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Short Communication

A New Technique for Development of Rabbit Ear Keloid Model

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ABSTRACT

Keloids and hypertrophic scars are an exaggeration of fibroplasia in dermal repair. Keloids are found solely in humankind, and not in animals. Thus far no practicable animal model of Keloid has been made. Recently a prototype rabbit ear keloid model has been produced by the authors by giving Transforming Growth Factor Beta 1 injection, and then skin punch excision on rabbit ear. In the present follow-up study consisting of eight refind techniques, best Keloid was produced by the technique where Transforming Growth Factor Beta 1 injection was followed by ventral skin, ventral perichondrium, cartilage, and dorsal perichondrium punch excision, leaving behind only dorsal skin.

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Cicatrix, Hypertrophic, Keloid, Transforming Growth Factor beta 1.

Keloids and hypertrophic scars are an exaggeration of fibroplasia in dermal repair. Keloids are basically benign tumours of skin. Hypertrophic scars represent a related entity, but they do not spread to surrounding tissue as do keloids (Rabello *et al*., 2014). Keloids and hypertrophic scars form as a consequence of wounds. They may form months or years after initial insult. They vary in size, and minor wounds can produce major keloids (Carmassi *et al*., 2015). Precise cause of keloids and hypertrophic scars is unknown. Nevertheless several causes are considered as risk factors. They may be internal or external (Babar, 2015a, b).

Keloids occur solely in humankind, and not in animals. This is the major obstacle against systematic enquiry on these lesions, as primary studies on humankind are not practical, and not ethical. Research on keloids has typically been clinical, with inadequate objective evidence. Thus far no practicable animal model of keloid has been made. Recently a prototype rabbit ear keloid model has been produced by the authors by giving transforming growth factor beta 1 injection, and then skin punch excision on rabbit ear (Nagi and Babar, 2015; Babar and Nagi, 2016).

Many types of rabbit ear hypertrophic scar have been produced until now by 5 mm (Xiao and Xi, 2013) and 6 mm circular ventral skin excision (Morris *et al*., 1997; Li *et al*., 2001), 6 mm circular dorsal skin excision (Li *et al*, 2001), 7 mm circular ventral skin excision (Kryger *et al*., 2007; Wang *et al*., 2015), 10 mm circular ventral skin excision (Diao *et al*., 2013), 15 mm x 45 mm rectangular ventral and dorsal skin excision (Li *et al*, 2001), 20 mm x 50 mm rectangular ventral skin excision (Xiang *et al*., 2004), 7 mm and 10 mm circular ventral skin and perichondrium excision (Jia *et al*., 2011), 7 mm circular ventral skin and perichondrium excision and nicking of cartilage (Kloeters *et al*., 2007), 6 mm circular ventral skin excision along with excision of two main sensory nerves (Yagmur *et al*., 2011) and circular ventral skin excision and applying pathogen-associated molecular patterns (PAMPs) of dead *Pseudomonas aeruginosa* and damage-associated molecular patterns (DAMPs) of homogenized dermis sample (Qian *et al*., 2015). These all are induced models. The purpose of this study was to make an ideal rabbit ear keloid model.

Material and methods

This was a quasi-experimental study done at Experimental Research Laboratory. Its duration was twelve months. Sample comprised of eight New Zealand White rabbits. Sampling technique was simple convenience.

Anaesthesia to rabbits was given with mixture comprising Ketamine and Xylazine. Study zone was cleaned using normal saline, and ethyl alcohol swab prior to creation of wound.

Eight rabbits were each given injection of Transforming Growth Factor Beta 1 (TGFB1) (Santa Cruz Biotechnology, 10410 Finnell Street Dallas, Texas 75220, USA; Tel +1-8004 57-3801; email: scbt@scbt.

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com; URL: www.scbt.com) 100 pg in 0.1 ml water, prepared in insulin syringe.Then one of the following eight different surgical techniques of punch excision was used: (a) ventral skin excision, (b) dorsal skin excision, (c) ventral skin and ventral perichondrium excision, (d) dorsal skin and dorsal perichondrium excision, (e) ventral skin, ventral perichondrium, and cartilage excision, (f) dorsal skin, dorsal perichondrium, and cartilage excision, (g) ventral skin, ventral perichondrium, cartilage, and dorsal perichondrium excision, and (h) dorsal skin, dorsal perichondrium, cartilage, and ventral perichondrium excision.

Skin punch excision was done using a 4 mm skin biopsy punch (Acu-Punch; Acuderm Inc., 5370 NW, 35 Terrace, Ft. Layderdale, FL 33309, USA; Tel +1-8003 27-0015; URL: www.acuderm.com). It is a pre-sterilized prepacked single-use skin punch biopsy gadget, which has a circular sharp edge acting as a knife. When pressure is applied at its back end, and circular movements are given, it cuts a disc of skin and underlying cartilage if required. Perichondrium is cut with a sharp pointed scissors. If there is bleeding, it is stopped with cotton gauze pressure for a short time.

Wounds were cleaned with sterilized gauze and dried with hot air dryer. Dressing was done with hydrocolloid sheet. Usual post-operative care was given. Standard wound care was given. Hydrocolloid sheet was permitted to drop off on its own. Results were recorded at twenty eight days.

Results and discussion

Rabbits were inspected at twenty eight days. Out of eight rabbits, best Keloid was created by technique (g) where injection of TGFB1 was given, followed by ventral skin, ventral perichondrium, cartilage, and dorsal perichondrium punch excision, leaving behind only dorsal skin. Gross height of this keloid was 1.355 mm. Scar elevation index (SEI) was calculated to be 3.45 (Table I).

Keloid was a clinically substantial. Its consistency was hard. It was dome-shaped, and colour was red. It had smooth surface, and there were no hair on it. Even after follow-up of one year, keloid was still present (Fig. 1A).

