Porcine acellular dermal graft with and without impermeable dressing to treat extensive wounds*

*Tratamento de feridas cutâneas extensas usando tecido dérmico acelular porcino com e sem cobertura impermeável**

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Abstract: BACKGROUND - The porcine acellular dermal graft is an alternative for the treatment of skin wounds.
OBJECTIVE - To evaluate the clinical and inflammatory response elicited by a porcine derived acellular dermal graft with or without an impermeable dressing.
METHODS - A matched-pair longitudinal study was conducted by creating two skin wounds along the dorsal midline of 16 rats (4 rats/group) with implantation of a porcine acellular dermal graft with or without an impermeable dressing . At the 7th, 15th, 30th and 60th postoperative days the animals were evaluated and sacrificed. The acellular tissue and surrounding tissues were removed for histological and morphometric analysis.
RESULTS - The impermeable dressing persisted on the wounds for up to approximately 15 days. The group without impermeable dressing presented more desiccation, fibrin crust, edema, and inflammatory reaction in the dermis. At the 60th postoperative day, the group without the impermeable dressing still showed ulcerations, epithelium thinning and lack of keratin, whereas in the group with impermeable dressing the skin was already normal.
CONCLUSION - The porcine acellular dermal tissue associated with the impermeable dressing presented better clinical and histological results compared to the porcine acellular dermal tissue without the impermeable dressing in extensive wound healing.
Keywords: Wound healing; Biocompatible materials; Tissue transplantation

Resumo: *FUNDAMENTOS - O tecido dérmico acelular porcino é alternativa para o tratamento de feridas cutâneas.*
OBJETIVO - Avaliar a resposta clínica e inflamatória do implante de tecido dérmico acelular porcino, com e sem cobertura impermeável.
MÉTODOS - Estudo pareado, longitudinal, criando-se duas feridas cutâneas no dorso de 16 ratos (quatro animais/grupo), em que foi implantado tecido dérmico acelular coberto ou não por impermeável. Os animais foram avaliados e sacrificados sete, 15, 30 e 60 dias após a cirurgia, sendo removidos os tecidos acelulares e adjacentes para avaliação histológica e morfométrica.
RESULTADOS - A cobertura impermeável permaneceu sobre o tecido acelular porcino até cerca de 15 dias. O grupo sem impermeável apresentou maior desidratação, com crosta fibrinoleucocitária, edema e reação inflamatória na derme. Sessenta dias após a cirurgia, animais do grupo sem impermeável ainda apresentavam ulcerações, afinamento do epitélio e ausência de queratina, enquanto nos do grupo com impermeável a pele já se encontrava normal.
CONCLUSÃO - O tecido dérmico acelular porcino com cobertura impermeável apresentou resultados clínicos e histológicos melhores do que os do tecido dérmico acelular porcino sem impermeável para tratamento de feridas cutâneas extensas.
Palavras-chave: Cicatrização de feridas; Matérias biocompatíveis; Transplante de tecidos

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INTRODUCTION

When tissue loss is extensive, the healing process may not be enough to cure, and treatment with grafts and flaps should be considered in these cases.[1] Regarding the origin of skin grafts, they may be classified as autologous, homologous or xenogenic, such as those of porcine or bovine origin.[2]

Several procedures for tissue replacement have been introduced with the purpose of reducing scar formation and speeding up healing time.[2]

Acellular dermal tissues are suggested for the treatment of extensive burns,[3,4] and several techniques to induce a more efficient healing are described.[2]

Acellular dermal tissues are obtained by removing cellular components that are present in the tissue, since the cells are responsible for the activation of the immunologic response. The absence of cellular structures results in little inflammation, and prevents rejection of the transplanted tissue.[5]

Because of technical difficulties to obtain acellular tissues and their high costs in Brazil, Dr. Romualdo Rossa developed a porcine acellular dermal tissue (Xenoderma® - Homus Biotecnologia Com. Ind. Ltda) and an impermeable dressing, using locally developed technology.

The objective of this study is to evaluate the clinical and inflammatory response to the implantation of porcine acellular dermal tissue manufactured in Brazil, associated or not with an impermeable dressing for the treatment of extensive skin wounds, using rats as experimental models.

