

of scores was made. To illustrate, according to Prinsen *et al.*, the cutoff scores for mildly, moderately, and severely impaired HRQoL on the emotions domain were ≥ 24 , ≥ 35 , and ≥ 39 , respectively, meaning that a patient with a score ≥ 24 can be categorized as having a mildly impaired HRQoL on this domain, a score ≥ 35 as “moderate”, etc. However, Sampogna and Abeni categorized “mild” as having a score between 0 and 23.9 and, as a consequence, misclassified all cutoff scores. Therefore, we would like to provide a correct overview of the categorization of Skindex-29 scores (Table 1).

Having said this, we fully agree with Sampogna and Abeni on the limitations of both methods, such as dependence on the distribution of HRQoL scores in estimation samples and biases when using prospective anchors. Nevertheless, we believe that, under the condition that the same scale or anchor question is being used, anchor-based methods may lead to less variant estimates of cutoff scores than distribution-based methods. In addition, anchor-based methods are less dependent on the sociocultural and clinical characteristics of the estimation sample. For example, patients in one sample, scoring themselves as having a severely impaired HRQoL on a global rating scale or anchor question (for instance, an anchor question such as “In your opinion, how severe is your skin con-

dition?”), are likely to have Skindex-29 scores in the same range of scores as patients of another sample who also score themselves as having a severely impaired HRQoL. Nevertheless, the phrasing of an anchor question is a great source of variation in the comparison of different cutoff scores. We therefore advocate the use of standardized anchors.

A clinically meaningful interpretation of Skindex-29 scores is of great value. At present, two studies on this intriguing subject are available. As already expressed by Sampogna and Abeni, the combination of an anchor-based and a distribution-based method in a subsequent study would allow an objective comparison of the results within one study population. In addition to this, we recommend including standardized anchors, and to conduct such a study on an international level. Eventually, such efforts will contribute to reaching consensus on the categorization of scores so that they can be applied in clinical practice.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Application of an Indoleamine 2,3-Dioxygenase-Expressing Skin Substitute Improves Scar Formation in a Fibrotic Animal Model

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TO THE EDITOR

Any delay in wound closure increases the probability of developing dermal fibrotic conditions such as hypertrophic

scars (Deitch *et al.*, 1983). For patients with extensive burn injuries, one of the most promising approaches is the application of an engineered skin sub-

stitute containing both epidermal and dermal cells (Coulomb *et al.*, 1998). Where allogeneic skin substitutes can provide a rapid, patient-ready wound coverage, they are susceptible to immune rejection. Our approach has been to engineer an allogeneic skin substitute

