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Light Microscopic, Electron Microscopic, and Immunohistochemical Comparison of Bama Minipig (*Sus scrofa domestica*) and Human Skin

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Here we sought to evaluate the possibility of using Chinese Bama miniature pig skin as a suitable animal model for human skin. Morphologic features of the skin of Bama miniature pigs resemble those of human skin, including skin layer thickness, development of a superficial vascular system, structure of the dermal–epidermal interface, and extracellular matrix. The characteristics and densities of Langerhans cells, fibroblasts, vascular endothelial cells, and mast cells were similar between Bama pig and human skin. Immunohistochemistry showed that miniature pigs and humans have the same antigenic determinants of human laminin, fibronectin, filaggrin, collagen I, collagen III, collagen IV, and keratin but not CD34, ICAM1, or S100. In addition, collagen type I from Bama miniature pig skin exhibited physicochemical characteristics resembling those of human skin, in regard to HPLC chromatography, UV spectroscopy, amino-acid composition, and SDS-PAGE analysis. Given these results, we concluded that Bama miniature pigs have great potential as a human skin model and for developing dermal substitute materials in wound repair. However, we also observed some disparities between the skin of Bama miniature pigs and humans, including pigment cell distribution, sweat gland types, and others. Therefore, further studies are needed to completely evaluate the effects of these interspecies differences on the actual application of the model.

Information from human skin pharmacologic and transplantation research is crucial for modern pharmaceutical analysis and wound healing applications. Because human skin for research purposes is not readily available, alternatives from mammals, rodents, and reptiles have been widely considered as suitable surrogates. However, the skin of most animals, including rats, mice, guinea pigs, dogs, rabbit, and other nonprimates, shows marked anatomic differences from human skin. In particular, the epidermis of these animals is too thin and the flat epidermal–dermal interface does not have rete ridges and papillary projections. Furthermore, in these animals, dermal structures are relatively loose, and the vascular system is underdeveloped[10] Therefore, the skin of most animals presents a much weaker barrier than does human skin.[1,22,24] Furthermore, reepithelization is a crucial step during wound healing of human, skin but rodent skin heals primarily through contraction.[23] In light of these reasons, the skin of nonhuman primates is a better model for human skin than are those of rodents and other animals. However, research in primates is more restricted than is that in rodents and other animals.

The skin of pigs is composed of an epidermis and dermis with characteristics like those of human skin.[14] The regeneration time of epidermal cells is 30 d for pig compared with 27 to 28 d for human.[26] Unfortunately, standard-size swine have a complex genetic background, great interindividual differences, and rapid growth, which are disadvantages for biomedical research models. However, miniature pigs, such as the Göttingen and Yucatan, with their more manageable size and defined background, are well-recognized animal models for dermal toxicology, transdermal drug delivery, and wound healing.[3,18,21] Bama miniature pigs, a distinct breed of miniature swine in China, is an ideal animal model of humans in many regards.[11,12] Nevertheless, basic research data regarding the skin of Bama miniature pigs are unavailable and its application in biomedicine is limited. In the present experiments, we studied various characteristics of the skin of Bama miniature pigs, including the morphology, ultrastructure, immunohistochemistry, and collagen physicochemical properties, were studied and compared with those of human skin.

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Materials and Methods

Chemicals. Antihuman serum: laminin (from rabbit, 1:500 dilution) was purchased from Sigma Chemicals (St Louis, MO); fibronectin (from mouse, 1:100 dilution), filaggrin (from goat, 1:50 dilution), and ICAM1 (from goat, 1:100 dilution) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); CD34 (from mouse, 1:100 dilution), collagen I (from mouse, 1:100 dilution), collagen III (from mouse, 1:60 dilution), collagen IV (from mouse, 1:50 dilution), and keratin (from mouse, 1:50 dilution) were purchased from Beijing Zhongshan Goldengridge Biotechnology (Beijing, China); and S100 (from rabbit, 1:100 dilution) was purchased from Dako (Glostrup, Denmark). 3,3′-Diaminobenzidine, type I collagen protein, and pepsin (1:2500 dilution) were purchased from Sigma Chemicals. Hydroxyproline and a total protein quantification kit (Coomassie brilliant blue) were pur-

Table 1. Cell density (mean ± 1 SD) in skin of Bama miniature pigs (age, 4 and 6 mo) and humans

	no. of cells/mm^2				
	Langerhans cells	Fibroblasts	Vascular endothelial cells	Mast cells	Pigment cells
Pig (n = 18)	587 ± 124	592 ± 115	369 ± 72	31.4 ± 7.9	0
Human (n = 20)	663 ± 107	573 ± 102	388 ± 85	40.2 ± 8.2	763 ± 77

Table 2. Thickness (mean ± 1 SD) of layers in skin of Bama miniature pigs and humans

	Horny layer (μm)	Epidermis (μm)	Dermis (mm)
1-mo-old pigs (n = 5)	14.483 ± 1.687	82.257 ± 5.934	1.153 ± 0.041[a]
4-mo-old pigs (n = 8)	14.903 ± 1.368	84.810 ± 3.345	1.270 ± 0.076
6-mo-old pigs (n = 10)	15.592 ± 1.474	97.083 ± 4.638[a]	1.932 ± 0.073[a]
Humans (n = 20)	15.110 ± 1.543	86.183 ± 6.832	1.332 ± 0.082

[a]$P < 0.05$ compared with thickness in humans.