MATERIALS AND METHODS

This was a matched-pair longitudinal study using 16 albino male rats, weighing approximately 200g. The rats were supplied by the Central Vivarium of Faculdade de Medicina de Botucatu (FMB)/UNESP.

Two 1.5x2.5cm wounds were surgically created along the dorsal midline region of each animal. In the upper wound porcine acellular dermal tissue associated with impermeable dressing was implanted (group with impermeable dressing), and in the lower wound only the porcine acellular dermal tissue was implanted (group without impermeable dressing).

The porcine acellular dermal tissue and the impermeable dressing were manufactured and supplied by Homus Biotecnologia Com. Ind. Ltda. - Sao Paulo, Brazil.

During the postoperative period, collagenase ointment (Iruxol® - Knoll Ltda., RJ) was applied twice a day on both wounds, until the day the animals were sacrificed.

Four animals in each group were sacrificed at four experimental time-points: the 7th, 15th, 30th, and 60th postoperative days.

Clinical evaluation - The animals were clinically evaluated regarding the appearance of the skin-graft healing using photodocumentation immediately before the sacrifice.

Anesthesia - Xylazine 10.66mg/kg associated with Ketamine 66mg/kg, intramuscular, was applied in the animals' lower limb.

Surgical procedure - The midline dorsal region was shaved with a razor blade, and then antisepsis was performed, followed by placement of a fenestrated surgical drape exposing the surgical site. Skin excision was then performed, creating two rectangular 1.5x2.5cm surgical wounds equal in size, one on the animal's upper dorsal region, and the other on the lower dorsal region, using a number 15 surgical blade and scissors. The porcine acellular dermal tissue and the impermeable dressing were placed on the upper wound, whereas the lower wound was only covered with the acellular tissue. The skin graft was sutured by direct approximation using a 5-0 nylon suture, in separate stitches. The impermeable dressing was also sutured to the skin with 5-0 nylon suture in separate stitches, fully covering the acellular tissue. All the procedures were performed by Dr. Hoyama, keeping constant the size of the grafts and of the impermeable dressing, and always following the same antisepsis and asepsis procedures.

Histology analysis - The specimens on the implantation site were removed and immersed in 10% formol, and stained with hematoxylin-eosin (HE), and examined by the same pathologist (Dr. Marques), who was blinded for the experimental time-point of each animal.

Morphometric analysis - The histological material was analyzed by image capture system, evaluating the thickness of the epidermis and dermis. The analysis of variance was used to perform the statistical analysis of the values obtained, considering the two-factor model for repeated measures complemented by the multiple comparison test.[6] The discussion was performed at a 5% significance level.

RESULTS

Clinical evaluation

The impermeable dressing was present in all animals at the seventh day, but it was absent in most of the animals at the 15th postoperative day, with the exception of two animals.

Clinically, at the 15th postoperative day, the group with impermeable dressing had a better appearance, that is, the wounds were less desiccated

and had less blood crusts around the margins. The healing area was also smaller in the group with impermeable dressing than in the group without impermeable dressing at the 30th postoperative day, keeping similar until the 60th day, when complete healing was observed in one animal in the group of acellular dermal tissue covered with impermeable dressing (Figure 1).

Histology analysis

An ulcerated area with cellular inflammatory infiltrate covered with blood crust and fibrin was observed in both groups at the 7th postoperative day. The inflammatory infiltrate decreased progressively during the study in both groups, with less inflammatory reaction in the group with impermeable dressing. At the 60th postoperative day, the group with impermeable dressing presented with normal epithelium, whereas the individuals in the group without impermeable dressing still presented with some ulcerated areas, decreased epithelial thickness and lack of keratin (Figures 2 and 3).

Morphometric analysis

The thickness of the epidermal and dermal layers in the animals receiving the acellular dermal tissue with impermeable dressing increased until the 15th postoperative day to gradually decrease from then on. No alteration in the thickness of the epidermis or the dermis was observed in the group without impermeable dressing throughout the study. The group with impermeable dressing presented a thicker epidermis and dermis than the group with impermeable dressing, both at the 7th and the 15th postoperative days (Tables 1 and 2).

