Research Article

Autologous cell therapy for aged human skin: a randomised, placebo-controlled, phase-I study

Susanne Grether-Beck[1], Alessandra Marini[1], Thomas Jaenicke[1], Petra Goessens-Rück[2], Kevin John McElwee[3,4], Rolf Hoffmann[3], Jean Krutmann[1,5]

https://doi.org/10.1159/000502240

[1] in vivo unit, IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany

[2] CATB, Hofheim, Germany

[3] Replicel Life Sciences Inc, Vancouver, Canada

[4] Centre for Skin Sciences, University of Bradford, Bradford, UK

[5] Medical Faculty, Heinrich Heine University, Düsseldorf, Germany

Drs Grether-Beck and Marini are co-first authors; Profs Krutmann and Hoffmann are co-last authors.

Corresponding Author

Prof. Dr. Jean Krutmann,

IUF – Leibniz Research Institute for Environmental Medicine

Auf'm Hennekamp 50

40225 Düsseldorf, Germany

Keywords: Autologous cell therapy • aged human skin • extracellular matrix • randomized, double-blind, placebo-controlled, phase-I study

Abbreviations used:

AE: adverse event

ALT: Alanine aminotransferase

AP: alkaline phosphatase

AST: Aspartate aminotransferase

BUN:	Blood urea nitrogen
cGMP:	current Good Manufacturing Practice
CRO:	Contract Research Organization
CRP:	C reactive protein
CTGF:	Connective tissue growth factor
COL1A1:	Collagen 1A1
COL1A2:	Collagen 1A2
COL3A1:	Collagen 3A1
DMSO:	Dimethyl sulfoxide
ECM:	Extracellular matrix
LUM:	Lumican
M-MLV RT:	Moloney Murine Leukemia Virus Reverse Transcriptase
MCHC:	Mean corpuscular hemoglobin concentration
MCV:	Mean corpuscular volume
mRNA:	messenger RNA
NBDS:	Non-bulbar dermal sheath
PCR:	Polymerase chain reaction
RBC:	Red blood cell
RCS-01:	Replicel skin (treatment) version 1
TGFß1:	Transforming growth factor beta 1
SAASP:	skin-ageing associated secretory phenotype
SNK:	Student-Newman-Keuls
WBC:	white blood cell

Abstract

Introduction: Skin ageing involves senescent fibroblast accumulation, disturbance in extracellular matrix (ECM) homeostasis, and decreased collagen synthesis.

Objective: to assess a cell therapy product for aged skin (RCS-01) consisting of ~25 x 10^6 cultured, autologous cells derived from anagen hair follicle non-bulbar dermal sheath (NBDS).

Methods: For each subject in the verum group, four areas of buttock skin were injected intra-dermally 1 or 3 times at monthly intervals with RCS-01, cryomedium, or needle penetration without injection; in the placebo group RCS-01 was replaced by cryomedium. The primary endpoint was assessment of local adverse event profiles. As secondary endpoints, expression of genes related to ECM homeostasis was assessed in biopsies from randomly selected volunteers in the RCS-01 group taken 4 weeks after the last injection.

Results: Injections were well tolerated with no severe adverse events reported 1 year after first injection. When compared with placebo treated skin, a single treatment with RCS-01 resulted in a significant upregulation of TGFß1, CTGF, COL1A1, COL1A2, COL3A1, and lumican mRNA expression.

Limitations: The cohort size was insufficient for dose ranging evaluation and subgroup analyses of efficacy.

Conclusions: RCS-01 therapy is well tolerated and associated with a gene expression response consistent with an improvement of ECM homeostasis.