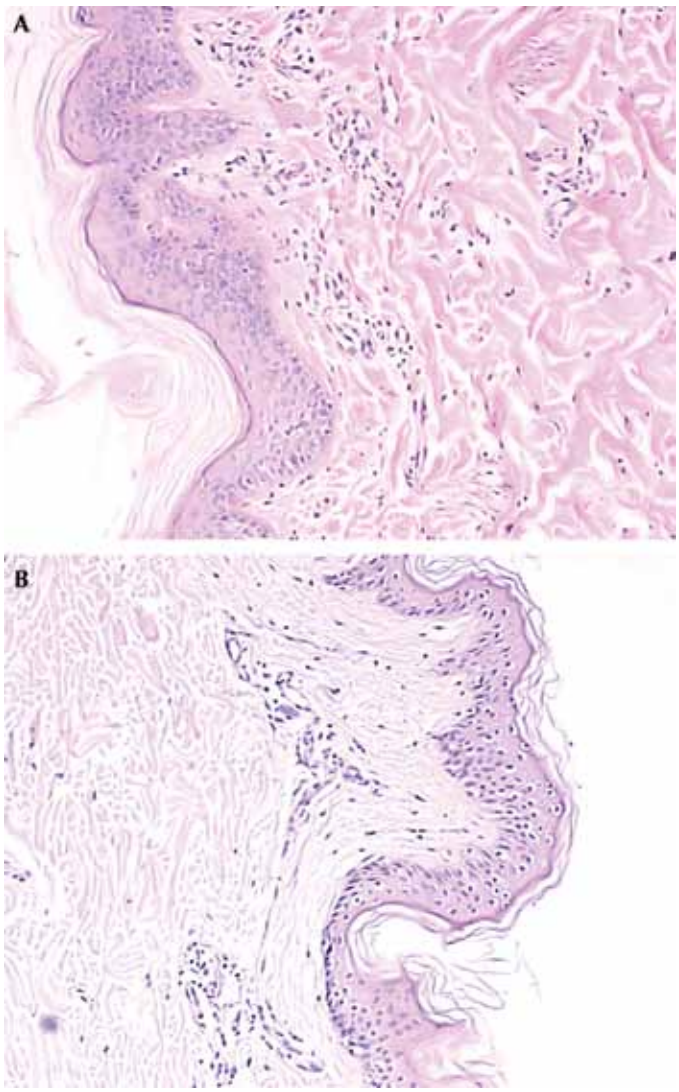

Figure 1. Light microscopy of skin of (A) Bama miniature pigs and (B) humans. Hematoxylin and eosin stain; Magnification, ×250.

chased from Nanjing Jiancheng Bioengineering Research Institute (Nanjing China). Spurr embedding kit was purchased from Haide Biochemicals Engineering (Beijing China); All other chemicals were the highest grades available from commercial suppliers.

Experimental animals and treatments. Bama miniature pigs (*Sus scrofa domestica*) were obtained from our laboratory. These animals (23 male, 23 female; age, 1 to 6 mo) were fed and housed as described previously.[11] Procedures involving animals were approved by the institutional animal care and use committee and complied with the Laboratory Animal Management Principles of China.[16] All animals were euthanized by intravenous injection of pentobarbital sodium (150 mg/kg body weight). Back skin was harvested from each minipig.

Normal human adult back skin was obtained with informed consent from 10 Chinese female donors and 10 Chinese male donors after plastic surgery (Chongqing Southwest Hospital, China). These tissues had been donated for research use, and this study was approved by the Ethics Committee of Third Military Medical University.

Light microscopy. Skin samples from Bama miniature pigs and humans were fixed in 4% formalin, dehydrated, embedded in paraffin, sectioned at a thickness of 4 μm, deparaffined, and stained with various staining methods.[2] Briefly fibroblasts and vascular endothelial cells were visualized by using hematoxylin and eosin stain; collagen fibers, Van Gieson stain; reticular fibers, Gordon and Sweet silver stain; elastic fibers, orcein stain; Langerhans cells, adenosine triphosphatase stain; melanin cell density, Fontana stain; and mast cells, toluidine blue stain. Samples were examined by light microscopy at magnifications of ×100 and ×400 to evaluate the various targets.

