;67:264-6.
- Dantzer E, Queruel P, Salinier L, Palmier B, Quinot JF. Integra, a new surgical alternative for the treatment of massive burns. Clinical evaluation of acute and reconstructive surgery: 39 cases. *Ann Chir Plast Esthet* 2001;46:173-89.
- Lee LF, Porch JV, Spenler W, Garner WL. Integra in lower extremity reconstruction after burn injury. *Plast Reconstr Surg* 2008;121:1256-62.
- Rindler R, Garcia C. Letter: The use of a dermal substitute and thin skin grafts in the cure of "complex" leg ulcers. *Dermatol Surg* 2010;36:426.
- Abai B, Thayer D, Glat PM. The use of a dermal regeneration template (Integra) for acute resurfacing and reconstruction of defects created by excision of giant hairy nevi. *Plast Reconstr Surg* 2004;114:162-8.
- Chalmers RL, Smock E, Geh JL. Experience of Integra® in cancer reconstructive surgery. *J Plast Reconstr Aesthet Surg* 2010;63:2081-90.
- Cherubino M, Scamoni S, Pellegatta I, Maggiulli F, Minuti A, Valdatta L. Massive de-gloving thigh injury treated by vacuum therapy, dermal regeneration matrix and lipografting. *Afr J Paediatr Surg* 2013;10:386-9.
- Moiemen NS, Vlachou E, Staiano JJ, Thawy Y, Frame JD. Reconstructive surgery with Integra dermal regeneration template: Histologic study, clinical evaluation, and current practice. *Plast Reconstr Surg* 2006;117 7 Suppl:160S-74.
- Unglaub F, Ulrich D, Pallua N. Reconstructive surgery using an artificial dermis (Integra): Results with 19 grafts. *Zentralbl Chir* 2005;130:157-61.
- Haertsch P. Reconstructive surgery using an artificial dermis (Integra). *Br J Plast Surg* 2002;55:362-3.
- Moiemen NS, Staiano JJ, Ojeh NO, Thway Y, Frame JD. Reconstructive surgery with a dermal regeneration template: Clinical and histologic study. *Plast Reconstr Surg* 2001;108:93-103.
- Xie WG, Tan H, Zhao CL, Wang H. The histological changes and the revascularization process in the grafted dermal substitutes. *Zhonghua Shao Shang Za Zhi* 2005;21:37-9.
- Moiemen NS, Vlachou E, Staiano JJ, Thawy Y, Frame JD. Reconstructive surgery with Integra dermal regeneration template: Histologic study, clinical evaluation, and current practice. *Plast Reconstr Surg* 2006;117 7 Suppl:160S-74.
- Kim KE, Sung HK, Koh GY. Lymphatic development in mouse small intestine. *Dev Dyn* 2007;236:2020-5.
- Zhou Q, Wood R, Schwarz EM, Wang YJ, Xing L. Near-infrared lymphatic imaging demonstrates the dynamics of lymph flow and lymphangiogenesis during the acute versus chronic phases of arthritis in mice. *Arthritis Rheum* 2010;62:1881-9.
- Wong AK, Schonmeyer B, Singh P, Carlson DL, Li S, Mehrara BJ. Histologic analysis of angiogenesis and lymphangiogenesis in acellular human dermis. *Plast Reconstr Surg* 2008;121:1144-52.
- Cross SE, Naylor IL, Coleman RA, Teo TC. An experimental model to investigate the dynamics of wound contraction. *Br J Plast Surg* 1995;48:189-97.
- Dorsett-Martin WA. Rat models of skin wound healing: A review. *Wound Repair Regen* 2004;12:591-9.
- Herlin C, Louhaem D, Bigorre M, Dimeglio A, Captier G. Use of Integra in a paediatric upper extremity degloving injury. *J Hand Surg Eur Vol* 2007;32:179-84.
- Chalmers RL, Smock E, Geh JL. Experience of Integra® in cancer reconstructive surgery. *J Plast Reconstr Aesthet Surg* 2010;63:2081-90.
- Popescu S, Ghetu N, Grosu O, Nastasa M, Pieptu D. Integra — A therapeutic alternative in reconstructive surgery. Our first experience. *Chirurgia (Bucur)* 2007;102:197-204.
- Dantzer E, Braye FM. Reconstructive surgery using an artificial dermis (Integra): Results with 39 grafts. *Br J Plast Surg* 2001;54:659-64.
- Wolter TP, Noah EM, Pallua N. The use of Integra in an upper extremity avulsion injury. *Br J Plast Surg* 2005;58:416-8.
- Gröger M, Loewe R, Holthöner W, Embacher R, Pillinger M, Herron GS, *et al.* IL-3 induces expression of lymphatic markers Prox-1 and podoplanin in human endothelial cells. *J Immunol* 2004;173:7161-9.
- Castro EC, Galambos C. Prox-1 and VEGFR3 antibodies are superior to D2-40 in identifying endothelial cells of lymphatic malformations — A proposal of a new immunohistochemical panel to differentiate lymphatic from other vascular malformations. *Pediatr Dev Pathol* 2009;12:187-94.
- McGovern S, Pan J, Oliver G, Cutz E, Yeger H. The role of hypoxia and neurogenic genes (Mash-1 and Prox-1) in the developmental programming and maturation of pulmonary neuroendocrine cells in fetal mouse lung. *Lab Invest* 2010;90:180-95.
- Petrova TV, Mäkinen T, Mäkelä TP, Saarela J, Virtanen I, Ferrell RE, *et al.* Lymphatic endothelial reprogramming of vascular endothelial cells by the Prox-1 homeobox transcription factor. *EMBO J* 2002;21:4593-9.
- Rodriguez-Niedenführ M, Papoutsis M, Christ B, Nicolaides KH, von Kaisenberg CS, Tomarev SI, *et al.* Prox1 is a marker of ectodermal placodes, endodermal compartments, lymphatic

