FINAL REPORT

Collagen Synthesis Test Using Hs27 Cells

Study Number: 23-VE-3046E

Sponsor: CGBIO Co., Ltd

CORESTEMCHEMON Inc

Nonclinical Research Institute, CORESTEMCHEMON Inc.
240, Nampyeong-ro, Yangji-myeon, Cheoin-gu, Yongin-si, Gyeonggi-do, 17162, Republic of Korea

Translation Verification Statement

This report is a translation of original Korean Final Report issued on Dec 06, 2023.

Hereby, I certify that the contents are accurately translated.

Yoon-Yi Kim, M.S. Date (MM/DD/YYYY)

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Approval

Collagen Synthesis Test Using Hs27 Cells

This study was performed in accordance with the SOPs of the Nonclinical Research Institute, CORESTEMCHEMON Inc. so, the issuance of this report has been approved.

Director, Nonclinical Research Institute,
CORESTEMCHEMON Inc. **(Signature)**

CORESTEMCHEMON Study No. 23-VE-3046E

승 인 서

Hs27 세포를 이용한 In vitro 콜라겐 생성 시험

상기 시험은 코아스템켐온㈜ 비임상연구소의
SOP에 준하여 수행되었음을 확인하였기에
본 보고서의 발행을 승인합니다.

코아스템켐온㈜ 비임상연구소장 (인)

Signature Page

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Director, Headquarters of New Drug Development Support

Nonclinical Research Institute, CORESTEMCHEMON Inc.

CORESTEMCHEMON Study No. 23-VE-3046E

서 명

김 윤 이, M.S. — 12/06/2023 (날 짜 (MM/DD/YYYY))

시험책임자

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이 훤 규, Ph.D. — 12/06/2023 (날 짜 (MM/DD/YYYY))

신약개발지원본부장

코아스템켐온㈜ 비임상연구소

Study Overview

Title Collagen Synthesis Test Using Hs27 Cells

Objectives This study was conducted using Hs27 cells to evaluate the collagen synthesis effect of the test substance.

Regulatory guideline This study was performed in accordance with the SOPs of Nonclinical Research Institute, CORESTEMCHEMON Inc. and reference[1-2.]

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Study Schedules

Aug 21, 2023	Protocol approval (study initiation)
Aug 21, 2023	Cell culture initiation (experimental initiation)
Aug 28, 2023	Test article treatment
Oct 06, 2023	Completion of CCK-8 assay
Oct 31, 2023	Completion of qPCR (Experimental completion)
Nov 14, 2023	Final report draft submission
Dec 06, 2023	Final report submission (due to, study completion)

Responsible staff	Cell culture:	Ye-Na Kim	Preparation/Storage of the test article:	Hyun-Ji Park
	Statistical analysis:	Min-Haeng Lee	Archives:	Hye-Jung Jung
	Main study person:	Ye-Na Kim		

Archives The study protocol, protocol amendment records, final report, raw data, and other relevant documents have been stored in the archives of CORESTEMCHEMON Inc.'s Non-clinical Research Institute for a period of three years after the completion of the experiments. Subsequent storage arrangements were determined through consultation between the testing institution and the sponsor.

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Summary

This study was conducted using Hs27 cells to evaluate the concentration-dependent effects on the synthesis of collagen COL1A1 and COL3A2 by test articles Sculptra, Juvelook, Facetem, Radiesse, and ELLANSE.

The experimental group consisted of a no-treatment group (G1), a test article Facetem treatment group (G2), a Scultra treatment group (G3), a Juvelook treatment group (G4), a Radiesse treatment group (G5), and a ELLANSE treatment group (G6). Collagen synthesis was evaluated by measuring COL1A1 and COL3A2 mRNA expression levels using the qPCR method.

The study results are as follows.

1. Non-cytotoxicity was defined as cell viability being 80% or higher in Hs27 cells for each test article. Non-cytotoxicity was observed at all concentrations of the test articles Facetem, Scultra, Juvelook, Radiesse, and ELLANSE (G2-G6).
2. After 24 hours of treatment, a concentration-dependent increase in COL1A1 expression was observed in the Facetem treatment group (G2), Sculptra treatment group (G3), Radiesse treatment group (G5), and ELLANSE treatment group (G6) at concentrations of 1, 2, and 5 mg/mL compared to the no-treatment group.

   In the Facetem treatment group (G2), statistically significant increases or increasing trends were observed when compared to the 1, 2 mg/mL concentrations of the Sculptra treatment group (G3), Radiesse treatment group (G5), and ELLANSE treatment group (G6). Additionally, statistically significant increases were observed when compared to the concentrations of 1, 2, and 5 mg/mL in the Juvelook treatment group (G4).

   At 48 hours of treatment, statistically significant increases or increasing trends in COL1A1 expression were observed in the Juvelook treatment group (G4) compared to the no-treatment group (G1) at all concentrations.

   At 24 hours of treatment, a concentration-dependent increase in COL3A2 expression was observed in the Facetem treatment group (G2), Sculptra treatment group (G3), Radiesse treatment group (G5), and ELLANSE treatment group (G6) at concentrations of 1, 2, and 5 mg/mL compared to the no-treatment group (G1).

   In the Facetem treatment group (G2), statistically significant increases were observed when compared to the concentrations of 2 mg/mL in the Sculptra treatment group (G3) and ELLANSE treatment group (G6), 2 and 5 mg/mL in the Radiesse treatment group (G5), and 1, 2, and 5 mg/mL in the Juvelook treatment group (G4).

At 48 hours of treatment, no statistically significant difference was observed in COL3A2 expression between the no-treatment group (G1) and the Juvelook treatment group (G4) at all concentrations. However, a decreasing trend was observed when compared to the Facetem treatment group (G2).

In summary, Facetem at a concentration of 2 mg/mL demonstrated higher collagen synthesis in Hs27 cells, that is, the expression levels of COL1A1 and COL3A2 than that of other test articles, and was determined to be more effective in collagen synthesis.

Materials and Methods

1. Test article, Medium and reference article

1.1 Test article I

Product name:	Facetem
Code No.:	EC-0062
Lot No.:	CIM423017
Acquisition Date:	Jul 31, 2023, Jul 16, 2023
Quantity:	1.5 mL/prefilled syringe X 1 ea, 1.5 mL/prefilled syringe X 2 ea
Date of receipt:	Prefilled syringe containing white particles with a gel carrier
Active Ingredient:	Hydroxy Apatite
Shelf life:	Apr 27, 2026
Storage Temperature:	2 - 25 ℃
Manufacturer :	CGBIO Co., Ltd.