Introduction

There is growing evidence that ageing of human skin is driven by the senescence of fibroblasts located in the upper dermis. Cells expressing markers of senescence are present at increased frequencies in aged skin of primates and humans [1, 2]. Primary skin fibroblasts isolated from intrinsically aged human skin exhibit molecular hallmarks of cellular senescence, including a secretory factor profile which is indicative of incipient cell senescence [3].This secretory phenotype of ~70 proteins, secreted in an age-dependent manner, are collectively termed the skin-ageing associated secretory phenotype (SAASP). Functional annotation analysis revealed that these proteins can be assigned to 14 different biological processes, among which extracellular matrix (ECM) organization was the most prominent. A detailed enrichment and network analysis using the "Reactome database" revealed that "ECM organization", "elastic fiber formation", "activation of matrix metalloproteinases" and "collagen degradation" categories had the strongest association with intrinsic skin ageing. These four biological processes are closely linked to ECM remodelling in general, and are of obvious relevance for defining the clinical, histological and molecular features of aged human skin. They are linked to a profound disturbance in collagen homeostasis because of a decrease in de novo collagen synthesis and an increase in collagen degradation [4]. The secretion of these proteins by senescent fibroblasts may be of pathogenetic relevance for the ageing process of human skin and, potentially, modulation of this secretion pattern might alter skin ageing.

The emerging field of regenerative medicine focuses on stem cell, progenitor cell and tissue-based therapies. It involves exploitation of cells and their proliferative capacity, potential to differentiate, paracrine signaling activity, and because they can be sourced autologously avoiding implant rejection or the need for immunosuppressive therapy [5].

To this end, we developed a cell-therapeutic approach based on the intradermal injection of autologous, cultured mesenchymal cells, isolated from the non-bulbar dermal sheath (NBDS) of hair follicles. The NBDS represents a unique source of fibroblasts, which express several proteins that potentially support ECM maintenance, most notably type 1 collagen [6-8]. Cultured NBDS cells provide a promising platform to treat intrinsically or extrinsically aged/damaged skin such as fine wrinkles by implanting UV naïve, collagen-producing NBDS cells directly to the affected area. As a first clinical step, we conducted a first-in-human clinical trial to determine, as a primary objective, the safety profile of this cell-therapeutic treatment, as defined by the incidence, causality, severity and seriousness of adverse events (AEs). In addition, as a secondary objective, we analyzed if and how this treatment affects gene expression in human skin. We focused on genes, which were previously found to be associated with skin ageing and linked to proteins, which are part of the SAASP. We

hypothesised that injecting autologous, cultured NBDS cells would be safe and would modify the local human skin gene expression profile consistent with increased collagen production and a reduced SAASP profile.

Materials and Methods

Study Design and Subjects

This clinical study was conducted at the IUF – Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany. It was approved by the ethical committee of the Heinrich Heine University in Düsseldorf, Germany, (study No MO-LKP-779; ClinicalTrials.gov Identifier: NCT02391935) and received clearance by the Paul Ehrlich Institute competent authority for cell therapy in Germany. The study design was a phase-I, randomized, double-blind, placebo controlled, single-centre study. Inclusion and exclusion criteria were as described (Table SI) A total of 26 subjects were screened, of which 21 were randomized and 17 completed the study protocol (demographic data in Table SII).

Treatments

The study was initiated on October 06th 2015 and the last study visit was conducted on May 16th, 2017. The study included a total of 14 visits, i.e. 3 screening visits and 11 visits during the treatment and observation period. At visit 1, visit 3 and visit 5 (Fig. 1, A), study subjects were injected intra-dermally with a cell therapy product (RCS-01; verum) manufactured from the subject's own (autologous) NBDS cells isolated from hair follicles, or they received the respective placebo. For this, a punch biopsy was taken at IUF from the back of the subject's scalp (occiput) at the second screening visit (visit S2; Fig. 1, A). The tissue sample was then transferred to biopsy media and subsequently shipped to the cGMP manufacturer of the RCS-01 product (Innovacell Biotechnologie AG, Innsbruck, Austria). At the manufacturing site, the tissue biopsy was dissected to isolate hair follicle non-bulbar dermal sheaths, which were then put into culture during which the NBDS cells were replicated to achieve 25.0 10^6 ± 3.0 x 10^6 NBDS cells per individual batch. The NBDS cells were then suspended in cryomedium composed of Ringer's lactate containing 2% human serum albumin and 5% DMSO. RCS-01, as well as placebo, were supplied by the manufacturer in coded, single-dose vials. Placebo vials contained cryomedium only. Vials containing RCS-01 or placebo were then shipped from the manufacturing site to the study site and kept below -130°C until administration. At the study site, verum and placebo products were injected in a volume of 1.0 ml evenly distributed in six injections throughout the approximately 2 cm^2 area. A syringe holder controlled depth, angle and volume of the injections into the dermis of the buttock. Sham injections involved only needle penetration with no fluid injected. (Figs 1-2).