- endothelium and lymphangioblasts. *Anat Embryol (Berl)* 2001;204:399-406.
35. Wilting J, Papoutsi M, Christ B, Nicolaidis KH, von Kaisenberg CS, Borges J, *et al.* The transcription factor Prox1 is a marker for lymphatic endothelial cells in normal and diseased human tissues. *FASEB J* 2002;16:1271-3.
 36. Park S, DiMaio TA, Scheef EA, Sorenson CM, Sheibani N. PECAM-1 regulates proangiogenic properties of endothelial cells through modulation of cell-cell and cell-matrix interactions. *Am J Physiol Cell Physiol* 2010;299:C1468-84.
 37. DiMaio TA, Sheibani N. PECAM-1 isoform-specific functions in PECAM-1-deficient brain microvascular endothelial cells. *Microvasc Res* 2008;75:188-201.
 38. Woodfin A, Voisin MB, Nourshargh S. PECAM-1: A multi-functional molecule in inflammation and vascular biology. *Arterioscler Thromb Vasc Biol* 2007;27:2514-23.
 39. Campitiello E, Della Corte A, Fattopace A, D'Acunzi D, Canonico S. The use of artificial dermis in the treatment of chronic and acute wounds: Regeneration of dermis and wound healing. *Acta Biomed* 2005;76 Suppl 1:69-71.
 40. Cole PD, Stal D, Sharabi SE, Hicks J, Hollier LH Jr. A comparative, long-term assessment of four soft tissue substitutes. *Aesthet Surg J* 2011;31:674-81.
 41. Upadhyaya M, Orford JE, Smith N, Barker A, Gollow I. Incorporation of Integra in tissue defects: A pilot study in the rat model. *Pediatr Surg Int* 2007;23:669-73.
 42. Greenwood J, Amjadi M, Dearman B, Mackie I. Real-time demonstration of split skin graft inosculation and Integra dermal matrix neovascularization using confocal laser scanning microscopy. *Eplasty* 2009;9:e33.
 43. Xie WG, Tan H, Zhao CL, Wang H. The histological changes and the revascularization process in the grafted dermal substitutes. *Zhonghua Shao Shang Za Zhi* 2005;21:37-9.
 44. Shimamura K, Nakatani T, Ueda A, Sugama J, Okuwa M. Relationship between lymphangiogenesis and exudates during the wound-healing process of mouse skin full-thickness wound. *Wound Repair Regen* 2009;17:598-605.
 45. Ji RC, Miura M, Qu P, Kato S. Expression of VEGFR-3 and 5'-nase in regenerating lymphatic vessels of the cutaneous wound healing. *Microsc Res Tech* 2004;64:279-86.
 46. Paavonen K, Puolakkainen P, Jussila L, Jahkola T, Alitalo K. Vascular endothelial growth factor receptor-3 in lymphangiogenesis in wound healing. *Am J Pathol* 2000;156:1499-504.
 47. Maruyama K, Asai J, Li M, Thorne T, Losordo DW, D'Amore PA. Decreased macrophage number and activation lead to reduced lymphatic vessel formation and contribute to impaired diabetic wound healing. *Am J Pathol* 2007;170:1178-91.
 48. Mallon EC, Ryan TJ. Lymphedema and wound healing. *Clin Dermatol* 1994;12:89-93.
 49. King WW, Lam PK, Liew CT, Ho WS, Li AK. Evaluation of artificial skin (Integra) in a rodent model. *Burns* 1997;23 Suppl 1: S30-2.
 50. Druecke D, Lamme EN, Hermann S, Pieper J, May PS, Steinau HU, *et al.* Modulation of scar tissue formation using different dermal regeneration templates in the treatment of experimental full-thickness wounds. *Wound Repair Regen* 2004;12:518-27.
 51. Vaillant L, Tauveron V. Primary lymphedema of limbs. *Presse Med* 2010;39:1279-86.
 52. Alitalo K, Carmeliet P. Molecular mechanisms of lymphangiogenesis in health and disease. *Cancer Cell* 2002;1:219-27.
 53. Liersch R, Detmar M. Lymphangiogenesis in development and disease. *Thromb Haemost* 2007;98:304-10.
 54. Ji RC. Characteristics of lymphatic endothelial cells in physiological and pathological conditions. *Histol Histopathol* 2005;20:155-75.
 55. Collier M. The use of advanced biological and tissue-engineered wound products. *Nurs Stand* 2006;21:68, 70, 72.

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Announcement

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<p>50th Annual Conference of the Association of Plastic Surgeons of India. Theme : Advocacy and Mass Education</p> <p>Dates : 28th to 31st December, 2015 Venue : The Renaissance, Mumbai, Hotel & Convention Centre.</p>		
Dr. Prabha Yadav Organizing Chairperson	Dr. Vinita Puri Organizing Secretary	Dr. Medha Bhawe Treasurer
Dr. Utpala Mulawkar Scientific Chairperson		