1.2 Test article II

Product name:	Scultra
Code No.:	EC-0060
Lot No.:	2J5015
Acquisition date.:	Jul 31, 2023, Jul 16, 2023
Quantity:	367.5 mg/mL/vial x 1 vial, 367.5 mg/mL/vial x 1 box
Appearance:	Vial with lyophilized white particles
Active Ingredients:	PLA
Shelf life:	Nov 2025
Storage Temperature:	1 - 30 ℃
Manufacturer:	GALDERMA

1.3 Test article III

Product name:	Juvelook
Code No.:	EC-0061
Lot No.:	VJ221221, VJ230623, VJ221221
Acquisition date.:	Jul 31, 2023, Aug 24, 2023, Jul 16, 2023
Quantity:	50 mg/mL/vial X 1 vial, 50 mg/mL/vial X 3 vials, 50 mg/mL/vial X 2

	vials
Appearance:	Vial with lyophilized white particles
Active Ingredients:	PLA/HA
Shelf life:	Dec 14, 2024, Jun 13, 2025, Dec 14, 2024
Storage Temperature:	1 - 30 ℃
Manufacturer:	VAIM GLOBAL INC.

1.4 Test article IV

Product name:	Radiesse
Code No.:	EC-0063
Lot No.:	A00065080
Acquisition date.:	Jul 31, 2023
Quantity:	1.5 mL/prefilled syringe X 1 ea
Appearance:	Prefilled syringe containing white particles with a gel carrier
Active Ingredients:	Hydroxy Apatite
Shelf life:	Jul 07, 2024
Storage Temperature:	15 - 32 ℃
Manufacturer:	MERZ North America, INC

1.5 Test article V

Product name:	ELLANSE
Code No.:	EC-0064
Lot No.:	001.05076
Acquisition date.:	Jul 31, 2023
Quantity:	1 mL/prefilled syringe x 1ea
Appearance:	Prefilled syringe containing white particles with a gel carrier
Active Ingredients:	PCL
Shelf life:	Aug 01, 2024
Storage Temperature:	15 - 25 ℃
Manufacturer:	Sinclair

1.6 Medium

Name:	Water for injection (WFI)
Lot No.:	55Z4F21

Storage conditions: Room temperature

Manufacturer: DAI HAN PHARM. co., Ltd

2. Preparation of dose formulation

Test articles Sculptra, Facetem, Radiesse, and ELLANSE were each measured at 100mg/ml and dissolved in 1 mL WFI. Juvelook was dissolved in 1 mL WFI to 50 mg/mL vial. Subsequently, they were warmed in a 37°C water bath for 24 hours and then filtered.

3. Cell information and culture method

3.1. Cell information

Cell: Hs27

Organism: Homo sapiens, Human

Tissue: Skin; Foreskin

Media: DMEM

Supplier: ATCC

Storage conditions: Cell Culture Room, Efficacy Evaluation Center, Nonclinical Research Institute, CORESTEMCHEMON Inc.

3.2. Cell culture method

3.2.1. The Hs27 cell stock vial was quickly dissolved (about 2 minutes) in a 37°C water bath.

3.2.2. Transfer the cell suspension in the vial to a centrifuge tube containing 10 mL DMEM medium with 10% FBS and centrifuge at approximately 1500 rpm for 5 minutes.

3.2.3. After centrifugation at 1500 rpm for 5 minutes, the supernatant was discarded, and the pellet was resuspended in 10 mL of 10% FBS, DMEM media

3.2.4. Resuspend cell pellet with the recommended complete medium and the number of cells was measured using trypan blue staining.

3.2.5. The cells were seeded to a T75 flask at 1 x 10^6 cells/20 mL and incubated at 37 ℃, 5% CO_2 incubator.

3.2.6. When the cells reached about 80% capacity, they were subcultured using 0.25% Trypsin-EDTA.

4. Evaluation

4.1. Cytotoxicity

To determine the highest non-toxic amount of the test articles, a cytotoxicity test was conducted as follows.

4.1.1. Hs27 cells were seeded at 1 X 10^4 cell/well in a 96 well plate and incubated for 24 hrs.

4.1.2. After 24 hrs, it was replaced with new culture medium and the volume was adjusted to 180 µL/well.

4.1.3. The final concentrations of the test article were diluted to 1, 5, 10, 20, and 50 mg/mL and then treated with 20 µL/well (four times).

Group	Test article	Concentration (mg/mL)				
G1	No treatment	-	-	-	-	-
G2	Facetem	0.1	0.5	1	2	5
G3	Scultra	0.1	0.5	1	2	5
G4	Juvelook	0.1	0.5	1	2	5
G5	Radiesse	0.1	0.5	1	2	5
G6	ELLANSE	0.1	0.5	1	2	5

4.1.4. After 24 hrs, the medium was replaced with new medium and treated with 190 µL of culture medium + 10 µL of CCK-8 solution.

4.1.5. The non-toxic concentration was confirmed by measuring the absorbance at 450 nm using a microplate reader.

4.2. Collagen formation (*COL1A1* and *COL3A2*)

4.2.1. The cells were seeded 1 X 10^6 cell/well in a 6 well plate and incubated for 24 hrs.

4.2.2. After 24 hrs, each well was replaced with 1.8 mL of fresh culture medium.

4.2.3. The test articles were set to three non-toxic concentrations confirmed in the cytotoxicity experiment above, treated to 200 µL/well, and incubated for 24 hrs.

* Scultra, Juvelook, and Facetem were additionally tested at concentrations of 0.1 mg/mL and 0.5 mg/mL to confirm the expression levels at lower concentrations.

* In case of Juvelook treatment, the expression level was confirmed after 48 hrs, not 24 hrs.

Group	Test article	Concentration (mg/mL)				
G1	No treatment	-	-	-	-	-
G2	Facetem	0.1	0.5	1	2	5
G3	Scultra	0.1	0.5	1	2	5
G4	Juvelook	0.1	0.5	1	2	5
G5	Radiesse	-	-	1	2	5
G6	ELLANSE	-	-	1	2	5

4.2.4. Remove the supernatant after culturing, analyze each gene for mRNA of COL1A1 and COL3A2 from the cell from Lysis buffer 1ml with qPCR (Total RNA Extraction kit, Intronbio, cat no.

17221).

COL1A1(size=153bp)	Forward:	5'-CCTGGATGCCATCAAAGTCT-3'
	Reverse:	5'-AATCCATCGGTCATGCTCTC-3'
COL3A2 (size=117bp)	Forward:	5'-TGGTCCCCAAGGTGTCAAAG-3'
	Reverse:	5'-GGGGGTCCTGGGTTACCATTA-3'
GAPDH (size=167bp)	Forward:	5'-GAAGGTCGGAGTCAACGGAT-3'
	Reverse:	5'-CGGTGCCATGGAATTTGCC-3'

5. Statistical analysis

The commercial statistical package SPSS Statistics 12.0K for Medical Science was used for all statistical analyses. The Kolmogorov-Smirnov test ensured that all data were normally distributed. Comparisons were made between G1 and G2-G6, and then between G2 and G3-G6 using Student's t-test (Welch's t-test in the case of unequal variance), with the significance level set at $P < 0.05$.