Transmission electron microscopy. Skin samples were sectioned at 0.5 mm and fixed in 2% glutaraldehyde solution. After fixation for 24 h, specimens were dehydrated through ethanol and infused with Spurr resin (Haide Biochemicals Engineering). Embedded tissue was cut into 5-μm slices[1,5] for investigation by transmission electron microscopy (Central Laboratory of Third Military Medical University).

Immunohistochemistry. Skin samples were shock-frozen in liquid nitrogen, cut into 4-μm paraffinated sections, and processed as described previously.[5] Diluted primary antibody (50 μL each; mouse antihuman collagen I, mouse antihuman collagen III, mouse antihuman collagen IV, goat antihuman fibronectin, rabbit antihuman laminin, mouse antihuman keratin, goat antihuman ICAM1, mouse antihuman CD34, rabbit antihuman S100) was used for each immunohistochemical test.

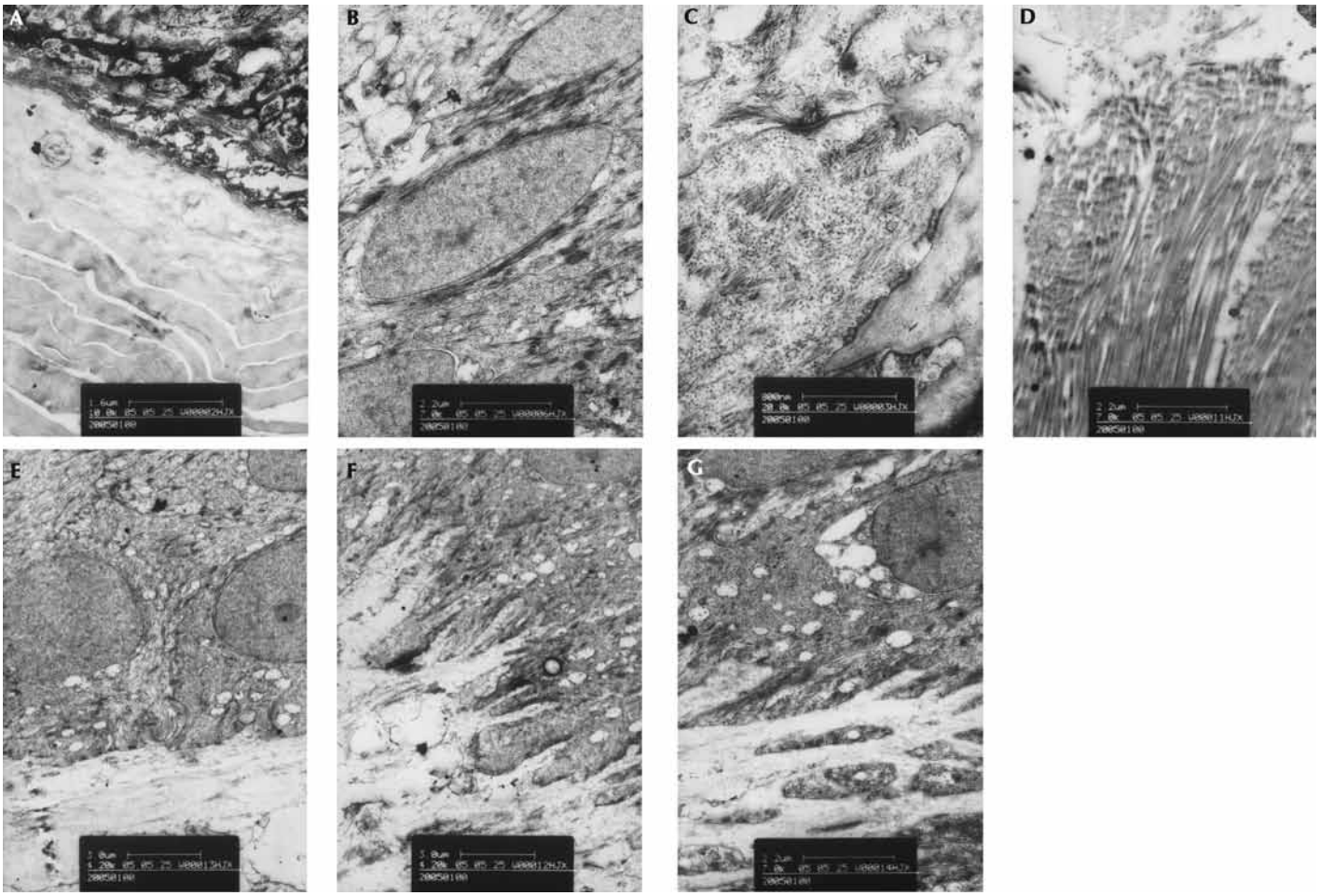

Figure 2. Transmission electron microscopy of skin of Bama miniature pig. (A) Horny cells and diaphanous cells; magnification, ×10,000. (B) Basal cells; magnification, ×7000. (C) Tonofilaments and desmosome junctions; magnification, ×20,000. (D) Collgen fibers; magnification, ×7000. (E) Flat epidermal–dermal interface; magnification, ×4200. (F) Serrated epidermal–dermal interface; magnification, ×4200. (G) Basal membrane; magnification, ×7000.

