MANNAN AND HANNAN TECHNIQUE FOR PRODUCTION OF RABBIT EAR KELOID MODEL

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ABSTRACT

Keloids & hypertrophic scars are a type of benign fibrous lesions formed during skin wound repair. Keloids occur exclusively in Homo sapiens, to the exclusion of animals. No operable keloid animal model has been built so far.The authors have developed a novel technique for rabbit ear keloid model creation using Transforming Growth Factor Beta 1 injection, a pro-fibrotic cytokine, followed by composite punch excision.

Objective of this study was to produce the best rabbit ear keloid model. Main outcome measure was the best rabbit ear keloid model. It was a quasi-experimental trial, carried out at Experimental Research/Pathology Laboratories, University of Health Sciences, Lahore, Pakistan. Its duration was twelve months, from April 2011 to March 2012.Sample consisted of one New Zealand White rabbit. Technique consisted ofgiving injection of Transforming Growth Factor Beta 1, and subsequently performing ventral skin, ventral perichondrium, cartilage, and dorsal perichondrium excision, leaving behind only dorsal skin.The best rabbit ear keloid model was produced by this technique, which was intact even after follow up of one year.This technique has been named as 'Mannan & Hannan Technique for production of rabbit ear keloid model'. This technique has not been used before. National and International patents are being applied for.

Key words: Cicatrix, Hypertrophic; Keloid; Models, Animal; Rabbit; Transforming Growth Factor Beta 1.

INTRODUCTION

Keloids are a type of benign fibrous lesions formed during skin wound repair. Keloids are actually benign dermal tumours. Hypertrophic scars denote an allied lesion, but they do not extend to neighbouring tissues (Hunasgi*et al*., 2013; Philandrianos*et al*., 2015). Keloids and hypertrophic scarsdevelop as a result of injuries. They occur immediately after injury, or may be delayed for months or years. They are of variable sizes, and small lesions may result in large keloids in prone patients (Mamalis *et al*., 2014; Carmassi*et al.*, 2015). Specific reason of development of keloids is not identified so far. Nonetheless some causes are thought as possible reasons. The causes may be inside or outside human body(Babar, 2015).

Keloidsoccur exclusively in Homo sapiens, to the exclusion of animals. This isthe chief impediment against organized probe regarding these lesions, as experiments on humans are not practicable, and not proper. Research on keloids has characteristically been patient-based, with poor neutral evidence. No operable keloid animal model has been built so far. The authors have developed a novel technique for rabbit ear keloid model creation.Technique consists of giving injection of Transforming Growth Factor Beta 1, and subsequently performing skin punch excision(Nagi& Babar, 2015; Babar &Nagi, 2016a, b).

There is akey function of Transforming Growth Factor Beta 1 in repair of wounds; it is a strong chemotactic factor for fibroblasts and activates them to produce main extracellular matrix components like collagen. Transforming Growth Factor Beta 1 is manufactured by macrophages as well as extracellular matrix. Transforming Growth Factor Beta 1 acts by activation of fibroblasts, causingenhanced collagen production. Transforming Growth Factor Beta 1 is produced in great quantities in keloids, and keloid fibroblasts respond to lower quantities of Transforming Growth Factor Beta 1 than normal fibroblasts. Addition of external Transforming Growth Factor Beta 1 stimulates fibroblasts, thereby increasing production of collagen, and formation of keloids (Chen *et al.*, 2014; Fan *et al.*, 2015; Wang *et al.*, 2015).

Morris *et al.* (1997), Xiang *et al.* (2004), Diao*et al.* (2013), Xiao and Xi (20130, and Wang *et al.* (2015) prepared rabbit ear hypertrophic scar models by full thickness skin excisions of various sizes, mostly circular in shape, on ventral surface of rabbit ears. Jia*et al.* (2011)created rabbit ear hypertrophic scar models by removing skin and perichondrium.Kloeters*et al.* (2007)created wounds on rabbit ear by excising ventral skin, and perichondrium. They deliberately injured the cartilage, but did not excise it. Li *et al.* (2001) built rabbit ear hypertrophic scar model by giving round or rectangular skin excisions on ventral or dorsal surface,

and studied effects of Transforming Growth Factor Beta 1 and Interferon Gamma on these scars.