Results

1. CCK-8 assay (Figure 1-2; Table 1; Appendix 1)

No cytotoxicity was observed at concentrations of 0.1, 0.5, 1, 2, and 5 mg/mL in Facetem.

No cytotoxicity was observed at concentrations of 0.1, 0.5, 1, 2, and 5 mg/mL in Scultra.

No cytotoxicity was observed at concentrations of 0.1, 0.5, 1, 2, and 5 mg/mL in Juvelook.

No cytotoxicity was observed at concentrations of 0.1, 0.5, 1, 2, and 5 mg/mL in Radiesse.

No cytotoxicity was observed at concentrations of 0.1, 0.5, 1, 2, and 5 mg/mL in ELLANSE.

2. qPCR result (Figure 3-4; Table 2-4; Appendix 2)

2.1. *COL1A1* mRNA

The Facetem treatment group (G2) showed a significant increase in COL1A mRNA expression at 1 mg/mL concentration compared to the no treatment group (G1) (P<0.01), however the Scultra treatment group (G3) was observed to have a 21% increase compared to the no-treatment group (G1). *COL1A*1mRNA expression of the Facetem treatment group (G2) showed a significant increase compared to the Juvelook treatment group (G4), the Radiesse treatment group (G5), and the ELLANSE treatment group (G6) (P<0.01), but a tendency to increase by 26 % compared to the Scultra treatment group (G3).

The Facetem treatment group (G2), the Scultra treatment group (G3), and ELLANSE treatment group (G6) showed a significant increase in COL1A mRNA expression at 2 mg/mL concentration compared to the no-treatment group (G1) (P<0.05 or P<0.01). The Radiesse treatment group (G5) showed a tendency to increase by 12 % compared to the no-treatment group (G1). The Facetem treatment group (G2) showed a significant increase in *COL1A1*mRNA expression compared to the Juvelook treatment group (G4) and the Radiesse treatment group (G5) (P<0.05), and tendencies to increase by 16 % and 36 % compared to the Scultra treatment group (G3) and the ELLANSE treatment group (G6), respectively.

The Facetem treatment group (G2), the Scultra treatment group (G3), the Radiesse treatment group (G5), and the ELLANSE treatment group (G6) showed a significant increase in *COL1A1*mRNA expression at 5 mg/mL concentration compared to the no-treatment group (G1) (P<0.05 or P<0.01). The Facetem treatment group (G2) showed a significant increase in *COL1A1*mRNA expression compared to the Juvelook treatment group (G4) (P<0.01).

The Juvelook treatment group (G4) showed a significant increase at 0.5 mg/mL, 1 mg/mL, 2 mg/mL, and 5 mg/mL concentration (P<0.05 or P<0.01) and a tendency to increase by 29% at 0.1 mg/ mL concentration in *COL1A1*mRNA expression compared to the no-treatment group (G1) at 48 hrs.

2.2. *COL3A2* mRNA

The Facetem treatment group (G2), the Radiesse treatment group (G5), and the ELLANSE treatment group (G6) showed a significant increase in *COL3A2* mRNA expression at 1 mg/mL concentration compared to the no-treatment group (G1) ($P<0.05$ or $P<0.01$). However, the Scultra treatment group (G3) showed a tendency to increase by 24 % compared to the no-treatment group (G1). The Facetem treatment group (G2) showed a significant increase in *COL3A2* mRNA compared to the Juvelook treatment group (G3) ($P<0.05$), and showed a significant decrease in *COL3A2* mRNA compared to the ELLANSE treatment group (G6) ($P<0.05$).

The Facetem treatment group (G2), the Scultra treatment group (G3), the Radiesse treatment group (G5), and the ELLANSE treatment group (G6) showed a significant increase in *COL3A2* mRNA expression at 2 mg/mL concentration compared to the no-treatment group (G1) ($P<0.05$ or $P<0.01$). The Facetem treatment group (G2) showed a significant increase in *COL3A2* mRNA compared to the Scultra treatment group (G3), the Juvelook treatment group (G4), the Radiesse treatment group (G5), and the ELLANSE treatment group (G6) ($P<0.01$ or $P<0.05$).

The Facetem treatment group (G2), the Scultra treatment group (G3), the Radiesse treatment group (G5), and the ELLANSE treatment group (G6) showed a significant increase in *COL3A2* mRNA expression at 5 mg/mL concentration compared to the no-treatment group (G1) ($P<0.01$). The Facetem treatment group (G2) showed a significant increase in *COL3A2* mRNA compared to the Juvelook treatment group (G4) and the Radiesse treatment group (G5) ($P<0.01$ or $P<0.05$), and a significant decrease in *COL3A2* mRNA compared to the ELLANSE treatment group (G6) ($P<0.05$).

COL3A2 mRNA expression showed no change between the no-treatment group (G1) and the Juvelook treated group (G4) at 48 hrs. However, the Juvelook treated group (G4) showed a tendency to decrease in *COL3A2* mRNA expression at all concentrations compared to the Facetem treatment group (G2) after 24 hrs.

Discussion and Conclusion

Collagen, a major component of the extracellular dermis (ECM), maintains the skin hydration and elasticity [1]and is a major structural protein that accounts for 75% of the skin's dry weight[2]. Therefore, when there is a lack of collagen, the skin becomes less elastic and moisturized, leading to wrinkles and other signs of skin aging[3]. Collagen in the body is divided into more than 20 types, and types I, III, V, VI, and VII collagen exist in the skin dermis. In particular, collagen type I accounts for 80-85 % of the total collagen, and collagen type III accounts for 10-15 %[2].

This study was conducted using Hs27 cells to evaluate the concentration-dependent effects on the synthesis of collagen COL1A1 and COL3A2 by test articles Sculptra, Juvelook, Facetem, Radiesse, and ELLANSE.

Non cytotoxicity observed at all concentrations of the test articles Facetem, Scultra, Juvelook, Radiesse, and ELLANSE.

In the COL1A1 mRNA expression level, as measured by the qPCR method after 24 hrs of treatment, the Facetem treatment group, the Scultra treatment group, the Radiesse treatment group, and the ELLANSE treatment group exhibited a dose-dependent increase at concentrations of 1, 2, and 5 mg/mL compared to the no-treatment group.

The Facetem treatment group exhibited a significant increase or tendencies to increase in COL1A1 mRNA expression at concentrations of 1 and 2 mg/mL compared to the Scultra treatment group, the Radiesse treatment group, and the ELLANSE treatment group

The Facetem treatment group exhibited a significant increase in COL1A1 mRNA expression at concentrations of 1, 2, and 5 mg/mL compared to the Juvelook treatment group. Additionally, the Juvelook treatment group demonstrated a significant increase and a tendency to increase in COL1A1 mRNA expression at all concentrations compared to the no-treatment group.