Extraction and characterization of collagen type I. According to conventional procedures,[15,27] collagen type I of skin was extracted with pepsin in 0.5 M acetic acid, salted out repeatedly with sodium chloride, and dialyzed in deionized water at 4 °C for 2 d. Collagen type I was further purified by semipreparative HPLC (1100 series, Agilent, Santa Clara, CA) by using a semipreparative column (9.4 × 250 mm, 5 µm; Zorbax 300SB-C18, Agilent). The column was eluted with a linear gradient of methanol (0% to 20%) at a flow rate of 1.0 mL/min and gradient change of 1% per minute. Eluates were monitored continuously at 220 nm, and the column temperature was set at 25 °C. The total run time for sample analysis was 20 min. Purity was analyzed by HPLC. The UV–visible spectra of the sample solutions were recorded in the wavelength range of 200 to 400 nm by using a UV–visible spectrophotometer (model 8453E, Agilent). The quantitative measurements of hydroxyproline were conducted at the specific wavelength of 560 nm. Other amino acids were analyzed on an amino-acid analyzer (System 6300, Beckman Coulter, Fullerton, CA). Collagen proteins were separated by SDS-PAGE as described previoiusly.[5]

Data analysis. The results were expressed as mean ± 1 SD. The Student *t* test was used to analyze differences between values, and a *P* value of less than 0.05 was considered statistically significant.

Results

Histologic analysis of skin structure. Like human skin, the skin of Bama miniature pig comprised all 5 strata: stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and stratum basale. The dermis contained the papillary and reticular layers. The epidermal interpapillary pegs and dermal papillae fitted together well to form a tight junction that was irregular at the epidermal–dermal interface. Many superficial venules and arteriolar plexuses were distributed around the boundary between the papillary and reticular layers. The dermis contained a variable amount of fat as well as collagen and elastic fibers. Elastic fibers, which often were intertwined with collagen fibers, primarily were located in the papillary dermis and surrounding vessels. These characteristics of the skin of Bama minipigs resembled those of human skin. In addition, the Langerhans cells, fibroblasts, vascular endothelial cells and mast cells of adult pigs did not display any marked differences from those in human skin (Table 1).

Despite the pronounced similarities of the skin from these 2 species, the skin of Bama miniature pigs and humans had several structural differences. First, the skin layer thickness of Bama miniature pig appeared to be age-dependent; the dermis from 1-mo-old pigs was thinner than human skin ($P < 0.05$), but that