Summing up, the techniques of rabbit ear keloid models in vogue are: 1) Circular excision of full thickness ventral skin of rabbit ear; 2) Circular excision of full thickness ventral skin plus ventral perichondrium of rabbit ear; 3) Circular excision of full thickness ventral skin plus ventral perichondrium, and nicking of cartilage of rabbit ear. The purpose of nicking of cartilage is not clear; 4) Circular excision of full thickness dorsal skin of rabbit ear; 5) Circular excision of ventral skin of rabbit ear, and application of bio-toxins; 6) Rectangular excision of full thickness ventral skin of rabbit ear; and 7) Rectangular excision of full thickness dorsal skin of rabbit ear.Diameter or length of these excisions ranged from 5 mm to 50 mm.

The purpose of this article to describe the best technique i.e. Mannan and Hannan Technique for production of rabbit ear keloid model.

MATERIALS AND METHODS

Approval was taken from institutional Ethical Review Committee.Universal protection was used i.e. surgical gown, surgical cap, surgical mask, surgical goggles, examination gloves, and surgical shoe covers.

Transforming Growth Factor Beta 1 Injection(Santa Cruz Biotechnology, 10410 Finnell Street Dallas, Texas 75220, USA; Tel +1-8004 57-3801; email scbt@scbt.com; URL www.scbt.com) 50 µg (stored at -40° C in ultra-low temperature freezer) was taken. It was prepared in 10 ml water for injection in 10 ml syringe. A 0.2 ml (1 µg) of it was taken in insulin syringe. It was put it in micro burette infusion set, diluting with water for injection 99.8 ml, making it 100 ml. A 0.1 ml (1 ng) of it was taken in insulin syringe.

Surgical instruments were taken and sterilized in hot air sterilizer at 160° C for 90 minutes.Surgeon washed-up with alcoholic surgical hand disinfectant, and wore surgical gloves.

One New Zealand White Rabbit (New Zealand White Rabbit, 1 year old, University of Health Sciences, Khayaban-e-Jamia-Punjab, Lahore 54600, Pakistan; Tel: +92-429 9231304; email: info@uhs.edu.pk; URL: www.uhs.edu.pk) was taken.

Rabbit was anaesthetized with Ketamine injection (Calypsol Injection 50 mg / ml, 10 ml, Medimpex, Budapest, Lehelu. 11, 1135 Hungary; Tel: +36 12 88 1400; email: trade@medimpex.hu; URL: www.medimpex.hu)(75 mg kg^{-1} intra-peritoneal), and Xylazine injection (Xylaz Injection 20 mg / ml, 25 ml, Farvet Laboratories B.V, Handelsweg 25, 5531 AE Bladel, Netherlands; Tel; +31-497 544 320; URL: urlm.co/www.eurovet-international.com)(15 mg kg^{-1} intra-peritoneal) mixed in 5 ml syringe.

Rabbit ear wasdisinfected with sterilized saline gauze and ethyl alcohol swab.Proposed point of keloid was marked on rabbit ear with gentian violet and Castroviejo caliper, saving major vessels.Thickness of ear at marked point was measured with digital micrometre gauge (Digital Micrometer Gauge, 0.001 mm, Interlabs, Cross Road 8, Ambala Cantt 133 001, Haryana. India; Tel: +91-171 2641604; email: info@interlabs-india.com; URL: www.interlabs-India.com). Transforming Growth Factor Beta 1 0.1 ml (1 ng), prepared in insulin syringe was injected at point marked, and waited for 1 minute.

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).8 mm was taken and a disc of ventral skin, ventral perichondrium, cartilage, and dorsal perichondrium was excised, leaving behind only dorsal skin, at point injected.If bleeding occurred, it was controlled with mosquito artery forceps.If inadvertently dorsal skin was injured, it was stitched with 4/0 surgical silk (Mersilk 4/0, Ethicon, Route 22 West Somerville, NJ 08876, USA; Tel: +1-9082 18-0707; URL: www.ethicon.com).

Thickness of dorsal skin was measured with digital micrometre gauge.Wound was dried with sterilized gauze and electric hot air dryer.Wound was dressed with hydrocolloid dressing(DuoDerm Extra Thin CGF Dressing, 10 cm x 10 cm, ConvaTec, 211 American Ave, Greensboro, NC 27409,USA; Tel +1-800-4228811; email: cic@convatec.com; URL: www.convatec.com).

General post-operative care was given. Analgesics were not required.Antibiotics were not used. Routine wound care was provided.If stitches had been applied, they were removed at one week.Dressing was allowed to fall off spontaneously.Keloids were formed at twenty eight days

RESULTS

Keloidproduced was considerable on examination. Its consistency was solid. It was of hemispherical shape, and red colour. Its surface was smooth, and without hair. Keloid could be palpated even after one year of follow-up (Table 1) (Figure 1).