After 24 hours of treatment, the COL3A2 mRNA expression levels, measured using the qPCR method, showed a dose-dependent increase in the Facetem treatment group, the Scultra treatment group, the Radiesse treatment group, and the ELLANSE treatment group at concentrations of 1, 2, and 5 mg/mL, compared to the no-treatment group.

The Facetem treatment group exhibited a significant increase in COL3A2 mRNA expression compared to the 2 mg/mL treatment groups of Scultra and ELLANSE, the 2 and 5 mg/mL treatment groups of Radiesse, and the 1, 2, and 5 mg/mL treatment groups of Juvelook. The Juvelook treatment group did not show a significant change in COL3A2 mRNA expression compared to the no-treatment group at all concentrations after 48 hours of treatment. Additionally, there was a tendency to decrease at all concentrations compared to the Facetem treatment group after 24 hours of treatment.

The tendency to decrease in the expression levels of COL1A1 and COL3A2 in the group administered with Facetem 5 mg/mL was judged to be due to the influence of the high concentration in the test system.

In summary, Facetem at a concentration of 2 mg/mL demonstrated higher collagen synthesis in Hs27 cells, that is, the expression levels of COL1A1 and COL3A2 than that of other test articles, and was determined to be more effective in collagen synthesis.

Reference

1. Wenging Zhu MM., Chengxhi Dong MM. Poly-L-Lactic acid increases collagen gene expression and synthesis in cultured dermal fibroblast (Hs68) through the TGF-β/Smad pathway. *J Cosmet Dermatol*. 2023 Apr; 22(4): 1213-1219.
2. Song P.Y., Jo H.S., et al., Emodin induces collagen type I synthesis in Hs27 human dermal fibroblasts. *EXPERIMENTAL AND THERAPEUTIC MEDICINE*. 2021; 21(5): 1792-0981.
3. Naoko T., Kentaro T.N., et al., Cinnamon Extract Promotes Type I Collagen Biosynthesis via Activation of IGF-I Signaling in Human Dermal Fibroblasts. *J. Agric. Food Chem.* 2012; 60(5): 1193–1200.

Units and Symbols

Note: The following lists of codes, abbreviations and units are used by CORESTEMCHEMON Inc.
Some, but not necessarily all, of this information may be needed for this report.

%	Percent	**hr**	Hour
º	Degree	**min**	Minute
C	Celsius	**sec**	Second
L	Liter	**rpm**	Revolution per Minute
dL	Deciliter	**RCF**	Relative Centrifugal Force
mL	Milliliter	**SE**	Standard Error
µL	Microliter	**CV**	Coefficient of Variation
g	Gram	**RE**	Relative Error
kg	Kilogram	**RH**	Relative Humidity
mg/mL	Milligram	**M**	Male
µg	Microgram	**F**	Female
ng	Nanogram	**NA**	Not Applicable
m	Meter	**N**	Number
cm	Centimeter	**SPF**	Specific Pathogen Free
mm	Millimeter	**TK**	Toxicokinetic
µm	Micrometer	**PK**	Pharmacokinetic
ppm	Parts per million	**AUC**	Area Under the Curve
ppb	Parts per billion	**C_{max}**	Maximum Concentration
wk	Week	**T_{max}**	Time at Maximum Concentration
d	Day	**$t_{1/2}$**	Half-life
GLP	Good Laboratory Practice Regu- lation	**SOP**	Standard Operating Procedures
QAU	Quality Assurance Unit	**ICH**	International Conference on Harmonization
OECD	Organization for Economic Co-operation and Development	**MFDS**	Ministry Food and Drug Safety
IACUC	Institutional Animal Care and Use Committee	**SPSS**	Statistical Package for the Social Sciences
HPLC	High-Performance Liquid Chro-matography	**LC-MS/MS**	Liquid Chromatography-Tandem Mass Spectrometry

FIGURES

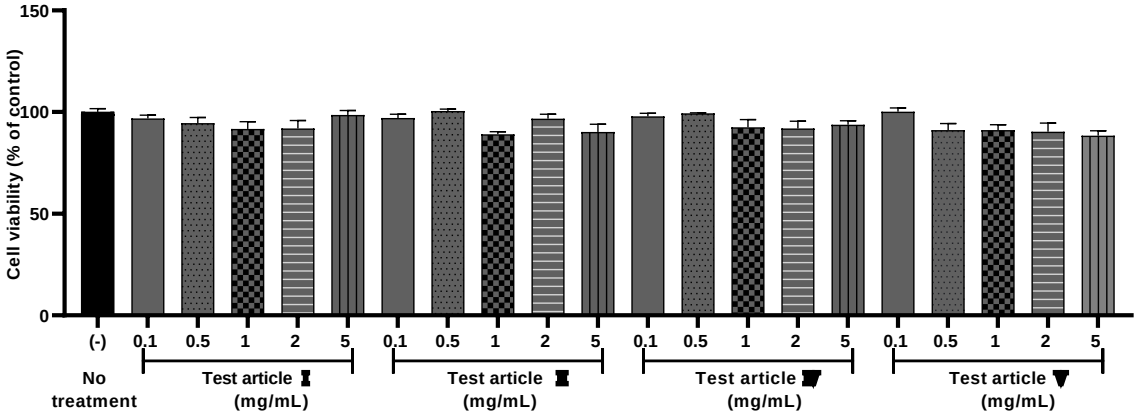

Figure 1. CCK-8 assay (%)
Data were represented by mean±S.E.

G1: No treatment (Cell only, n=4)
G2: Test article I (Facetem, n=4)
G3: Test article II (Scultra, n=4)
G5: Test article IV (Radiesse, n=4)
G6: Test article V (ELLANSE, n=4)

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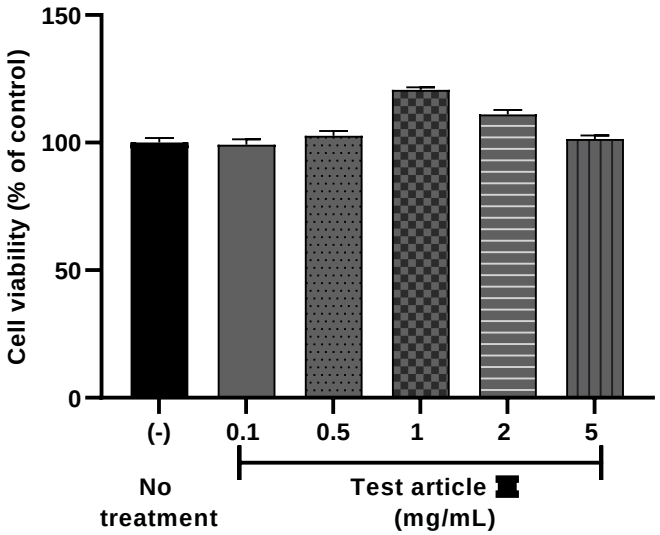

Figure 1. CCK-8 assay (%)
Data were represented by mean±S.E.