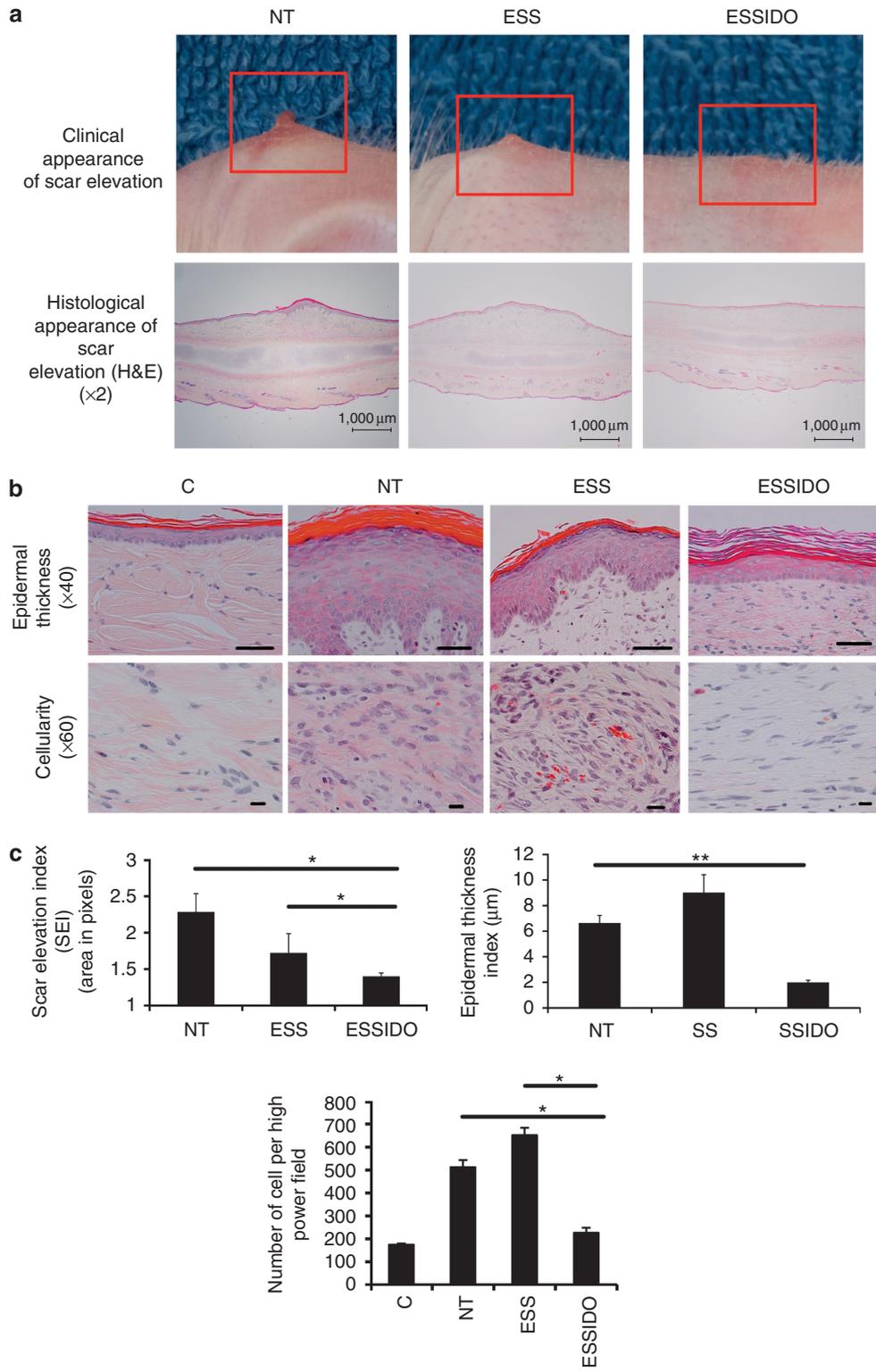


Figure 1. Morphological and histological evaluation of skin substitutes in rabbit ear model. Skin substitutes containing indoleamine 2,3-dioxygenase (IDO)-transduced (ESSIDO) and non-transduced (ESS) fibroblasts were applied on rabbit wounds. (a) Clinical appearance of wounds showed an increased scar elevation in nontreated (NT) and ESS wounds compared with those of ESSIDO wounds, which showed a flatter scar (original magnification × 2 bar = 1,000 μm). (b) Tissue samples were histologically analyzed for epidermal thickness (original magnification × 40 bar = 40 μm) and cellularity (original magnification × 60 bar = 10 μm) using hematoxylin and eosin staining (H&E). Normal rabbit skin was used as control (C). (c) Quantification and statistical analysis was performed for scar elevation index (SEI), epidermal thickness, and cellularity. The results revealed a statistically significant decreased SEI, epidermal thickness, and cellularity in ESSIDO samples compared with those of NT and ESS samples (*P < 0.05 and **P < 0.001).

that expresses an immunomodulating enzyme to protect the graft.

Indoleamine 2,3-dioxygenase (IDO) is the rate-limiting enzyme of tryptophan catabolism that converts tryptophan to kynurenine (Taylor and Feng, 1991), and is well known to have a key role in producing immunoprivileged tissue environments. For example, it has been shown that IDO has a role in the prevention of the immune rejection of the semi-allogeneic fetus (Munn *et al.*, 1998) and in the immune resis-

tance of tumors (Uyttenhove *et al.*, 2003). In our earlier work, we demonstrated that the expression of IDO can not only downregulate the expression of MHC class I in IDO-transduced keratinocytes (Li *et al.*, 2004), but also that it can prevent immune rejection of grafted, simple xenogeneic fibroblast-populated scaffolds (Li *et al.*, 2006). Conversely, depletion of tryptophan by IDO jeopardizes the survival of CD4⁺ lymphocytes and THP-1 monocytes (Ghahary *et al.*, 2004).