Grossly, keloid tissue sample was hard in consistency, and was palpable as definite nodule. It had red colour and smooth surface. It gave a gritty sensation on cutting. Cut surface was of pale colour. Microscopically, keloid showed glassy, hyalinized,homogeneous, brilliantly eosinophiliccollagen. There were scant fibroblasts, and they lied in the line of collagen (Figure 2).

Table 1. A Comparison of Rabbit Ear Keloid Model production Techniques

Researcher Feature	Babar & Nagi(2016)	Morris *et al.*(1997)	Li *et al.*(2001)	Kloeters*et al.*(2007)	Krygeret *al.*(2007)	Jia *et al.*(2011)	Diao *et al.*(2013)	Xiao & Xi(2013)	Wang *et al.*(2015)
Clinical Examination:									
Period	365+ days	288 days	262 days	NA	NA	NA	NA	180 days	NA
Gross Examination:									
Scar Elevation Index (SEI)	3.45	2.72	NA	1.70	1.67	2.10	2.07	1.73	2.93
Consistency	Hard	Palpable	NA	NA	NA	Protrusive	Palpable	NA	NA
Microscopic Examination:									
Hyalinized Collagen	Abundant	Absent	NA	Absent	Absent	NA	Absent	Absent	Absent
Fibroblasts	Scant	NA	Increased	NA	Decreased	NA	NA	NA	NA

NA = Not Available

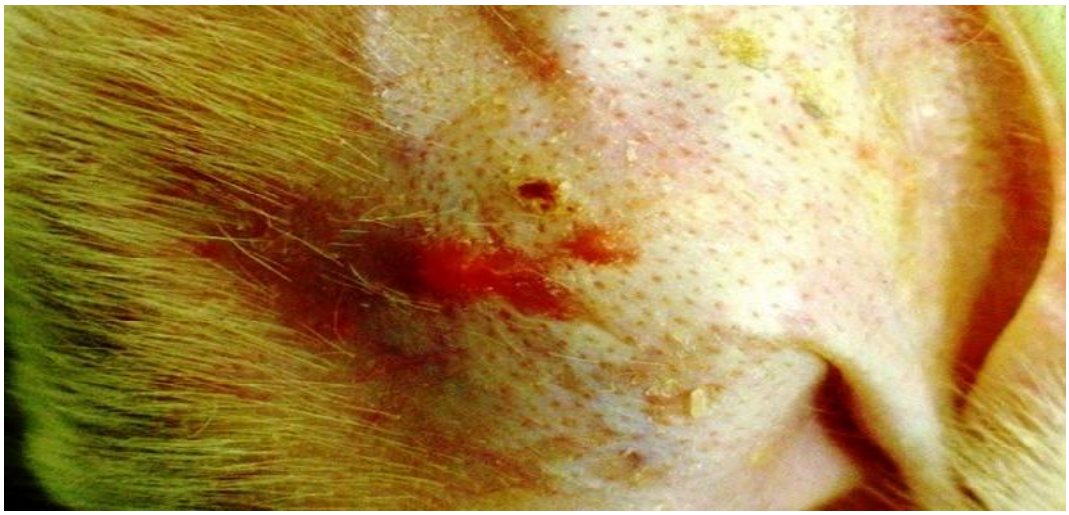

Figure 1. Rabbit Ear Keloid Model produced using Mannan&Hannan Technique.