G1: No treatment (Cell only, n=4)
G4: Test article III (Juvelook, n=4)

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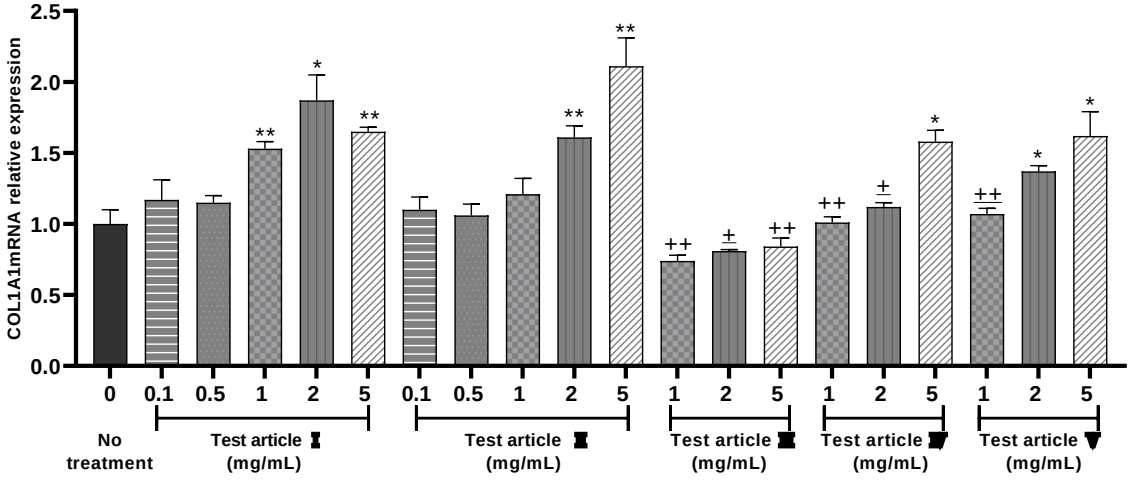

Figure 2. mRNA espression levels of *COL1A1*

Data were represented by mean±S.E. The results were statistically analyzed by *Student's t-test.*

**: significantly different from G1, $P<0.01$
*: significantly different from G1, $P<0.05$
++: significantly different from G2, $P<0.01$
+: significantly different from G2, $P<0.05$
Underline: not equal variance *(Welch's t-test)*

G1: No treatment (Cell only, n=3)
G2: Test article I (Facetem, n=3)
G3: Test article II (Scultra, n=3)
G4: Test article III (Juvelook, n=3)
G5: Test article IV (Radiesse, n=3)
G6: Test article V (ELLANSE, n=3)

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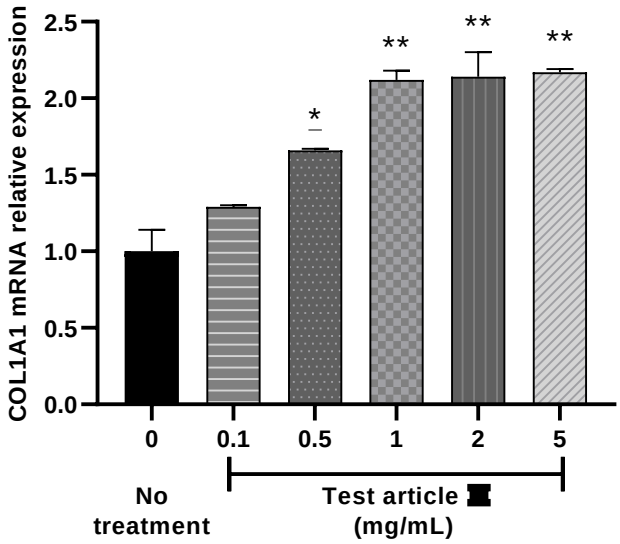

Figure 2. mRNA espression levels of *COL1A1*

Data were represented by mean±S.E. The results were statistically analyzed by *Student's t-test.*

**: significantly different from G1, $P<0.01$
*: significantly different from G1, $P<0.05$
Underline: not equal variance (Welch's t-test)

G1: No treatment (Cell only, n=3)
G4: Test article II (Juvelook, n=3)

<END>

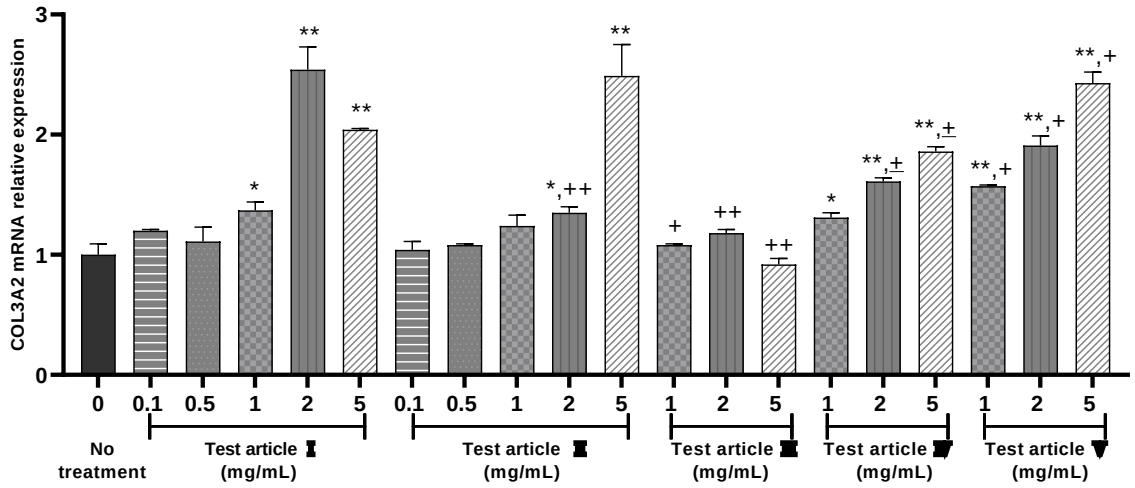

Figure 3. mRNA espression levels of *COL3A2*

Data were represented by mean±S.E. The results were statistically analyzed by *Student's t-test.*

**: significantly different from G1, $P<0.01$
*: significantly different from G1, $P<0.05$
++: significantly different from G2, $P<0.01$
+: significantly different from G2, $P<0.05$
Underline: not equal variance *(Welch's t-test)*

G1: No treatment (Cell only, n=3)
G2: Test article I (Facetem, n=3)
G3: Test article II (Scultra, n=3)
G4: Test article III (Juvelook, n=3)
G5: Test article IV (Radiesse, n=3)
G6: Test article V (ELLANSE, n=3)