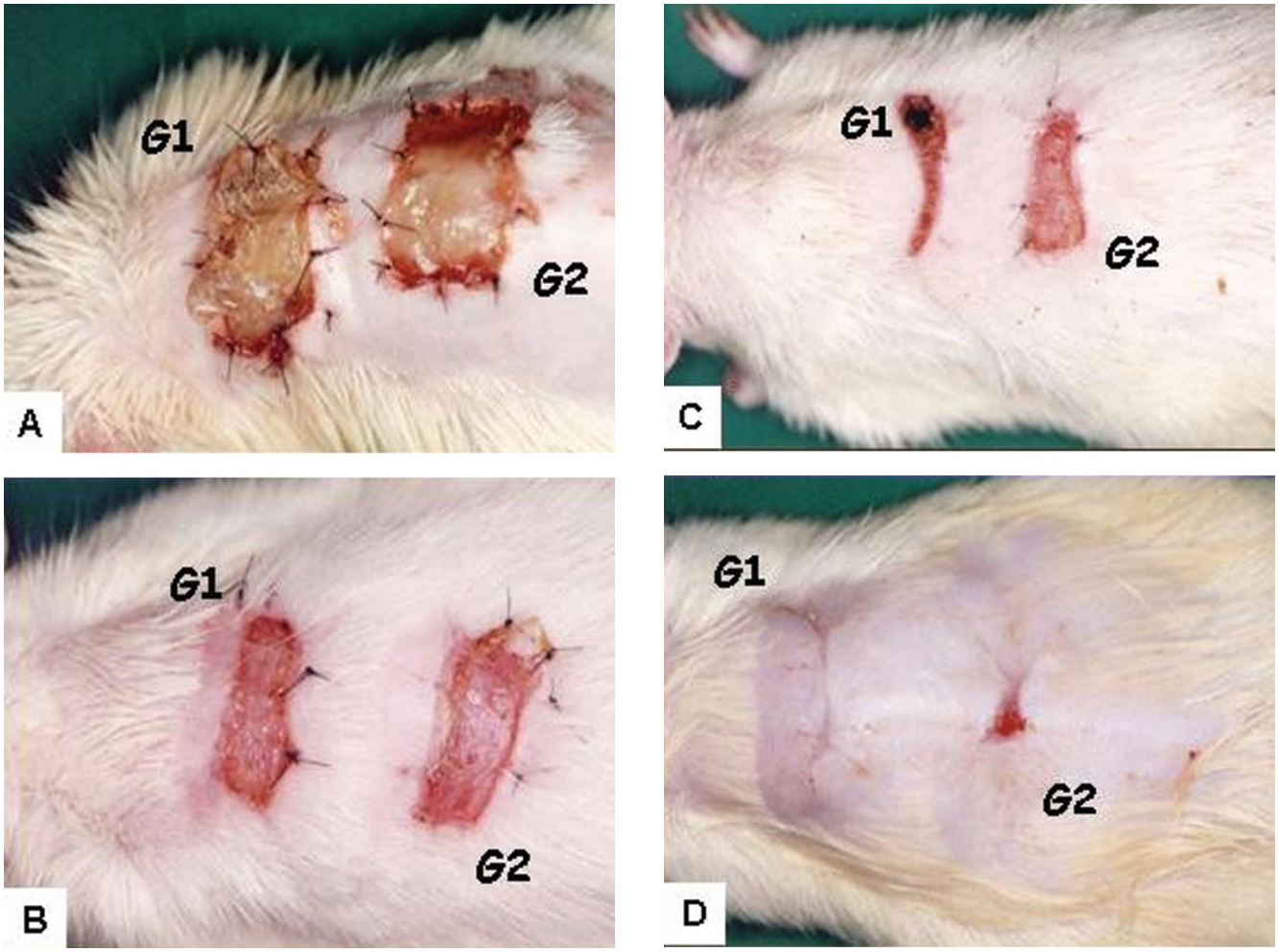

Figure 1: Clinical appearance of the animals at the 7th (A), 15th (B), 30th(C) and 60th(D) postoperative days. Observe superior healing pattern of the wounds treated with the porcine acellular dermal tissue associated with impermeable dressing (G1) compared to that of the animals which did not receive impermeable dressing (G2), in which an ulcerated area can still be observed at the 60th postoperative day.

