Pain-related Mechanism Analysis by rolling with Microneedle Rollers according to Miconeedle Types (Pre-clinical test)

December 3, 2012

CHUNG ANG University Industry
Academic Cooperation Foundation

중앙대학교
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- List -

Introduction

Title	Pain-related Mechanism Analysis by rolling with Microneedle Rollers according to Miconeedle Types (Pre-clinical test)		
IACUC No.	12-0025	Reception Date	August 1, 2012
Principal Investigator	Kim Beom Joon	Tel.	Tel. 02) 6299-1525 Fax. 02) 6359-9573
Testing Institution	CHUNG ANG University Industry Academic Cooperation Foundation	Testing Period	August 3, 2012 ~ November 30, 2012
	Address: Department of Dermatology, Chung-Ang University Hospital, 224-1, Heukseok-dong, Dongjak-gu, Seoul		
Testing Client	DTS MG Co.,Ltd. Representatives : Han Gi Soo	Tel.	Tel. 02)558-5480 Fax. 02)558-5482
	Address: #402, Daegun Indus Town, 275-29, Seongsu-Dong 2-ga, Seongdong-Gu, Seoul		
Testing objective and contents	▣ **Objective** The comparison study for occurred pain levels after rolling with DTS microneedle rollers(DTS MG Co., Ltd, Korea) or MTS microneedle rollers(MTS Roller™) is performed using back skin of hairless(HR-1) mice. Conditions of all microneedle rollers as follows: microneedle length is 0.5mm and roller type is manual. ▣ **Contents** Testing Name: Pre-clinical test for microneedle rollers; Testing animal: Hairless mice (HR-1) *number: 28; Testing methods: Rolling of microneedle rollers; Testing tools: Microneedle rollers (manual type, 0.5mm) ▣ **Methods** After rolling with DTS or MTS microneedle rollers on back skin of hairless mice, we photographed using digital camera or folliscope to confirm damage of skin tissue by microneedle rollers. Histological analysis is performed by H&E staining. Also, water content and transepidermal water loss(TEWL) of mouse skin are measured. Difference of occurred pain level by rolling of microneedle rollers according to microneedle types is confirmed though expression levels of pain-related factors(COX-2, PGE_2, SP, CGPR, TRPV1, S100A8) using IF, western blot, and IHC. ▣ **Results** When rolling with MTS or DTS microneedle rollers on mouse skin, water contents are more decreased by rolling of MTS microneedle rollers than DTS microneedle rollers because of damage of skin barrier by increased contact side of skin by microneedle type of MTS microneedle rollers. Also, expression level of COX-2, PGE_2, and TRPV1 are more increased immediately after rolling with MTS microneedle roller than other groups. Therefore, we confirmed that when miconeedle roller of two types is applied to the skin, fine pain is more occurred immediately after rolling with MTS microneedle roller than DTS microneedle roller.		
Declaration	All animal test is performed according to regulation of Institutional Animal Care and Use Committee(IACUC) of Chung-Ang University. **Principal Investigator Beom Joon Kim (인)**		

Principal Investigator Profile

Beom Joon Kim, M.D. PhD. and Dermatologist

Present : Associate Professor, Department of Dermatology, College of Medicine, Chung-Ang University,Seoul,Korea

- Education -

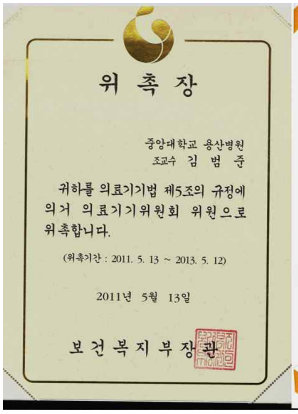
1998 Subintern, Dept. of Internal Medicine, Samsung Seoul Hospital,Korea
1999 Subintern, The National Institute of Scientific Investigation, Korea
2000 Graduated College of Medicine,Chung-AngUniversity,Korea
2000-2001 Internship, Chung-Ang University Hospital,Korea
2001-2005 Resident, Chung-Ang University Hospital,Korea
2003 Master of Science, Graduate school of Chung-Ang University,Korea
2007 Doctorate of Philosophy, Dermatology, Chung-Ang University,Korea