On gross examination, keloid specimen was hard, and was palpated as distinct nodule. Its colour was red, and surface smooth. On dissection, there was a gritty sensation. Colour of cut surface was pale. Microscopically, keloid depicted homogeneous, brilliantly eosinophilic, haphazardly dispersed, glassy, hyalinized collagen. Fibroblasts were scarce, and lied parallel to collagen (Fig. 1B).

In this study, the authors used eight different techniques to find out ideal method for production of rabbit ear keloid model. Out of eight techniques, best keloid was produced by the technique where TGFB1 injection 1 was followed by ventral skin, ventral perichondrium, cartilage, and dorsal perichondrium punch excision, leaving behind only dorsal skin.

TGFB1 is a strong chemotactic factor for fibroblasts and excites them to build main extracellular matrix constituents like collagen. It is manufactured by macrophages, and also by extracellular matrix itself. It is formed in greater amounts in keloids and keloid fibroblasts react to lesser amounts of TGFB1 than non-keloid fibroblasts. Adding exogenous TGFB1 results in stimulation of fibroblasts, which enhances secretion of collagen, and ultimately production of keloids.

Our technique of production of rabbit ear keloid model is superior to previous techniques, as keloids produced by us had maximum scar elevation index, hard texture, long duration, abundant hyalinized collagen, and scant fibroblasts. Hyalinized collagen, which is pathognomonic, characteristic and hallmark of keloid was not reported by

Table I.- Size of keloids produced by different techniques.

Technique Code	Tissues excised with 4 mm Skin Punch, after injection of 100 pg TGF Beta 1	Gross Keloid Height (mm)	Ranking No
a	Ventral skin	0.512	8
b	Dorsal skin	0.550	7
c	Ventral skin and ventral perichondrium	0.939	6
d	Dorsal skin and dorsal perichondrium	0.954	4
e	Ventral skin, ventral perichondrium, and cartilage	1.049	3
f	Dorsal skin, dorsal perichondrium, and cartilage	1.253	2
g	Ventral skin, ventral perichondrium, cartilage, and dorsal perichondrium	1.355	1 (Best)
h	Dorsal skin, dorsal perichondrium, cartilage, and ventral perichondrium	0.918	5

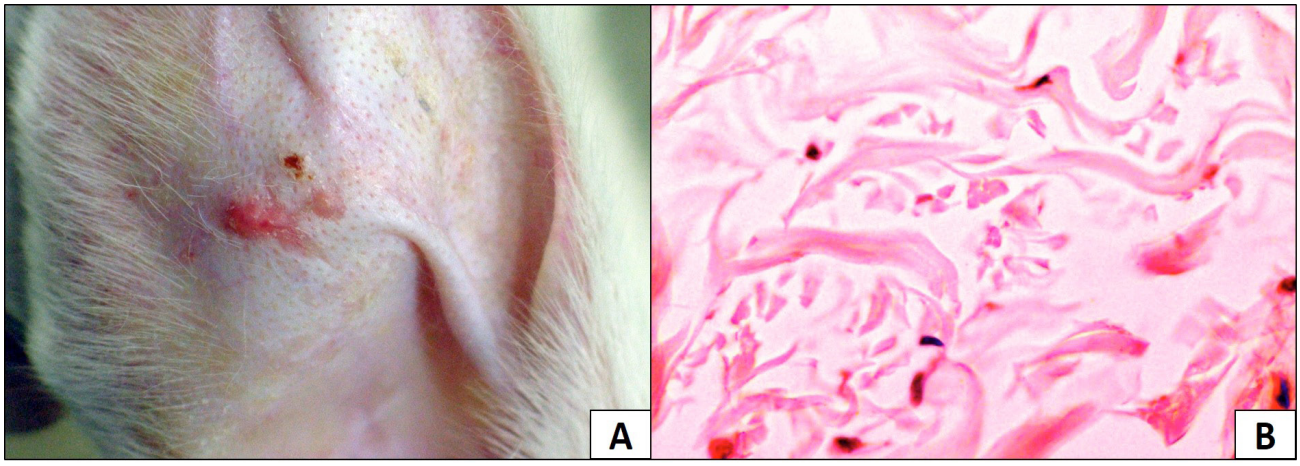

Fig. 1. Rabbit ear keloid: **A,** gross view; **B,** microscopic view

Table II.- Comparison of techniques of production of rabbit ear keloid models.

Author / Parameter	Babar and Nagi, (2016)	Morris *et al*. (1997)	Li *et al.* (2001)	Kloeters *et al.* (2007)	Kryger *et al.* (2007)	Jia *et al.* (2011)	Diao *et al.* (2013)	Xiao and Xi (2013)	Wang *et al.* (2015)
Clinical									
Duration	365+ days	288 days	262 days	NR	NR	NR	NR	180 days	NR
Gross									
Scar elevation index (SEI)	3.45	2.72	NR	1.70	1.67	2.10	2.07	1.73	2.93
Texture	Hard	Palpable	NR	NR	NR	Protrusive	Palpable	NR	NR
Microscopic									
Hyalinized collagen	Abundant	Absent	NR	Absent	Absent	NR	Absent	Absent	Absent
Fibroblasts	Scant	NR	Increased	NR	Decreased	NR	NR	NR	NR

NR, Not reported.

any of the previous authors. Morris *et al*, whose technique has been considered as gold standard so far, reported: 'No hyalinized collagen such as seen in keloids was present.' Comparison with previous keloid production techniques is given in Table II.

Conclusion

Best rabbit ear Keloid model can be produced by giving TGFB1 injection, followed by ventral skin, ventral perichondrium, cartilage, and dorsal perichondrium excision

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Conflict of interest statement

We declare that we have no conflict of interest.

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