In this study, primary dermal fibroblasts were transduced with *IDO* gene using a lentiviral vector (Rezakhanloo *et al.*, 2010). Kynurenine catabolite concentration was found to be 36.5 times higher in conditioned media from IDO-transduced cells compared with controls (14.6 ± 0.103 vs. 0.4 ± 0.007 , $n=4$; $P<0.001$). Bilayered skin substitutes were engineered to have both an epidermal (primary keratinocytes) and dermal layer containing either IDO-transduced fibroblasts (ESSIDO)

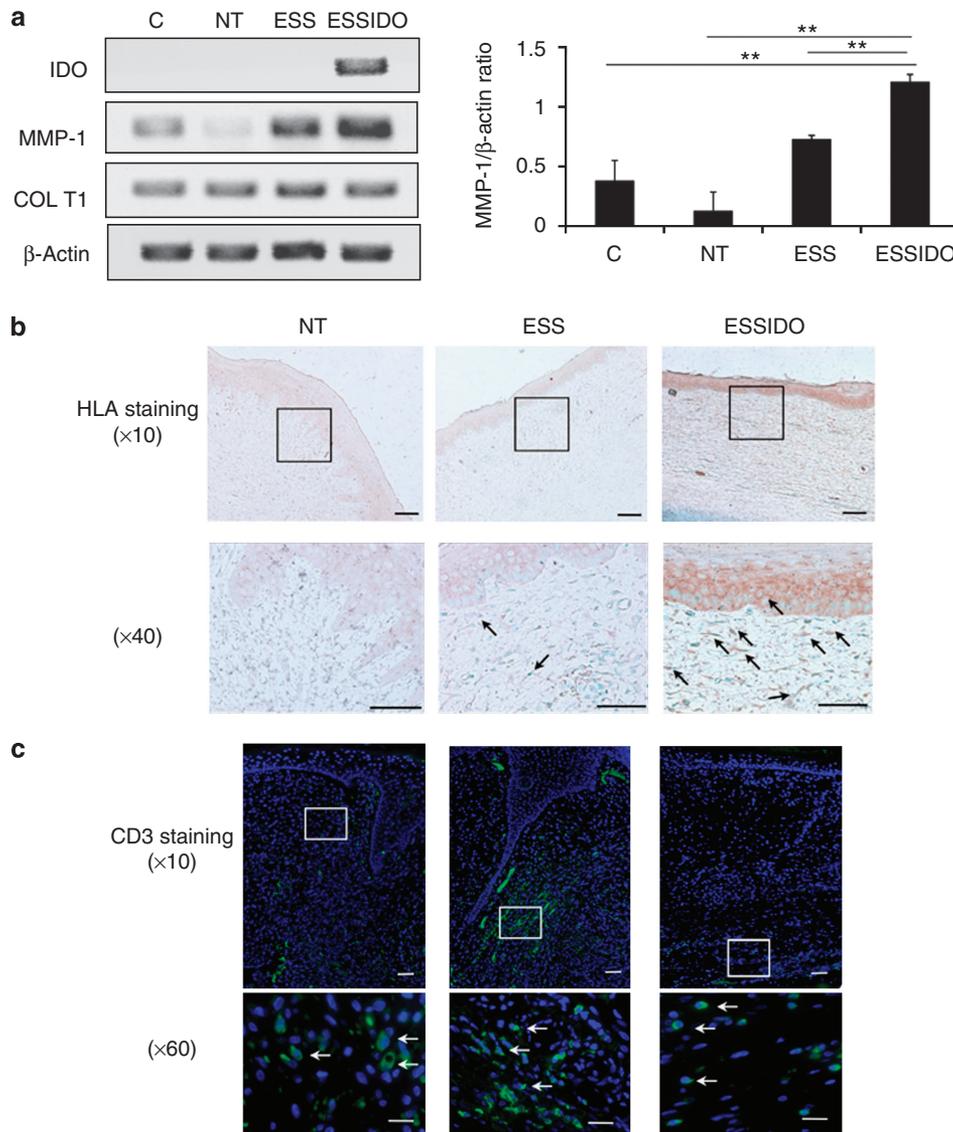


Figure 2. Molecular and histological characteristics of the tissue samples after 35 days post-transplantation. (a) Collagen and MMP-1 gene expression in nontreated (NT), ESS, and ESSIDO tissue samples were evaluated. After 35 days, indoleamine 2,3-dioxygenase (IDO) was only expressed in the tissue samples treated with ESSIDO (first row). A significant increase in MMP-1 expression was observed in ESSIDO-treated samples when compared with those of control, NT, and ESS-treated samples (second row and graph). β -Actin was used as a loading control (fourth row; $**P<0.001$). (b) Tissue sections were stained for HLA-DR (original magnification $\times 10$, bar = 100 μ m; and original magnification $\times 40$, bar = 100 μ m) and (c) CD3 (T-lymphocyte marker; original magnification $\times 10$, bar = 100 μ m; and original magnification $\times 60$, bar = 50 μ m). Only ESSIDO tissue samples showed the presence of HLA-DR; moreover, ESSIDO sections revealed the presence of T cells only in the deep dermis. Arrows show positive cell staining.