Randomization and Masking

Randomization of subjects to the two treatment groups (RCS-01 group and placebo group) in a 4:1 ratio and the two post-treatment biopsy groups (visit 7 biopsy group and visit 9 biopsy group; Fig. 1,

B) used two separate randomization lists, which were computer generated by Pharmalog Institut für Klinische Forschung GmbH, Munich, Germany, which served as the CRO in this study. The vehicle treated area in the group of RCS-01 receiving volunteers served as an intraindividual control for evaluation of local AEs as compared to the verum treated areas in the same volunteer, while the smaller group only receiving placebo served as a control for systemic AEs.

The randomization ratio used for allocation to one of the injections sites of the four (RCS-group) / two (placebo group) treatment patterns was 1:1:1:1 and 1:1, respectively (Fig. 2). As RCS-01 and placebo were visually different, an unblinded study team member not involved in any post administration evaluations performed all injections, including sham injections, at visits 1, 3 and 5. Related information was kept separately from other records and was accessible only to the unblinded study team member.

Safety Parameters

Clinical safety assessments included height, body weight, vital signs, an abbreviated clinical examination, serology laboratory assessments according to the exclusion criteria, safety laboratory assessments including haematology (RBC, haematocrit, MCV, MCHC, reticulocytes, WBC with differential, platelet count), biochemistry (sodium, potassium, creatinine, total protein, AP, total cholesterol, ALT, AST, BUN, CRP), and dipstick urinalysis (protein, glucose, ketones, bilirubin, blood, urobilinogen, pH, specific gravity, nitrite, hemoglobin and leukocytes), investigator assessment of the treatment evaluation sites for local intolerance (solicited local adverse events: erythema, bruising / haemorrhage, pyoderma / infection, eczema, granuloma / papules / nodules, hypertrichosis / hair density, hypopigmentation, hyperpigmentation, scar), and adverse events (AEs). A detailed definition of the AEs is given in Table 1. In addition, histopathological evaluation of treatment sites was performed on biopsies obtained at visit 9 (Fig. 1, A).

Efficacy Parameters

Gene expression studies used total RNA which was extracted from punch biopsies obtained from all four treatment sites at visit 7 (Fig. 1, A) as previously described [9-12]. Frozen biopsies were disrupted in lysis buffer from an RNA isolation kit (Peq-Gold Total RNA Kit (Peqlab, Erlangen, Germany) using a Mixer Mill MM300 (Retsch, Haan, Germany) three times for 3 minutes with 30Hz. Isolated RNA was photometrically quantified and 50 ng total RNA was used for cDNA synthesis with M-MLV reverse transcriptase (Life Technologies, Karlsruhe, Germany). An aliquot was used for pipetting PCR reactions with ABsolute QPCR SYBR Green Mix (Thermo Fisher Scientific, St. Leon, Germany) with the help of an epMotion 5070 pipetting device (Eppendorf, Wesseling-Berzdorf). PCR

reactions, using primer sequences as described (Supplemental Table SIII), were performed in a CFX384 Real-Time PCR Detection System (Bio-Rad Laboratories GmbH, München, Germany) after 15 minutes at 94° C for activation of hot start Taq polymerase. Cycles consisted of 20 seconds denaturation (95° C), 20 seconds annealing (55° C) and 20 seconds extension (72° C). For comparison of relative gene expression in real time PCR the $2(-\Delta\Delta C(T))$ method was used [13].