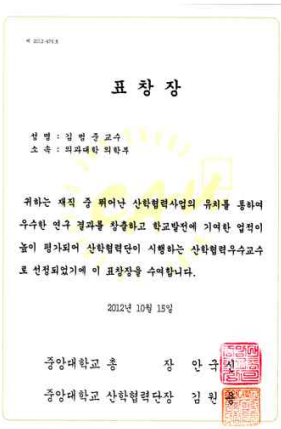
- Professional Career -

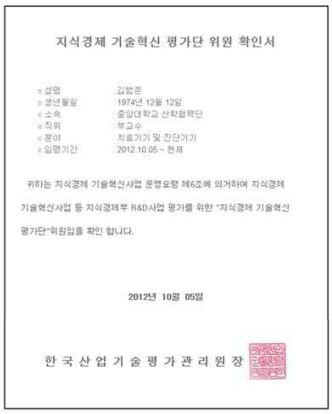
2003 -Secretary general, The 11th annual meeting of the SHSR(Society for Hair Science Research, Japan) joint with KHRS(Korean Hair Research Society)
2005-present -Invited reviewer of 'British Journal of Dermatology'
-Reviewer of 'Journal of American Academy of Dermatology'
-Reviewer of 'International Journal of Dermatology'
2005-2006 -Clinical fellow, Seoul National University Hospital,Korea
2006-present -Instructor, international staff, Eastern University,Pennsylvania,USA
2006-2007 -Assistant Professor,Department of Dermatology,Dong-Guk University International Hospital, Gyeunggi,Korea
2007-present -Assistant Professor, Department of Dermatology,Chung-Ang University Yongsan Hospital,Seoul,Korea
-Invited reviewer, Clinical and experimental dermatology, Dermatologic Surgery, Pediatric dermatology
-Editorial Board, Chung-Ang Journal of Medicine
2008-present -Editorial Board, Journal of the AmericanAcademyofDermatology
-Editorial Board, International Journal of Dermatology
2009-present -Vice Editor in Chief, Korean Journal of Medical Mycology
-Editorial board, The Journal of Neural Regeneration Research
2010-present -Editorial board, Asian Aesthetic Guide
2011-present -Associate Professor, Department of Dermatology, Chung-Ang University Hospital, Seoul, Korea
-Committee for Medical Equipment Board, Department of Health and Human Services

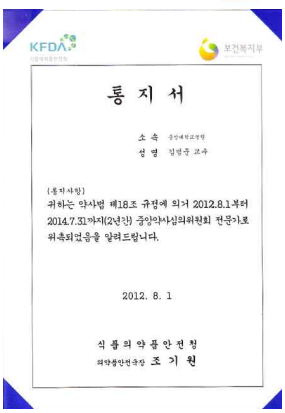
-Planning Committee for National Project, Ministry of Knowledge Economy
-Clinical specialist, Korea Food & Drug Administration,
-Committee member for Medical Device, Ministry of Health & Welfare, Korea
-Project Manager board member, Korea Health Industry Development Institute, Korea
-Chairman of hearing, Korea Food & Drug Administration, Korea

2012-present -Editorial board, Journal of Cosmetics, Dermatological Sciences and Applications
-Editorial board, Annals of Dermatology
-International Advisory Board, Aesthetics Asia 2012









Testing Contents

1. Title

Pain-related Mechanism Analysis by rolling with Microneedle Rollers according to Miconeedle Types (Pre-clinical test)

2. Objective

: The comparison study for occurred pain levels after rolling with DTS microneedle rollers(DTS MG Co., Ltd, Korea) or MTS microneedle rollers(MTS Roller™) is performed on back skin of hairless(HR-1) mice. Conditions of all microneedle rollers as follows: microneedle length is 0.5mm and roller type is manual.

3. Materials and methods

1) Materials

(1) Material name : Microneedle rollers(manual type, microneedle length: 0.5mm)
(2) Source of supply

MTS Roller™

DTS MG Co., Ltd

2) Animal test (pre-clinical test)

(1) Animal : Hairless mice (HR-1)
(2) Source of supply : Central Lab. Animal Inc., Seoul, Korea
: The skin of hairless mouse is likely to rolling by microneedle roller because of the mouse had rarely bodily hair. And changes of skin are easy to check after rolling by microneedle roller.
(3) Weight range: 16~24kg

2) Facility condition

: The animal study using hairless mice(6W, Female) is performed in conventional system. The condition are as follows: Temperature 23±3℃, Humidity 55±15%, Light is blocked range from 8pm to 8am.