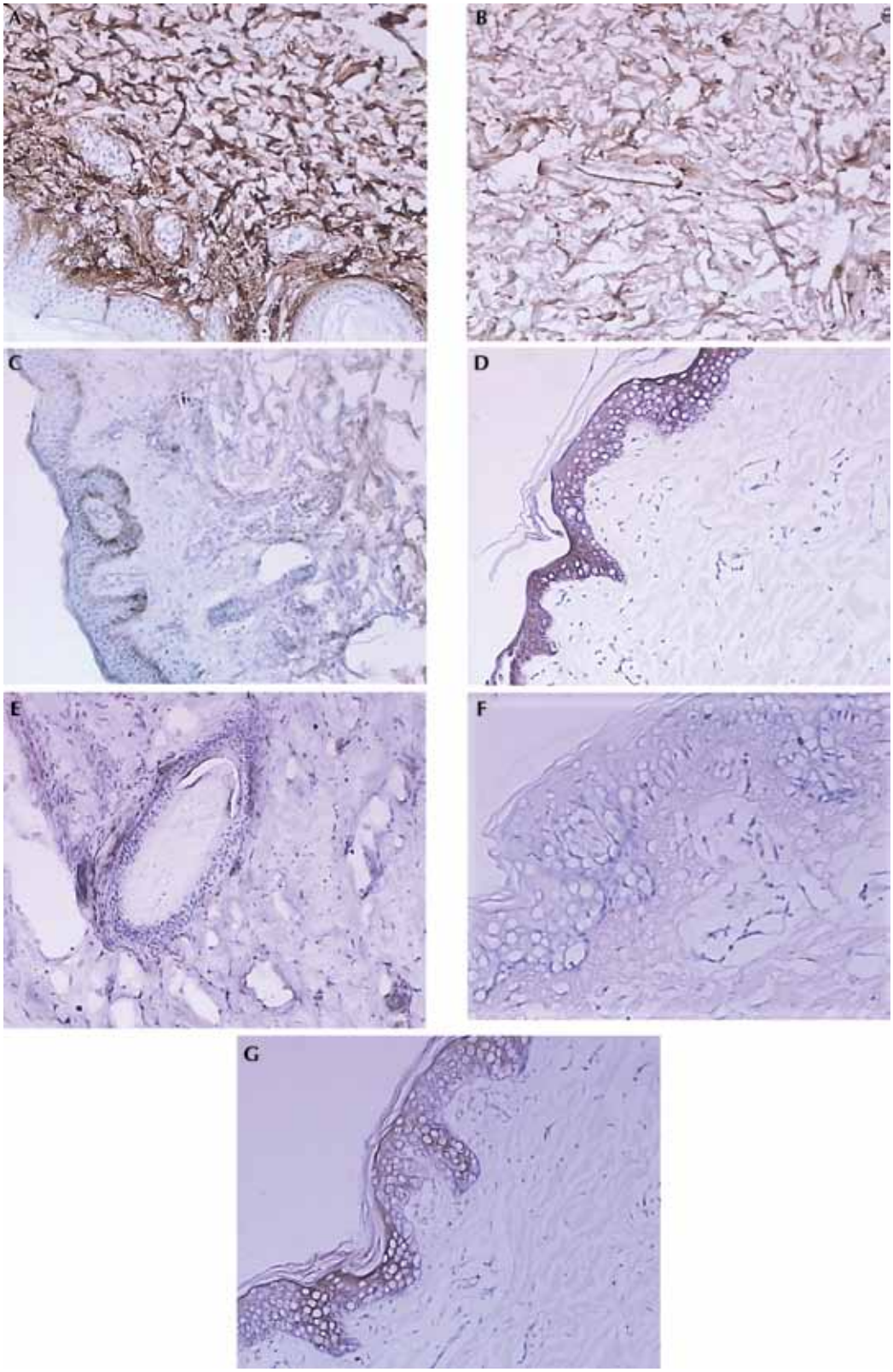

Figure 3. Immunohistochemistry of skin of Bama miniature pig. Antihuman antibodies used against: (A) Collagen I, (B) collagen III, (C) collagen IV, (D) keratin, (E) fibronectin, (F) laminin, and (G) filaggrin. Visualized by using Envision reagent (Dako); magnification, ×250.

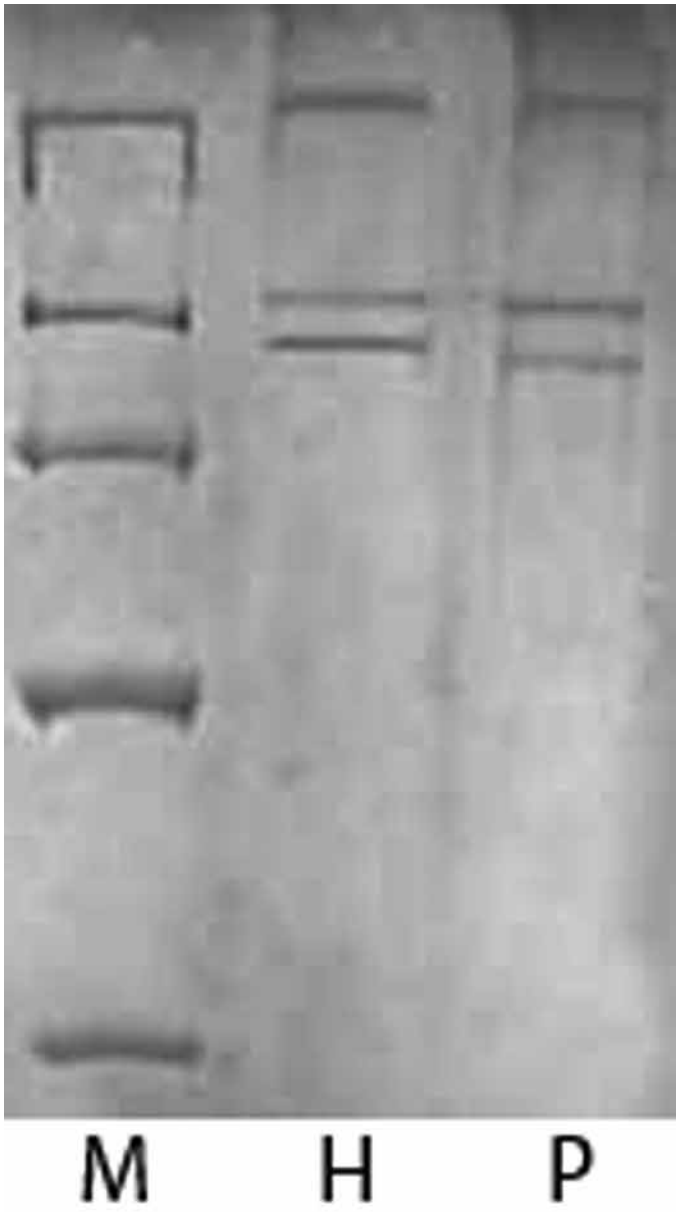

Figure 4. SDS–PAGE of collagen I extracted from Bama miniature pig (P) and human (H) skin. M, Molecular weight marker proteins: 130, 97.4, 66.2, 43, and 31 kD.