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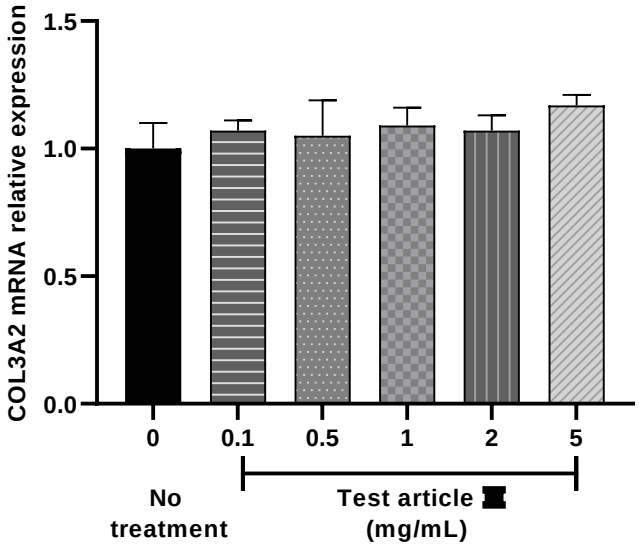

Figure 3. mRNA espression levels of *COL3A2*

Data were represented by mean±S.E. The results were statistically analyzed by *Student's t-test.*

**: significantly different from G1, $P<0.01$
*: significantly different from G1, $P<0.05$

G1: No treatment (Cell only, n=3)
G4: Test article III (Juvelook, n=3)

<END>

TABLES

Table 1. CCK-8 assay (%)

CONCENTRATION (mg/mL)		OD_{450nm}	CELL VIABILLITY (%)
No treatment		1.125±0.031	100.00±2.75
Test article I (Facetem)	0.1	1.089±0.019	96.76±1.66
	0.5	1.063±0.032	94.47±2.82
	1	1.032±0.039	91.69±3.48
	2	1.034±0.044	91.91±3.90
	5	1.108±0.025	98.49±2.24
Test article II (Scultra)	0.1	1.092±0.021	97.04±1.87
	0.5	1.131±0.010	100.53±0.88
	1	1.001±0.014	88.98±1.29
	2	1.088±0.024	96.73±2.17
	5	1.015±0.043	90.18±3.87
Test article IV (Radiesse)	0.1	1.101±0.017	97.82±1.50
	0.5	1.117±0.002	99.31±0.22
	1	1.040±0.042	92.47±3.78
	2	1.034±0.039	91.93±3.50
	5	1.054±0.022	93.71±1.98
Test article V (ELLANSE)	0.1	1.127±0.019	100.18±1.73
	0.5	1.025±0.035	91.11±3.13
	1	1.025±0.029	91.09±2.59
	2	1.016±0.048	90.33±4.25
	5	0.993±0.028	88.29±2.45

Data were represented by mean ± S.E.

G1: No treatment (Cell only, n=4)
G2: Test article I (Facetem, n=4)
G3: Test article II (Scultra, n=4)
G5: Test article IV (Radiesse, n=4)
G6: Test article V (ELLANSE, n=4)

<CONTINUED>

Table 1. CCK-8 assay (%)

CONCENTRATION (mg/mL)		OD_{450nm}	CELL VIABILLITY (%)
No treatment		1.430±0.026	100.00±1.85
Test article III (Juvelook)	0.1	1.418±0.031	99.14±2.20
	0.5	1.469±0.026	102.71±1.80
	1	1.725±0.015	120.67±1.07
	2	1.588±0.025	111.03±1.72
	5	1.451±0.019	101.45±1.30

Data were represented by mean ± S.E

G1: No treatment (Cell only, n=4)
G4: Test article III (Juvelook, n=4)

<END>

Table 2. mRNA expression levels of *COL1A1*

CONCENTRATION (mg/mL)		mRNA EXPRESSION LEVEL
No treatment		1.000±0.096
Test article I (Facetem)	0.1	1.171±0.140
	0.5	1.152±0.048
	1	1.532±0.023 **
	2	1.866±0.177 *
	5	1.646±0.026 **
Test article II (Scultra)	0.1	1.095±0.091
	0.5	1.064±0.083
	1	1.215±0.113
	2	1.609±0.080 **
	5	2.106±0.200 **
Test article III (Juvelook)	1	0.736±0.044 ++
	2	0.812±0.014 ±
	5	0.836±0.064 ++
Test article IV (Radiesse)	1	1.006±0.044 ++
	2	1.123±0.029 ±
	5	1.577±0.079 *
Test article V (ELLANSE)	1	1.072±0.039 ++
	2	1.367±0.063*
	5	1.618±0.165 *

Data were represented by mean±S.E. The results were statistically analyzed by *Student's t-test.*

**: significantly different from G1, $P<0.01$
*: significantly different from G1, $P<0.05$
++: significantly different from G2, $P<0.01$
+: significantly different from G2, $P<0.05$
Underline: not equal variance (*Welch's t-test*)

G1: No treatment (Cell only, n=3)
G2: Test article I (Facetem, n=3)
G3: Test article II (Scultra, n=3)
G4: Test article III (Juvelook, n=3)
G5: Test article IV (Radiesse, n=3)
G6: Test article V (ELLANSE, n=3)

<CONTINUED>

Table 2. mRNA expression levels of *COL1A1*(48 hr)

CONCENTRATION (mg/mL)		mRNA EXPRESSION LEVEL
No treatment		1.000±0.140
Test article II (Juvelook)	0.1	1.289±0.012
	0.5	1.656±0.010 *
	1	2.116±0.060 **
	2	2.137±0.165 **
	5	2.167±0.023 **

Data were represented by mean±S.E. The results were statistically analyzed by *Student's t-test*.

**: significantly different from G1, $P<0.01$
*: significantly different from G1, $P<0.05$
Underline: not equal variance (*Welch's t-test*)

G1: No treatment (Cell only, n=3)
G4: Test article III (Juvelook, n=3)

<CONTINUED>

Table 3. mRNA expression levels of *COL3A2*

CONCENTRATION (mg/mL)		mRNA EXPRESSION LEVEL
No treatment		1.000±0.092
Test article I (Facetem)	0.1	1.196±0.013
	0.5	1.106±0.121
	1	1.365±0.069 *
	2	2.536±0.192 **
	5	2.044±0.014 **
Test article II (Scultra)	0.1	1.040±0.065
	0.5	1.081±0.008
	1	1.236±0.087
	2	1.351±0.051 *, ++
	5	2.487±0.261 **
Test article III (Juvelook)	1	1.078±0.011 +
	2	1.177±0.032 ++
	5	0.920±0.078 ++
Test article IV (Radiesse)	1	1.308±0.040 *
	2	1.608±0.028 **, +
	5	1.862±0.040 **, +
Test article V (ELLANSE)	1	1.572±0.009 **, +
	2	1.910±0.077 **, +
	5	2.427±0.094 **, +