3) Methods

(1) Testing groups

: Total twenty-eight hairless mice are used as control or test groups(seven mice/group). Control group is one, test group is two.

(2) method of anesthesia

- insufflation narcosis : Gerolan (0.81mg/kg) is used.

(3) Testing methods

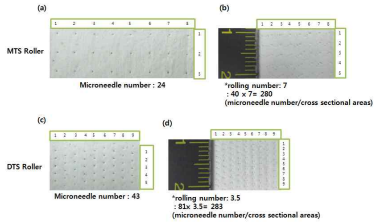
: All animal test is performed according to regulation of Institutional Animal Care and Use Committee(IACUC) of Chung-Ang University. (IRB No.12-0025)

: Animal test is performed as total three groups(control, MTS microneedle roller, DTS microneedle roller) using hairless mice.

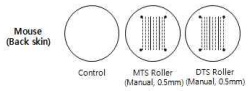
: Water contents and transepidermal water loss(TEWL) are measured using comeometer and tewameter and changes of skin are confirmed using folliscope immediately(0hr), at 30minutes and 24hours after rolling with microneedle roller according to microneedle types on back skin of hairless mice. Also, histological analysis is performed using hematoxylene and eosin(H&E) staining through prepared skin tissue according to various times such as 0minutes, 30minutes, and 24hours. Biological analysis is performed using immunofluorescence assay(IF), western blot, and immunohistochemistry(IHC) to confirm expression level of cyclooxygenase-2(COX-2), prostaglandin E_2(PGE_2), substance P(SP), calcitonin gene-related peptide(CGRP), transient receptor potential cation channel subfamily V member1(=capsaicin receptor)(TRPV1), S100 calcium binding protein A8(S100A8) as pain related factors.

(4) Testing conditions

: Groups divided into 1 control groups and 2 test groups (MTS microneedle roller, DTS microneedle roller). All microneedle roller is manual type and microneedle length is 0.5mm. The differences between MTS microneedle roller and DTS microneedle roller are microneedle shape and the microneedle number per unit area. So, this study is set the different rolling number of test groups to have identical the microneedle number per unit area between MTS microneedle roller and DTS microneedle roller(Fig.1). The objective of this study is the comparison for occurred pain level between various microneedle types when rolling with microneedle rollers on back skin of mice. We confirmed skin changes and occurred pain levels by microneedle rollers through visiual assessment, histological assessment, and molecular biological assessment using various equipments for 0, 0.5(30minutes), and 24hours after rolling with various microneedle rollers according to setting rolling numbers(MTS microneedle roller: 7, DTS microneedle roller: 3.5) to apply identical microneedle numbers on mouse skin(Fig.2).



(Fig 1. Comparison for inserted microneedle numbers between microneedle rollers according to microneedle types)



(Fig 2. Group classification as control and test groups according to microneedle types)

(5) Evaluation methods

: Equipments: comeometer/tewameter, folliscope, digital camera, Scanning electron microscope(SEM)

Histological evaluation: H&E staining, western blot, IF, IHC

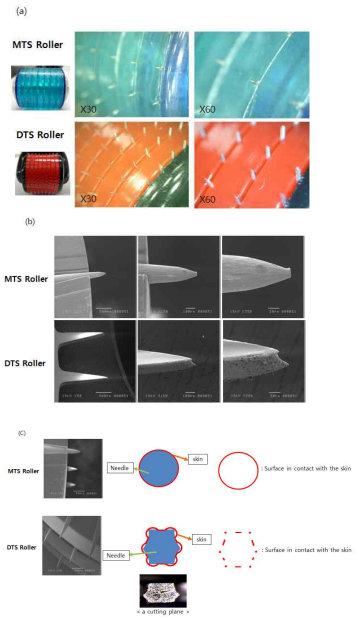
4. Results

1) Characteristic comparison for microneedle rollers according to microneedle types

(1) Microneedle observation of microneedle rollers with various types

: Microneedle shape of each microneedle rollers(MTS, DTS) is confirmed using folliscope and SEM. The images of folliscope are 30x or 60x magnification(Fig.3(a)). And the images of SEM are 50x, 150x and 350x magnification(Fig.3(b)). The

microneedle shape of MTS microneedle roller has tapered at the end as cylindricality. The tip of microneedle is blunt. Whereas, which of DTS microneedle roller has more tapered at the end than MTS microneedle as hexagon. But, we expect that when rolling with microneedle roller, cross sectional areas of microneedle of MTS microneedle roller are in contact with the skin in a moment are more bigger than which of DTS microneedle roller(Fig.3(c)).