of 4-mo-old pigs was the same thickness as in humans. The epidermis and dermis from 6-mo-old pigs was thicker than those in humans, except for the horny layer (Table 2). Second, pig epidermis had fewer pigment cells ($P < 0.05$; Table 1), and the dermis had a lower elastic fiber content than did human skin ($P < 0.05$). The distribution of pigment cells of Bama minipig skin differed with the location of the sample. Specifically, samples from white areas lacked pigment cells but those from black positions, such as the head and hindquarters, had higher densities of pigment cells than those in humans. Third, unlike other pigs,[19] Bama miniature pigs have only apocrine sweat glands, which cannot adjust body temperature, but the sweat glands in human skin are primarily eccrine sweat glands. Fourth, the sebaceous glands of Bama minipig skin were smaller than those of human skin from similar locations (Figure 1).

Table 3. Amino acid composition of collagen I in skin of Bama miniature pigs and humans

Amino acid	Pig	Human
Asp	5.75	5.98
Thr	0.86	1.04
Ser	2.51	3.03
Glu	9.46	9.38
Pro	12.97	12.10
Gly	29.44	30.52
Ala	7.36	6.14
Cys	0.21	0.17
Val	1.89	2.03
Met	0.83	0.78
Ile	1.51	1.62
Leu	2.97	3.09
Tyr	0.56	0.51
Phe	2.17	2.23
Lys	2.82	2.77
His	0.75	0.70
Arg	5.50	5.41

Data are given as the no. of the amino acid of interest per 100 amino-acid residues.

Ultramicrostructural analysis. Transmission electron microscopy revealed the presence of keratin filaments, membrane-coating granules, desmosomes, and keratohyaline granules in the horny cells of Bama minipig skin. As in humans, the Langerhans cells of Bama minipigs contained Birbeck granules, and their endothelial cells contained Weibel–Palade bodies. Two ultrastructurally heterogeneic types of dermal–epidermal junctions were present in Bama minipig skin. Basal cells facing the bottom of the epidermal staple had flat dermal–epidermal interfaces, whereas basal cells facing the top of dermal papillae had serrated dermal–epidermal interfaces. The dermis of Bama miniature pigs mainly consisted of extracellular matrix and a superficial vascular system, which contained endothelial, mononuclear, and mast cells. Moreover, the elastic fibers in the Bama minipig dermis had the same triaxial arrangement and thickness as those of humans, although pig skin had far fewer elastic fibers than did human skin (Figure 2).

Immunohistochemistry. Immunohistochemical analysis of the components of Bama minipig skin yielded positive staining of collagen I, collagen III, collagen IV, keratin, laminin, fibronectin, and filaggrin; for all of these components, the distribution was similar between Bama minipig and human skin. The results showed collagen I and Collagen III were detected in all layers of dermis but collagen IV only in basal membrane and around vasculature. Laminin and fibronectin located in basal membrane, dermal–epidermal junction and around vasculature. Keratin and filaggrin was observed in epidermal keratinocytes. However, antihuman antibodies to CD34, ICAM1, and S100 did not positively stain any component of Bama minipig skin (Figure 3).

Extraction and comparison of collagen type I from Bama pig skin. As reported by other researchers,[17,25] collagen type I extracted and purified from Bama minipig skin was dissoluble in PBS at 4 °C and became solid at 37 °C. Results from SDS-PAGE showed col-

Table 4. Characteristics of skin of Bama miniature pigs and humans

	Pig	Human
Epidermis		
Layers	Five layers	Five layers
Corneous layer thickness	Similar	Similar
Cuticular layer thickness	Similar	Similar
Skin staple	Yes	Yes
Basal cell heterogeneity	Yes	Yes
Keratohyaline granule	Yes	Yes
Tonofilaments	Yes	Yes
Bridge corpuscles	Yes	Yes
Filaggrin	Yes	Yes
Langerhans cells	Yes	Yes
Pigment cells	Yes, nonuniform distribution	Yes
Sweat glands	Apocrine	Eccrine and apocrine
Sebiferous glands	Small volume	Large volume
Dermis		
Layers	Papillar and reticular layers	Papillar and reticular layers
Papillae	Yes	Yes
Collagen I	Similar disposition	Similar disposition
Collagen III	Similar disposition	Similar disposition
Elastic fibers	Small quantity	Large quantity
Reticular fiber	Yes	Yes
Fibroblasts	Similar density	Similar density
Laminin	Similar disposition	Similar disposition
Fibronectin	Similar disposition	Similar disposition
Mast cells	Yes	Yes
S100 reaction	No	Yes
Developed microvascular system	Yes	Yes
Endothelial cells	Similar density	Similar density
Weibel–Palade bodies	Yes	Yes
CD34 reaction	No	Yes

lagen type I from human skin and Bama minipig skin had nearly the same molecular weight (Figure 4). A 0.05% solution of Bama minipig skin collagen type I had a λ_{max} at 223 nm, and its hydroxyproline concentration was 11.68%. The amino acids contents of Bama minipig and human skin are shown in Table 3.