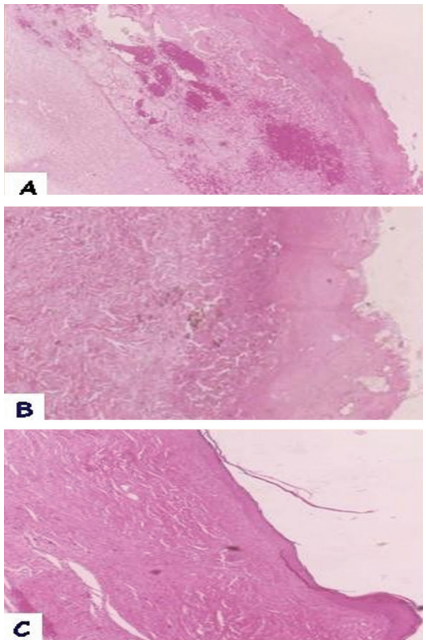

FIGURE 2: Photomicrographs of the histological exams of G1 animals at the 7th (A), 15th (B), and 60th (C) postoperative days

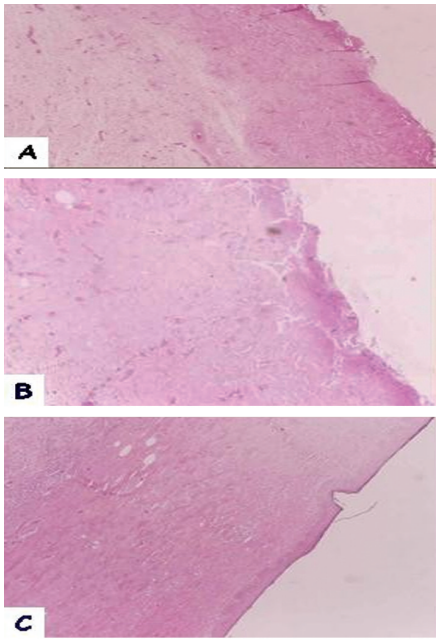

FIGURE 3: Photomicrographs of the histological exams of G2 animals at the 7th (A), 15th (B), and 60th (C) postoperative days. At the 60th postoperative day, G1 animals had normal epithelium, unlike G2 animals, which still had a thinner epithelium and lack of keratin.

DISCUSSION

Of all animals, the domestic pig was described as the most appropriate model for all types of dermatologic and surgical investigations, since human and porcine skins have remarkable similarities.[7,8] This fact, in addition to the difficulty in obtaining human tissue in our country, made us choose the use of porcine acellular dermal tissue in our study.

In a previous pilot study using only porcine acellular dermal tissue for the treatment of extensive wounds in rats, a significant desiccation of the graft was observed, with its early loss. Thus, the utilization of an impermeable dressing was thought to be necessary to prevent water loss from the acellular dermal tissue to the external environment.

One of the major effects of occlusive materials is to prevent desiccation of the wound surface. When crust formation and desiccation occur, the superficial portion of the dermis also desiccates and is incorporated into the supradjacent crust. The more intense the dermal desiccation, the deeper and thicker is the crust formation, and the longer it takes for the reepithelialization process to occur.[4]

TABLE 1: Descriptive measures of the epidermal layer thickness and statistical test results

Material	Experimental time-point				Time-point statistical result
	7 days	15 days	30 days	60 days	
Acellular tissue + impermeable	361.2aB (247.9-555.8)	531.5bC (440.1-627.8)	50.3aA (30.9-259.7)	65.7aA (52.6-114.9)	15(P<0.005)
Acellular tissue	267.7aA* (219.5-428.0)	260.8 aA (43.8-471)	102.0 aA (50.1-249.7)	119.9 aA (98.8-151.4)	7.1(P>0.05)
Material statistical	0.7(P>0.05)	2(P<0.05)	0.7(P>0.05)	1.8(P>0.05)	

*Lower case letters compare materials, that is, column variables.
Capital letters compare time-points, that is, line variables.