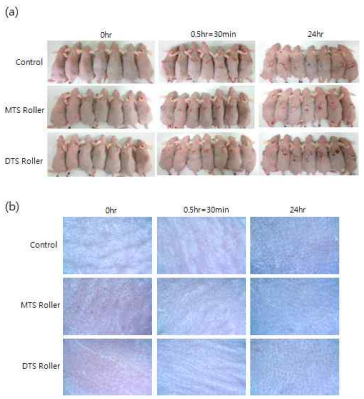
(Fig 3. microneedle analysis of microneedle rollers with various types)

2) Skin safety evaluation for microneedle rollers with various types

(1) Visual evaluation

: All evaluation is performed through the skins after rolling with various microneedle roller according to time such as 0, 0.5(30minutes), and 24hours.

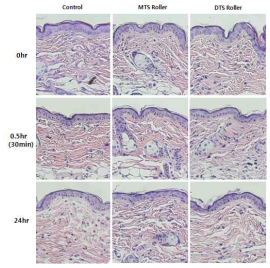
: The results of skin surface photograph using folliscope are confirmed that cutaneous adverse reactions including skin surface damage, erythema, and heat injury reaction by rolling with microneedle roller aren't verified(Fig. 4).



(Fig 4. (a) group photograph and (b) photograph for cutaneous adverse reactions before and after rolling of microneedle rollers using folliscope. Overall, cutaneous adverse reactions aren't verified.)

(2) Histological evaluation using hematoxylin & eosin (H&E) staining

: After rolling with various microneedle roller on skin, each skin tissues are prepared according to time. All skin tissues are incubated in 10% formalin solution(fixing solution). And then, paraffin blocks using the skin tissues are prepared. We are confirmed through H&E staining that cutaneous adverse reactions of the skin tissues aren't verified(Fig.5).



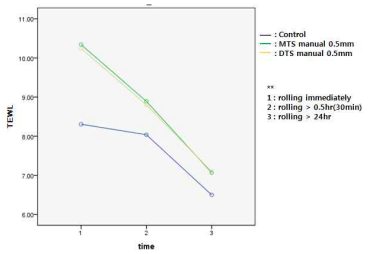
(Fig 5. After rolling with microneedle roller according to microneedle types, cutaneous adverse reactions aren't verified compare to control group.)

3) After rolling with microneedle roller according to microneedle types on skin, measurements of transepidermal water loss(TEWL) and water contents

(1) Mesaurement of transepidermal water loss (TEWL)

: Transepidermal water loss(TEWL) is a value for water loss by occurred vaporization on the skin itself contains water because of damage of skin barrier by external stimulus. TEWL can be analyzed that the bigger value is, the more water loss is increase.

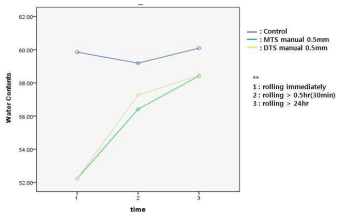
: We confirmed damage of skin barrier by rolling with microneedle roller through increased TEWL value of test groups that it was performed rolling with various microneedle roller than control groups that it wasn't performed rolling by time zone. But, TEWL values between test groups (MTS microneedle roller, DTS microneedle roller) aren't changed significantly($p>0.5$). At 24hours after rolling with microneedle roller, TEWL values are tended to decrease in baseline way(Fig. 6).



(Fig 6. the results for measurement of transepidermal water loss(TEWL) according to the time after rolling with various microneedle rollers)

(2) Mesaurement of water contents

: Unlike in the results of TEWL, water contents are more increased in control groups than test groups. This results do support the damage of skin barrier by microneedle roller. Also, at 30minutes after rolling with microneedle roller, water contents are more increased in DTS microneedle roller group than MTS microneedle roller group. But, this results aren't significantly($p>0.5$). However, this results could be analyzed that water loss is occurred temporarily because of microscopic holes by rolling with microneedle roller, water recovery process of water loss through restoring force of skin is performed rapidly in DTS microneedle roller group than MTS microneedle roller group. At 24hours after rolling with microneedle roller, water contents of all test group are tended to increase in baseline way(Fig. 7).