Data were represented by mean±S.E. The results were statistically analyzed by *Student's t-test.*

**: significantly different from G1, $P<0.01$
*: significantly different from G1, $P<0.05$
++: significantly different from G2, $P<0.01$
+: significantly different from G2, $P<0.05$
Underline: not equal variance (*Welch's t-test*)

G1: No treatment (Cell only, n=3)
G2: Test article I (Facetem, n=3)
G3: Test article II (Scultra, n=3)
G4: Test article III (Juvelook, n=3)
G5: Test article IV (Radiesse, n=3)
G6: Test article V (ELLANSE, n=3)

<CONTINUED>

Table 3. mRNA expression levels of *COL3A2* (48 hr)

CONCENTRATION (mg/mL)		mRNA EXPRESSION LEVEL
No treatment		1.000±0.096
Test article II (Juvelook)	0.1	1.067±0.037
	0.5	1.052±0.143
	1	1.086±0.066
	2	1.068±0.058
	5	1.167±0.041

Data were represented by mean±S.E. The results were statistically analyzed by *Student's t-test.*

G1: No treatment (Cell only, n=3)
G4: Test article III (Juvelook, n=3)

<END>

APPENDICES

Appendix 1. Individual optical density$_{450nm}$ by CCK-8 assay

CCK-8 ASSAY (OD_{450nm})	
WELL ID	No treatment (Cell only)
1	1.191
2	1.162
3	1.104
4	1.146
MEAN	1.125
S.E.	0.031
N	4
WELL ID	Test article I (Facetem) 0.1 mg/mL
1	1.033
2	1.103
3	1.105
4	1.113
MEAN	1.089
S.E.	0.019
N	4
WELL ID	Test article I (Facetem) 0.5 mg/mL
1	1.113
2	0.985
3	1.037
4	1.116
MEAN	1.063
S.E.	0.032
N	4

<CONTINUED>

Appendix 1. Individual optical density$_{450nm}$ by CCK-8 assay

CCK-8 ASSAY (OD_{450nm})	
WELL ID	Test article I (Facetem) 1 mg/mL
1	0.965
2	1.083
3	0.964
4	1.114
MEAN	1.032
S.E.	0.039
N	4
WELL ID	Test article I (Facetem) 2 mg/mL
1	0.995
2	0.938
3	1.142
4	1.061
MEAN	1.034
S.E.	0.044
N	4
WELL ID	Test article I (Facetem) 5 mg/mL
1	1.079
2	1.17
3	1.126
4	1.057
MEAN	1.108
S.E.	0.025
N	4

<CONTINUED>

Appendix 1. Individual optical density$_{450nm}$ by CCK-8 assay

CCK-8 ASSAY (OD_{450nm})	
WELL ID	Test article II (Scultra) 0.1 mg/mL
1	1.104
2	1.057
3	1.146
4	1.060
MEAN	1.092
S.E.	0.021
N	4
WELL ID	Test article II (Scultra) 0.5 mg/mL
1	1.111
2	1.142
3	1.153
4	1.118
MEAN	1.131
S.E.	0.010
N	4
WELL ID	Test article II (Scultra) 1 mg/mL
1	1.021
2	0.963
3	0.994
4	1.026
MEAN	1.001
S.E.	0.014
N	4
WELL ID	Test article II (Scultra) 2 mg/mL
1	1.150
2	1.089
3	1.083
4	1.031
MEAN	1.088
S.E.	0.024
N	4
WELL ID	Test article II (Scultra) 5 mg/mL
1	1.102
2	0.949
3	0.931
4	1.076
MEAN	1.015
S.E.	0.043
N	4

<CONTINUED>

Appendix 1. Individual optical density$_{450nm}$ by CCK-8 assay

CCK-8 ASSAY (OD_{450nm})	
WELL ID	Test article IV (Radiesse) 0.1 mg/mL
1	1.128
2	1.052
3	1.118
4	1.104
MEAN	1.101
S.E.	0.017
N	4
WELL ID	Test article IV (Radiesse) 0.5 mg/mL
1	1.111
2	1.118
3	1.117
4	1.123
MEAN	1.117
S.E.	0.002
N	4
WELL ID	Test article IV (Raiesse) 1 mg/mL
1	0.915
2	1.097
3	1.089
4	1.060
MEAN	1.040
S.E.	0.042
N	4
WELL ID	Test article IV (Radiesse) 2 mg/mL
1	1.075
2	0.928
3	1.109
4	1.025
MEAN	1.034
S.E.	0.039
N	4
WELL ID	Test article IV (Radiesse) 5 mg/mL
1	1.072
2	1.009
3	1.028
4	1.108
MEAN	1.054
S.E.	0.022
N	4

<CONTINUED>

Appendix 1. Individual optical density$_{450nm}$ by CCK-8 assay

CCK-8 ASSAY (OD_{450nm})	
WELL ID	Test article V (ELLANSE) 0.1 mg/mL
1	1.155
2	1.086
3	1.102
4	1.165
MEAN	1.127
S.E.	0.019
N	4
WELL ID	Test article V (ELLANSE) 0.5 mg/mL
1	1.089
2	0.990
3	0.943
4	1.078
MEAN	1.025
S.E.	0.035
N	4
WELL ID	Test article V (ELLANSE) 1 mg/mL
1	1.108
2	1.022
3	0.983
4	0.986
MEAN	1.025
S.E.	0.029
N	4
WELL ID	Test article V (ELLANSE) 2 mg/mL
1	1.099
2	1.099
3	0.937
4	0.930
MEAN	1.016
S.E.	0.048
N	4
WELL ID	Test article V (ELLANSE) 5 mg/mL
1	1.062
2	0.994
3	0.990
4	0.927
MEAN	0.993
S.E.	0.028
N	4

<CONTINUED>

Appendix 1. Individual optical density$_{450nm}$ by CCK-8 assay

CCK-8 ASSAY (OD_{450nm})	
WELL ID	No treatment (Cell only)
1	1.500
2	1.409
3	1.435
4	1.375
MEAN	1.430
S.E.	0.026
N	4
WELL ID	Test article III (Juvelook) 0.1 mg/mL
1	1.419
2	1.346
3	1.406
4	1.499
MEAN	1.418
S.E.	0.031
N	4
WELL ID	Test article III (Juvelook) 0.5 mg/mL
1	1.498
2	1.415
3	1.436
4	1.525
MEAN	1.469
S.E.	0.026
N	4

<CONTINUED>

Appendix 1. Individual optical density$_{450nm}$ by CCK-8 assay

CCK-8 ASSAY (OD_{450nm})	
WELL ID	Test article III (Juvelook) 1 mg/mL
1	1.697
2	1.757
3	1.746
4	1.701
MEAN	1.725
S.E.	0.015
N	4
WELL ID	Test article III (Juvelook) 2 mg/mL
1	1.624
2	1.546
3	1.636
4	1.544
MEAN	1.588
S.E.	0.025
N	4
WELL ID	Test article III (Juvelook) 5 mg/mL
1	1.469
2	1.492
3	1.408
4	1.433
MEAN	1.451
S.E.	0.019
N	4