TABLE 2: Descriptive measures of dermal layer thickness and statistical test results

Material	Experimental time point				Time point statistical result
	7 days	15 days	30 days	60 days	
Acellular tissue + impermeable	1797.3bB* (1576.1-1803.6)	1666.4bB (1352.6-2226.6)	596aA (94.4-1320.3)	739.2aA (506.9-960)	14.7(P<0.005)
Acellular tissue	362.8aA (268.3-583.5)	316.8aA (98.3-474.5)	367.5aA (307.4-840.7)	584.9aA (308.3-723.7)	4.9(P>0.05)
Material statistical result	2(P<0.05)	2(P<0.05)	0.9(P>0.05)	0.9(P>0.05)	

*Lower caser letters compare materials, that is, column variables.
Capital letters compare time-points, that is, line variables.
Two different letters mean statistical difference.

Collagenase ointment was used to decrease crust formation and necrosis of the collagenous tissue surrounding the implants, thus providing better conditions for the healing process.

The present study showed the incorporation of the porcine acellular dermal tissue into the rat's dermal tissue, with mild to moderate inflammatory host response.

Early research with acellular dermal tissues was based on the theory that, if an appropriate matrix existed, the normal cells of the normal adjacent tissue would migrate towards the matrix, attaching themselves to the site and performing their functions as if they were in normal tissues.[9] Further studies corroborated these expectations, showing the presence of phenotypically normal fibroblasts, collagen and elastin with normal maturity and orientation, active keratinocytes with uniform distribution of hemidesmosomes and of anchoring fibrils in the basal membrane, neovascularization from preexisting channels and reappearance of immunohistocompatibility complexes (IHC) types I and II indicating that cell repopulation in the acellular dermal graft occurred.[4,5]

The use of impermeable dressing aiming at reducing water loss in the porcine acellular dermal tissue showed positive results, since the wounds in the group with impermeable dressing healed with a better clinical and histological appearance than those in the group without impermeable dressing, in agreement with prior studies reporting increased collagen synthesis, reepithelialization rate, and better cosmetic results when excessive water loss in the wound bed is prevented. In addition, dressed wounds are known to heal faster because the crust formation occurring on the bloody area is prevented or minimized, with subsequent reduction of the inflammatory process and formation of a less apparent scar.[4]

CONCLUSION

The results obtained in the present study supported the use of porcine acellular dermal tissue associated with an impermeable membrane as a satisfactory option for the treatment of full-thickness skin loss. ❑

REFERENCES

1. Pigossi N, Raia A, Lex A, et al. Estudo experimental e clínico sobre o emprego, como implante, da dura-máter homógena conservada em glicerina à temperatura ambiente. Rev Ass Med Brasil. 1971;17:263-80.
2. Ruszczak Z, Schwartz RA. Modern aspects of wound healing: an update. Dermatol Surg. 2000;26:219-29.
3. Wainwright DJ. Use of an acellular allograft dermal matrix (AlloDerm) in the management of full-thickness burns. Burns. 1995;21:243-8.
4. Bennett RG. Dressings and miscellaneous surgical materials. In: Bennett RG, editors. Fundamentals of cutaneous surgery. St Louis: Mosby; 1988. p.310-51.
5. Livesey SA, Herndon DN, Hollyoak MA, Matalas SL, Nelson GS. Transplanted acellular allograft dermal matrix. Transplantation.1995;60:1-9.
6. Zar JH. Biostatistical analysis. New Jersey: Prentice Hall; 1999. 663p.
7. Vardaxis NJ, Brants TA, Boon ME, Kreis RW, Marres LM. Confocal laser scanning microscopy of porcine skin: implications for human wound healing studies. J Anat. 1997;190:601-11.
8. Bernatchez SF, Parks PJ, Grussing DM, et al. Histological characterization of a delayed wound healing model in pig. Wound Rep Reg. 1998;6:223-33.
9. Reichley ML. New skin growth/graft technology: improves prognosis for patients with severe burns. Adv Phys Therap. 1995;1-2.

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