Discussion

Here we compared the skin of Bama miniature pigs and with that of humans in terms of histologic structure, ultrastructure, immunohistochemical response characteristics, and collagen physicochemical properties (Table 4). Most physicochemical features of Bama miniature pig skin were very similar to those of human.

Skin is composed of the epidermis and dermis. The thickness of the horny layer in Bama miniature pigs was very similar to that of humans and did not change with age. Because this layer is the main barrier to drug penetration, mini pig skin offers a unique advantage as an in vitro model in drug research.[3] The epidermal and dermal thicknesses of 4-mo-old pigs were similar to those of human adults, although some discrepancies were present depending on the body region from which the sample was obtained. Some scientists have proposed that the epidermis:dermis ratio is a more precise index of evaluating skin similarity than is comparing the thicknesses of the epidermis and dermis directly.[21] In that case, 4- and 6-mo-old pigs both were similar to humans because their epidermis:dermis ratios were 10:1 compared with approximately 13:1 for humans. Moreover, the collagen fibers, distributions of elastic and reticular fibers, capillary plexus from the superficial microvascular plexus, and ultrastructure of dermal cells of Bama minipigs were all similar to those of human skin. Furthermore, several features are present only in the skin of humans, nonhuman primates, and pigs, including basal cell heterogeneity, serrated epidermal–dermal interface pattern, well-developed superficial vascular system, and mast cell granules. In addition, various antihuman sera against skin proteins (for example, collagen types I and 3, keratin) recognize the cognate proteins in pigs but rarely in other animals, indicating that some skin proteins of pigs have the same antigen determinants as those of humans. But some components of pig skin (for example, S100, CD34) differed so markedly from those of humans that human antibodies could not efficiently recognize the pig proteins.[9]

Our results indicated that the phenotypes and densities of fibroblasts, endothelial cells, and mast cells from Bama pig skin are similar to those in human skin. These similarities suggest that, when confronted with the same stimulus, Bama minipig skin will mount reactions similar to those in human skin. In particular, the repair of traumatic damage is an important function of human skin. This process involves complex interactions among cells, cytokines, and extracellular matrix, and various inflammatory and tissue-repair cells participate in wound healing.[20] We expect that Bama pig skin will manifest the same responses for traumatic healing as does human skin. For example, Langerhans cells are important skin cells that are involved in cell-mediated immune reactions and graft reaction.[7] The Langerhans cells density of Bama pig skin did not differ significantly from that of human skin, and this finding prompts us to predict the recipient pig's immune response after skin graft operation (for example, antigen presentation in the induction of contact hypersensitivity) will be similar to that of human graft recipients.

Type I collagen is the predominant collagen of the dermis and forms collagen fibers which maintain dermal configuration.[4,13] Collagen is an effective matrix that helps to protect abraded skin surfaces. Collagen also is beneficial for endoepidermal growth to promote healing.[13] The use of collagen in the treatment of large burns is gaining attention.[27] Collagen is the main constituent of acellular dermal matrix and can induce an undesirable immune response in recipients. Interspecies differences at the amino- and carboxy-termini of the collagen molecule have been postulated to cause the immunologic rejection after transplantation of heterogeneous acellular dermal matrix.[8] However, the collagen from pig is unlikely to cause immune rejection in human recipients because the α1 and α2 subunits of pig collagen are very similar to those of human skin.[6] Our results showed that the amino acid composition of collagen type I from Bama miniature pig is almost identical to that of human skin. Therefore, donor pig collagen likely will not cause immune rejection in human recipients.

Together, our results suggest that Bama minipigs are a suitable animal model for studies of human skin. However, because of the

various differences we identified between Bama minipig and human skin, additional studies are required to determine the true applicability of miniature pig skin as an alternative for human skin.