(Fig 7. the results for measurement of water contents according to the time after rolling with various microneedle rollers)

4) A Comparison of occurred pain level according to time after rolling with various microneedle roller on skin tissue

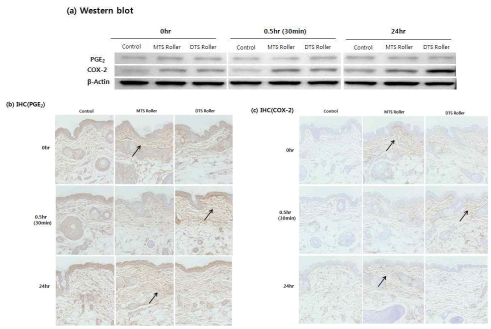
: We confirmed expression level of well-known pain-related factors such as COX-2, PGE_2, substance P, CGRP, TRPV1, and S100A8 though IF, western blot, and IHC.

(1) A comparison of expression level of pain-related factors(COX-2, PGE_2) using Western blot and IHC

: After rolling with various microneedle roller on back skin meet the conditions, we confirmed expression level of COX-2 and PGE_2 that influences expression of neuropeptides such as SP, CGRP, and TRPV1(Ref.6) and are known as factors of pain-related mechanisms(Ref.4,5) through western blot and IHC according to time.

: In the results of IHC, expression level of COX-2 and PGE_2 are more increased in MTS microneedle roller group immediately or at 24hours after rolling with microneedle rollers than DTS microneedle roller group. But, at 30minutes after rolling with microneedle rollers, its expression levels are more increased in DTS microneedle roller group than MTS microneedle roller group(Fig.8).

: We verified that expression level of PGE2 in results of western blot is identical to that of IHC. But, expression level of COX-2 in results of western blot differs from that of IHC. Because penetration depth by microneedle according to angle or force of each microneedle roller can vary in rolling process on back skin, we can predict as method error for western blot through protein extraction from applied skin selectively.

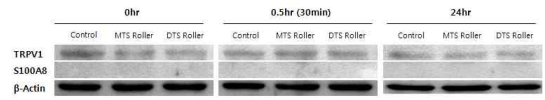


(Fig 8. the expression levels of COX-2 and PGE_2 in skin according to the time after rolling with various microneedle rollers)

(2) A comparison of expression level of pain-related factors(TRPV1, S100A8) using Western blot

: After rolling with various microneedle roller on back skin meet the conditions, we confirmed expression level of TRPV1 as capsacin receptor and S100A8 through western blot according to time.

: The expression level of TRPV1 is similar in all groups, the expression level of S100A8 isn't appeared. The expression level of TRPV1 and S100A8 between all groups aren't changed significantly(Fig 9).