<END>

Appendix 2. Individual *COL1A1*, *COL3A2* mRNA expression levels

mRNA EXPRESSION LEVEL		
	G1: No treatment (Cell only)	
WELL ID	*COL1A1*	*COL3A2*
1	0.823	0.840
2	1.027	1.003
3	1.151	1.157
MEAN	1.000	1.000
S.E.	0.096	0.092
N	3	3
	G2: Test article I (Facetem) 0.1 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	0.970	1.206
2	1.103	1.212
3	1.441	1.169
MEAN	1.171	1.196
S.E.	0.140	0.013
N	3	3
	G2: Test article I (Facetem) 0.5 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.239	1.343
2	1.147	1.030
3	1.072	0.945
MEAN	1.152	1.106
S.E.	0.048	0.121
N	3	3

<CONTINUED>

Appendix 2. Individual *COL1A1*, *COL3A2* mRNA expression levels

mRNA EXPRESSION LEVEL		
	G2: Test article I (Facetem) 1 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.485	1.374
2	1.554	1.242
3	1.557	1.479
MEAN	1.532	1.365
S.E.	0.023	0.069
N	3	3
	G2: Test article I (Facetem) 2 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.519	2.161
2	1.978	2.653
3	2.100	2.793
MEAN	1.866	2.536
S.E.	0.177	0.192
N	3	3
	G2: Test article I (Facetem) 5 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.608	2.034
2	1.635	2.071
3	1.695	2.025
MEAN	1.646	2.044
S.E.	0.026	0.014
N	3	3

<CONTINUED>

Appendix 2. Individual *COL1A1*, *COL3A2* mRNA expression levels

mRNA EXPRESSION LEVEL		
	G3: Test article II (Scultra) 0.1 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	0.956	0.931
2	1.063	1.031
3	1.266	1.156
MEAN	1.095	1.040
S.E.	0.091	0.065
N	3	3
	G3: Test article II (Scultra) 0.5 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	0.918	1.094
2	1.068	1.081
3	1.206	1.067
MEAN	1.064	1.081
S.E.	0.083	0.008
N	3	3
	G3: Test article II (Scultra) 1 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	0.994	1.150
2	1.364	1.410
3	1.286	1.149
MEAN	1.215	1.236
S.E.	0.113	0.087
N	3	3
	G3: Test article II (Scultra) 2 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.450	1.272
2	1.703	1.447
3	1.673	1.333
MEAN	1.609	1.351
S.E.	0.080	0.051
N	3	3
	G3: Test article II (Scultra) 5 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.711	2.217
2	2.360	3.008
3	2.246	2.237
MEAN	2.106	2.487
S.E.	0.200	0.261
N	3	3

<CONTINUED>

Appendix 2. Individual *COL1A1*, *COL3A2* mRNA expression levels

mRNA EXPRESSION LEVEL		
	G4 Test article III (Juvelook) 1 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	0.743	1.098
2	0.657	1.059
3	0.808	1.077
MEAN	0.736	1.078
S.E.	0.044	0.011
N	3	3
	G4: Test article III (Juvelook) 2 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	0.826	1.113
2	0.784	1.212
3	0.826	1.207
MEAN	0.812	1.177
S.E.	0.014	0.032
N	3	3
	G4: Test article III (Juvelook) 5 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	0.712	0.824
2	0.924	0.972
3	0.870	0.965
MEAN	0.836	0.920
S.E.	0.064	0.048
N	3	3

<CONTINUED>

Appendix 2. Individual *COL1A1*, *COL3A2* mRNA expression levels

mRNA EXPRESSION LEVEL		
	G5: Test article IV (Radiesse) 1 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	0.997	1.244
2	0.935	1.299
3	1.086	1.381
MEAN	1.006	1.308
S.E.	0.044	0.040
N	3	3
	G5: Test article IV (Radiesse) 2 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.064	1.661
2	1.151	1.567
3	1.153	1.596
MEAN	1.123	1.608
S.E.	0.029	0.028
N	3	3
	G5: Test article IV (Radiesse) 5 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.554	1.811
2	1.454	1.940
3	1.724	1.836
MEAN	1.577	1.862
S.E.	0.079	0.040
N	3	3

<CONTINUED>

Appendix 2. Individual *COL1A1*, *COL3A2* mRNA expression levels

mRNA EXPRESSION LEVEL		
	G6: Test article V (ELLANSE) 1 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.088	1.559
2	0.998	1.589
3	1.129	1.568
MEAN	1.072	1.572
S.E.	0.039	0.009
N	3	3
	G6: Test article V (ELLANSE) 2 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.320	1.771
2	1.290	1.924
3	1.491	2.035
MEAN	1.367	1.910
S.E.	0.063	0.077
N	3	3
	G6: Test article V (ELLANSE) 5 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.791	2.601
2	1.287	2.278
3	1.774	2.401
MEAN	1.618	2.427
S.E.	0.165	0.094
N	3	3

<CONTINUED>

Appendix 2. Individual *COL1A1*, *COL3A2* mRNA expression levels

mRNA EXPRESSION LEVEL		
	G1: No treatment (Cell only)	
WELL ID	*COL1A1*	*COL3A2*
1	1.272	1.192
2	0.806	0.905
3	0.921	0.904
MEAN	1.000	1.000
S.E.	0.140	0.096
N		
	G4: Test article III (Juvelook) 0.1 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.266	1.112
2	1.301	1.094
3	1.301	0.994
MEAN	1.289	1.067
S.E.	0.012	0.037
N	3	3
	G4: Test article III (Juvelook) 0.5 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.658	0.875
2	1.677	0.946
3	1.642	1.335
MEAN	1.659	1.052
S.E.	0.010	0.143
N	3	3

<CONTINUED>

Appendix 2. Individual *COL1A1*, *COL3A2* mRNA expression levels

mRNA EXPRESSION LEVEL		
	G4: Test article III (Juvelook) 1 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	2.148	1.079
2	2.000	0.975
3	2.199	1.202
MEAN	2.116	1.086
S.E.	0.060	0.066
N	3	3
	G4: Test article III (Juvelook) 2 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	1.809	0.951
2	2.276	1.133
3	2.325	1.119
MEAN	2.137	1.068
S.E.	0.165	0.058
N	3	3
	G4: Test article III (Juvelook) 5 mg/mL	
WELL ID	*COL1A1*	*COL3A2*
1	2.131	1.094
2	2.209	1.171
3	2.160	1.236
MEAN	2.167	1.167
S.E.	0.023	0.041
N	3	3

<END>