or non-transduced fibroblasts (ESS). Using the rabbit ear model (Morris *et al.*, 1997), we created four 8-mm punch wounds on the ventral surface of each ear and either covered it with skin substitutes or left it untreated (non-treated (NT)). Wounds were evaluated over a period of 35 days. Untreated wounds were considered as the control for wound healing progression. Clinical and histological examination of the wounds showed an increase in scar elevation for NT and ESS wounds compared with those of ESSIDO wounds, which showed a flatter scar (Figure 1a). Wounds were analyzed for epidermal thickness and cellularity using hematoxylin and eosin staining. Normal rabbit skin was used as control (C) (Figure 1b top panel). Epidermal thickness was found to be greater in NT and ESS samples compared with that of control and ESSIDO (Figure 1c). In addition, both NT and ESS displayed increased cellularity compared with ESSIDO, which was more comparable to normal skin (Figure 1b lower panel). A decreased scar elevation index was evident in ESSIDO wounds compared with those of NT and ESS samples (1.4 ± 0.04 vs. 2.3 ± 0.25 and 1.72 ± 0.26 , respectively; $n=6$; $P<0.05$). Moreover, ESSIDO wounds showed a thinner epidermal layer when compared with those of ESS and NT wounds (1.99 ± 0.16 vs. 9.01 ± 1.4 and 6.63 ± 0.58 , respectively; $n=6$, $P<0.001$). A significant decrease in cellularity was also found in the ESSIDO wounds compared with either NT or ESS wounds (108.25 ± 5.34 vs. 238.88 ± 24.01 and 164.30 ± 4.68 , respectively; $n=6$, $P<0.001$; Figure 1c). To further elucidate the role that ESSIDO might have in mitigating fibrotic conditions, biopsies were examined for *MMP-1* and *pro- $\alpha 1$ collagen* messenger RNA. At the gene level, we found that *MMP-1* expression in the ESSIDO-treated wounds was significantly higher compared with control, NT, and ESS-treated wounds (1.21 ± 0.06 vs. 0.38 ± 0.16 , 0.13 ± 0.15 , and 0.73 ± 0.03 , respectively; $n=3$, $P<0.001$; Figure 2a). In contrast, there was no significant difference in *pro- $\alpha 1$ collagen* gene expression. Therefore, these data suggest that local IDO expression *in vivo* is

correlated with an increase in *MMP-1*, without changing the production of collagen (*pro- $\alpha 1$*). To ensure that grafted cells were present in the rabbit tissue for 35 days, biopsy sections were stained with a mAb for HLA-DR, revealing the presence of human keratinocytes from the ESSIDO in rabbit tissue but not in those from NT or ESS tissue samples (Figure 2b). In comparison, wound sections were also stained with a CD3 lymphocyte mAb demonstrating a random distribution of T cells throughout the dermis of both NT and ESS. Interestingly, ESSIDO-treated tissue sections were infiltrated with fewer T cells, present only in the deep dermis and as if to form a linear distribution (Figure 2c). These results suggest that the expression of IDO by the ESSIDO creates a barrier-impeding migration of T cells into the transplanted graft.

Using a well-documented rabbit ear fibrotic model, we showed that the application of a skin substitute containing IDO-transduced cells improves scar elevation, epidermal thickness, and cellularity, which are the known characteristics of dermal fibroproliferative disorders. In addition, we demonstrated that IDO-expressing cells significantly stimulated *MMP-1* expression in bystander fibroblasts (Supplementary Figure S1 online). This finding suggests that IDO likely induces *MMP-1* expression in the host fibroblasts. Although it remains to be further elucidated, the mechanism for this response could be partly due to depletion of tryptophan or an increase in IDO metabolites. Again, we are able to demonstrate that IDO is able to protect a multicellular xenograft from rejection, similar to our previous study (Jalili *et al.*, 2010). CD3 staining in the ESSIDO samples revealed the presence of these cells only in the deep dermis, corresponding with our previous *in vitro* findings (Forouzandeh *et al.*, 2008) and suggesting that the IDO may create a niche that restricts T-cell infiltration.

In conclusion, this study describes a possible alternative role for IDO in scar improvement through *MMP-1* stimulation in host cells. Moreover, it demonstrates the survival of an ESSIDO graft for up to 35 days, (Supplementary Figures S2, S3 online) suggesting the possibility of serving as a permanent

wound coverage. Further studies are suggested to address the mechanism through which these phenomena occur.