Acknowledgments

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References

1. **Chen RN, Ho H, Tsai YT, Sheu MT.** 2004. Process development of an acellular matrix (ADM) for biomedical applications. Biomaterials **25:**2679–2686.
2. **Chinese Medical Association.** 2004. Pathematology technical manipulation, p 130–136. In: Chinese Medical Association, editors. Clinical technical specification, vol Pathology. Beijing (China): People's Military Medical Press.
3. **Ferry LL, Argentieri G, Lochner DH.** 1995. The comparative histology of porcine and guinea pig skin with respect to iontophoretic drug delivery. Pharm Acta Helv **70:**43–56.
4. **Fitzgerald AMP, Kirkpatrick JJR, Foo ITH, Naylor IL.** 1996. Human skin histology as demonstrated by Herovici's stain: a guide for the improvement of dermal substitutes for use with cultured keratinocytes. Burns **22:**200–202.
5. **Ge L, Zheng S, Wei H.** 2009. Comparison of histological structure and biocompatibility between human acellular dermal matrix (ADM) and porcine ADM. Burns **35:**46–50.
6. **Heinrich W, Lange PM, Stirz T, Iancu C, Heidermann E.** 1971. Isolation and characterization of the large cyanogen bromide peptides from the α1 and α2 chains of pig skin collagen. FEBS Lett **16:**63–67.
7. **Hoefakker S, Balk HP, Boersma WJ, van Joost T, Notten WR.** 1995. Claassen E migration of human antigen-presenting cells in a human skin graft onto nude mice model after contact sensitization. Immunology **86:**296–303.
8. **Jiang DY, Chen B, Jia CY, Tao K.** 2003. [An experimental study on the difference of the antigenicity of xenogenic acellular dermal matrix]. Zhonghua Shao Shang Za Zhi **19:**155–158. Article in Chinese.
9. **Jiang DY, Zhang H, Zhou AZ, Chen B.** 2003. [Determination of skin antigen marker of pig and human with immunohistochemical method]. Chinese Medical Journal of Metallurgical Industry **20:**236–239. Article in Chinese.
10. **Lavker RM, Dong G, Zheng PS, Murphy GF.** 1991. Hairless micropig skin. A novel model for studies of cutaneous biology. Am J Pathol **138:**687–697.
11. **Li J, Liu Y, Zhang JW, Wei H, Yang L.** 2006. Characterization of hepatic drug-metabolizing activities of Bama miniature pigs (*Sus scrofa domestica*): comparison with human enzyme analogs. Comp Med **56:**286–290.
12. **Liu Y, Zeng BH, Shang HT, Cen YY, Wei H.** 2008. Bama miniature pigs (*Sus scrofa domestica*) as a model for drug evaluation for humans: comparison of in vitro metabolism and in vivo pharmacokinetics of lovastatin. Comp Med **58:**580–587.
13. **Medalie DA, Tompkins RG, Morgan JR.** 1996. Evaluation of acellular human dermis as a dermal analog in a composite skin graft. Am Soc Artif Intl Organs J **42:**M455–M462.
14. **Meyer W, Scharz R, Neurand K.**1978. The skin of domestic mammals as a model for the human skin with special reference to the domestic pig. Curr Probl Dermatol **7:**39–52.
15. **Miller EJ, Rhodes RK.** 1982. Preparation and characterization of the different types of collagen. Methods Enzymol **82:**33–64.
16. **Ministry of Science and Technology of China.** [Internet]. The laboratory animal management principles of China. 2005 update [Cited 21 Mar 2010]. Available at http://www.most.gov.cn/bszn/new/dwjk/wjxz/200512/t20051225_ 55325.htm.
17. **Morimura S, Nagata H, Uemura Y, Fahmi A. Shigematsu T, Kida K.** 2002. Development of an effective process for utilization of collagen from livestock and fish waste. Process Biochem **37:**1403–1412.
18. **Qvist MH, Hoeck U, Kreilgaard B, Madsen F, Frokjaer S.** 2000. Evaluation of Gottingen minipig skin for transdermal in vitro permeation studies. Eur J Pharm Sci **11:**59–68.
19. **Simon GA, Maibach HI.** 2000. The pig as an experimental animal model of percutaneous permeation in man: qualitative and quantitative observations —an overview. Skin Pharmacol Appl Skin Physiol **13:**229–234.
20. **Stadelmann WK, Digenis AG, Tobin GR.** 1998. Physiology and healing dynamics of chronic cutaneous wounds. Am J Surg **176**(2A Suppl):26S–38S.
21. **Sullivan TP, Eaglstein WH, Davis SC, Mertz P.** 2001. The pig as a model for human wound healing. Wound Repair Regen **9:**66–76.
22. **Van Ravenzwaay B, Leibold E.** 2004. The significance of in vitro rat skin absorption studies to human risk assessment. Toxicol In Vitro **18:**219–225.
23. **Vardaxis NJ, Brans TA, Boon ME, Kreis RW, Marres LM.** 1997. Confocal laser scanning microscopy of porcine skin: implications for human wound healing studies. J Anat **190:**601–611.
24. **Walker M, Dugard PH, Scott RC.** 1983. In vitro percutaneous absorption studies: a comparison of human and laboratory species. Hum Toxicol **2:**561–562.
25. **Wang W, Sun JL, Liu JL, Pan YL.** 1988. [The preparation of soluble acidic collagen and clinical application]. J Biomed Eng **5:**224–227. Article in Chinese.
26. **Weinstein GD.** 1965. Autoradiographic studies of turnover time and protein synthesis in pig epidermis. J Invest Dermatol **44:**413–419.
27. **Wu ZG, Sheng ZY, Sun TZ, Geng M, Zhou BT, Li JY, Huang ZX.** 2003. [Preparation of collagen-based materials for wound dressing]. Chinese Med J **16:**147–150. Article in Chinese.