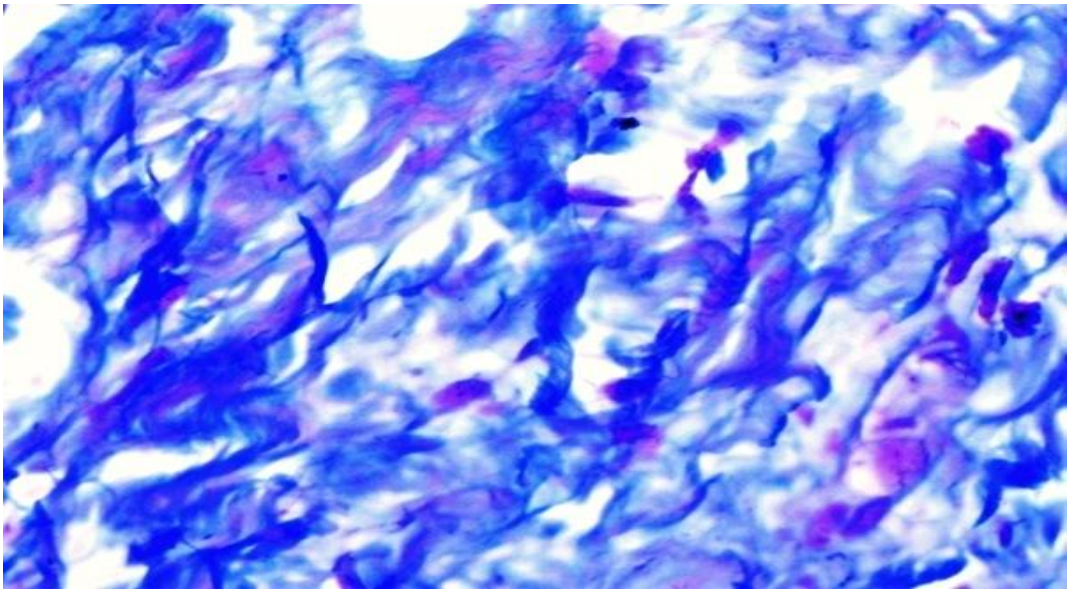

Figure 2. Histopathology of Mannan&Hannan Keloid, using Periostin stain.

DISCUSSION

Keloids occur exclusively in Homo sapiens, to the exclusion of animals. This is the chief impediment against organized probe regarding these lesions, as experiments on humans are not practicable, and not proper. Research on keloids has characteristically been patient-based, with poor neutral evidence. No operable keloid animal model has been built so far. The authors have developed a novel technique for rabbit ear keloid model creation. Previous techniques could only produce hypertrophic scars.

There is a key function of Transforming Growth Factor Beta 1 in repair of wounds; it is a strong chemotactic factor for fibroblasts and activates them to produce main extracellular matrix components like collagen. Transforming Growth Factor Beta 1 is manufactured by macrophages as well as extracellular matrix. Transforming Growth Factor Beta 1 acts by activation of fibroblasts, causingenhanced collagen production. Transforming Growth Factor Beta 1 is produced in great quantities in keloids, and keloid fibroblasts respond to lower quantities of Transforming Growth Factor Beta 1 than normal fibroblasts. Addition of external Transforming Growth Factor Beta 1 stimulates fibroblasts, thereby increasing production of collagen, and formation of keloids (Chen *et al.*, 2014; Fan *et al.*, 2015; Wang *et al.*, 2015).

Morris *et al.*(1997) were first to create a rabbit ear hypertrophic scar model. They created this model by 6 mm skin excisional wounds on ventral surfaces of rabbit ears. They treated these scars with triamcinolone acetonide. Li*et al.* (2001) builtrabbit ear hypertrophic scar model by giving round or rectangular skin excisions on ventral or dorsal surface. They reported that rectangular wound created more hypertrophy than round wounds. They studied effects of Transforming Growth Factor Beta 1 and Interferon gamma on these scars.

Xiang *et al.* (2004) prepared rabbit ear hypertrophic scar model by skin excisionsof 2 cm x 5 cm on ventral surface of rabbit ears. They applied 1% silver sulfadiazine dressing on it.Kloeters*et al.* (2007)created wounds reaching cartilage, on ventral side of ear. They removed skin, and perichondrium. They deliberately injured the cartilage, but did not excise it. They applied liquid adhesive to adjacent skin, and covered it with polyurethane dressing.

Jia *et al.* (2011) built rabbit ear hypertrophic scar model by giving7 mm and 10 mm punch wounds reaching cartilage, combined with removal of perichondrium.Diao*et al.* (2013) produced rabbit ear hypertrophic scar model by full thickness skin excision. Xiao and Xi (2013) made full thickness wounds to produce rabbit ear hypertrophic scar model. Wang *et al.* (2015) produced rabbit ear hypertrophic scar model by making full thickness circular wounds.

Our method of creation of rabbit ear keloid model is better thanpriormethods, as keloids created by us had greatest scar elevation index, hard consistency, long time of stay, copious hyalinized collagen, and negligible fibroblasts. Hyalinized collagen, which is pathognomonic, characteristic and hallmark of keloid was not produced by any of the prior authors. Morris et al, whose technique is thought as gold standard method, reported: 'No hyalinized collagen such as seen in keloids was present.' Comparison with past keloid creation methods is given in Table 1.

Conclusion: This technique has been named as 'Mannan & Hannan Technique for production of rabbit ear keloid model'. National and International patents are being applied for.

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