CONFLICT OF INTEREST

STB is the named inventor on patents and patent applications assigned to the University of Cincinnati and Shriners Hospitals for Children according to their intellectual property policies. Patents, patent applications, and other intellectual property pertaining to engineered skin substitutes are licensed to Cutanogen Corporation, which was founded by STB, and in which he has past and present financial interests. STB resigned as an officer of Cutanogen's current activities. STB also serves currently as a paid consultant to Aderans Research.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/jid>

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Validation of GWAS Loci for Atopic Dermatitis in a Singapore Chinese Population

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TO THE EDITOR

Atopic dermatitis (AD) is a complex trait resulting from an interaction between a large variety of environmental and genetic factors (Irvine and McLean, 2006; Rodríguez et al., 2009; van den Oord and Sheikh, 2009). Research on the filaggrin gene (*FLG*) in Asian populations including Singapore has confirmed that allelic variants in this gene influence risk to AD (Akiyama, 2010), as in Europe, while also strengthening the evidence for other factors involved in the disease etiology (Chen et al., 2011). Recently, Sun et al. (2011) reported a genome-wide association study on AD in which they identified two new susceptibility loci at 5q22.1 (rs7701890) and 20q13.33 (rs6010620), and one suggestive locus at 10q21.2 (rs2393903) in the Chinese population. The previously unreported associations were validated in Northern and Southern Chinese, but only the 20q13.33 (rs6010620) locus was validated in a German population, supporting the presence of ethnic differences or environmental influences on the observed genetic associations.

Here, we analyzed these three loci in 827 AD cases and 1,104 controls (Supplementary Information online), all of which were Singaporean Chinese (Andiappan et al., 2011). All three

single-nucleotide polymorphisms (SNPs) were directly assayed by Taqman genotyping and were found to be in Hardy–Weinberg equilibrium in controls ($P > 0.05$). The chromosome (chr)10 and chr20 SNPs showed significant association in our population (odds ratio (OR) = 1.26 (95% confidence interval (CI) 1.11–1.44), $P = 0.0004$; and OR = 1.18 (95% CI 1.02–1.36), $P = 0.024$, respectively). The effect sizes of these associations are similar to that reported in Sun et al. (Table 1). However, the chr5 SNP rs7701890 showed no evidence of association (OR = 0.96 (95% CI 0.81–1.13), $P = 0.59$). With our current sample size and the minor allele frequency of 20% in Singaporean Chinese, we estimate that we had close to 80% power to detect the effect observed by Sun et al. (OR 1.24; Purcell et al., 2003).

The well-known association of atopy with allergic phenotypes (Bousquet et al., 2001, 2008) prompted us to investigate the association of the three SNPs with positive skin prick test (SPT) results in our cohort. We analyzed a total of 2,627 SPT-positive and 389 SPT-negative individuals, which included both AD cases and controls. There was no association with positive SPT status (Supplementary Table S1 online), and adjustment for this SPT

status had little or no impact on the AD association results for the chr10 and chr20 SNPs. We then tested the three above-mentioned SNPs (rs7701890, rs2393903, and rs6010620) for association with allergic rhinitis (AR; 472 cases) and asthma (323 cases), in the absence of AD. The rs6010620 SNP on 20q13.33 was associated with AR ($P = 0.008$), although further validation of this result is required. Although the 5q22.1 locus has recently been reported to be associated with AR (Ramasamy et al., 2011), the SNP we tested was not in linkage disequilibrium with any of the reported SNPs ($r^2 = 0$ with rs17513503 and rs1898671).

The AD cases were separated into two groups (i) AD only and (ii) AD with other associated atopic conditions (asthma, AR, or both). We found the risk C allele of the chr10 locus showed a significantly stronger association with the group with AD only compared with the group with AD and other atopic conditions (OR = 1.45 (95% CI 1.21–1.79) vs 1.19 (1.03–1.38), $P = 0.038$; Table 1). We then performed a quantitative trait analysis in a subset of 397 AD cases whose disease severities were determined by the SCORing Atopic Dermatitis (SCORAD) index (Oranje et al., 2007). Consistently, we found the CC genotype of rs2393903 at chr10 was significantly associated with a lower SCORAD score (mean 33.99 ± 1.568 ; $n = 94$), compared with

Abbreviations: AD, atopic dermatitis; AR, allergic rhinitis; CI, confidence interval; OR, odds ratio; SCORAD, SCORing Atopic Dermatitis; SNP, single-nucleotide polymorphism; SPT, skin prick test