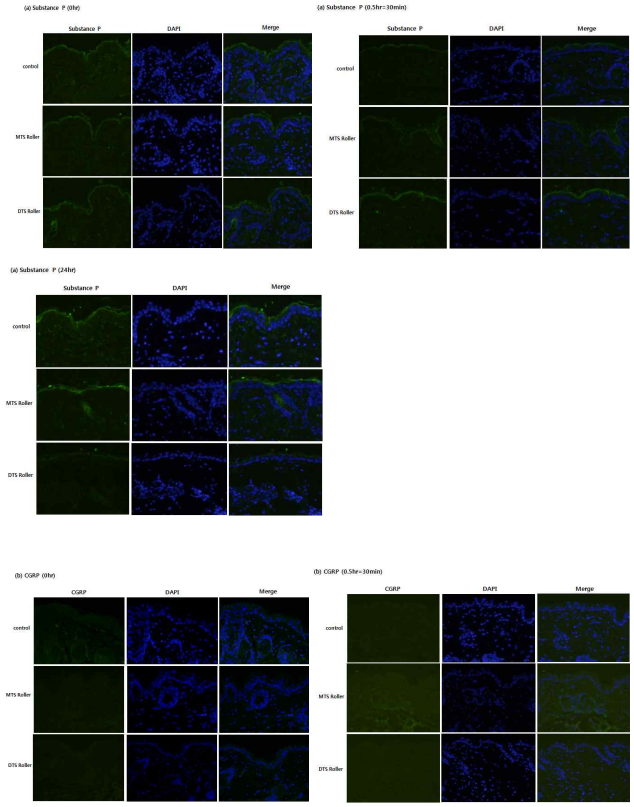


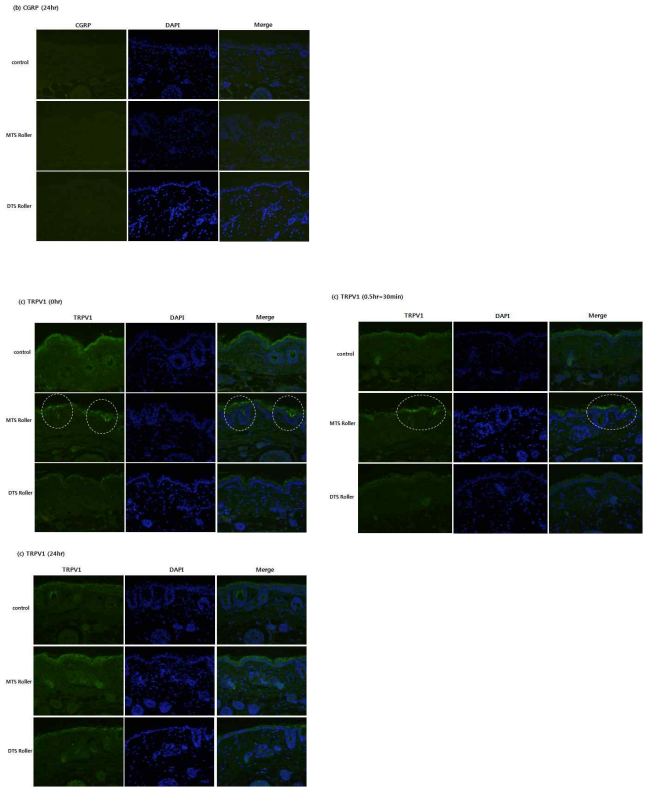
(Fig 9. the expression levels of TRPV1 and S100A8 in skin according to the time after rolling with various microneedle rollers)

(3) A comparison of expression level of pain-related factors(Substance P, CGRP, TRPV1) using IF

: After rolling with various microneedle roller on back skin meet the conditions, we confirmed expression level of SP, CGRP, and TRPV1 through IF according to time.

: In expression of SP, at 30minutes after rolling, DTS microneedle roller group is minutely increased than MTS microneedle roller or control group. On the other hand, at 24hours after rolling, MTS microneedle roller group is minutely increased than other groups. The expression of TRPV1 is largely increased than other groups immediately or at 30minutes after rolling. But, the expression of CGRP isn't changed between groups(Fig.10).





(Fig 10. the expression levels of SP, CGRP, and TRPV1 in skin according to the time after rolling with various microneedle rollers)

5. Discussions

: We performed that comparison study for occurred pain by various microneedle type through rolling with MTS or DTS microneedle roller on condition of identical microneedle number per unit area. The results for safety evaluation of microneedle rollers(2 types), cutaneous adverse reactions aren't confirmed from the results of folliscope and H&E staining. After rolling with various microneedle roller, TEWL values of all test group for various microneedle roller are similar. But, the value of water content for DTS microneedle roller group is more higher than that of MTS microneedle roller group.

Skin has elasticity because of it is composed with fibrous tissue. So, damaged skin by external stimulus is tended to recovery by restoring force. In figure 3, the microneedle shape of DTS microneedle roller is hexagon that has slopes of edge of both sides. And structure of MTS microneedle is cylindrical. As a results, when rolling with various microneedle roller on back skin, MTS microneedle is more increased surface in contact with the skin than DTS microneedle. And then, water content of skin is more decreased by damage of skin barrier.

Also, the expression level of COX-2, PGE_2, and TRPV1 as pain-related factors in MTS microneedle roller group are slightly increased than other groups immediately after rolling with mircroneedle roller. Therefore, we confirmed that when rolling with microneedle roller of two types on skin, the expression of pain-related factors by MTS microneedle roller is more increased than DTS microneedle roller. Generally, the more increase the expression level of pain-related factors are, the bigger the pain level is. On the basis of this contents, we verified that at least, immediately after rolling, occurred pain level by DTS microneedle roller is more weaker than MTS microneedle roller.

6. References

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