Pro-collagen ELISA Assay

Cultures of NBDS cells were examined for expression of pro-collagen (n=5). Briefly, NBDS cells were cultured using the clinical trial protocols. Cells were plated post collection at the end of passage 4 of primary culture (8×10^4 cells per well in a 6-well plate; Corning, Tewksbury, MA) in duplicates; equivalent to the point at which cells are injected in the clinical trial. Culture supernatants were sampled at 0, 3, and 6 hours post plating, and the concentration of pro-collagen type I was quantified according to manufacturer's instructions (Procollagen Type I C-Peptide EIA Kit, Takara-Bio, Mountain View, CA).

Statistical Analysis of Gene Expression

Data are given as x-fold induction as compared to a placebo control as median [25% to 75% percentiles]. Statistical evaluation and graphical presentation was performed with SigmaPlot 12.3 (Systat Software GmbH, Erkrath, Germany). Normality of the data was tested using Shapiro-Wilk. For comparison of significant differences, Kruskal-Wallis one-way analysis or variance on ranks was employed. As post hoc analysis, the SNK test was used. A probability level of $p<0.05$ was considered significant. Data are presented as box plots with median and corresponding percentiles.

Results

A total of 26 subjects (Supplemental Table SII) were screened at screening visit 1. From these, five were not randomized due to the presence of exclusion criteria (n=3), withdrawal of consent (n=1) or onset of an adverse event (n=1) that prevented the subject returning for screening visit 2. The remaining 21 subjects had a scalp biopsy and were randomized to the two treatment groups at screening visit 2 (Fig. 2). From these, 4 subjects, which had been randomized to the verum treatment group, did not receive any intradermal injections of study products because NBDS cell cultivation was not successful due to poor biopsy quality.

Of the 17 subjects (15 females and 2 males, mean age of 55.1 years) which completed the study, 13 (76.5%) were in the RCS-01 group (Fig. 2), i.e. they received intradermal injections of RCS-01 and placebo and sham injections, whereas 4 subjects (23.5 %) were in the placebo group (Fig. 2), i.e. they received intradermal injections of placebo and sham injections, but not RCS-01 injections. This approximately reflects the intended ratio of 4:1. All 17 subjects also completed the 44-weeks post injection follow-up period.

The safety data of the 13 individuals in the RCS-01 subgroup demonstrated a good local and systemic safety profile of RCS-01 injections up to 1 year after single and triple intradermal injections. Serious local or systemic AEs did not occur. There was no evidence of systemic toxicity. No clinically relevant changes were observed in mean values of systolic / diastolic blood pressure, pulse rate, body temperature and body weight of the treated subjects. There were neither clinically significant abnormal laboratory test results shortly after study treatment, nor any clinically relevant changes during the course of the study. Fourteen out of 17 treated subjects reported at least 1 local and / or systemic AE (Table 1). One out of 13 subjects treated in the RCS-01 group and one out of 4 subjects treated in the Placebo group experienced at least 1 systemic AE after the first treatment (treatment-emergent AEs). All systemic AEs were considered unrelated or unlikely related to the study treatment and had resolved by the end of the observation period.

Ten subjects out of 13 in the RCS-01 group, and all 4 subjects in the placebo group, presented with at least one solicited local AE of mild to moderate intensity, which were all transient in nature. Mild bruising (haemorrhage) at the injection site was the most common local AE with a similar occurrence after intradermal administration of RCS-01 and placebo and sham injections.

Repeated intradermal injection of RCS-01 was associated with a higher occurrence of granuloma, papules or nodules compared to control injection of cryomedium (placebo). Hyperpigmentation, eczema and erythema was observed rarely and showed no increased occurrence after repeated

treatment with RCS-01 and placebo. There were no signs of pyoderma (infection), hypertrichosis and hypopigmentation at the injection sites of RCS-01 or placebo. In addition, histopathological assessment of 32 treatment sites from 8 subjects, which were biopsied 4 months after the last injection, revealed no abnormalities or structural changes (data not shown).

In terms of pro-collagen type C peptide expression post production (after passage 4), NBDS cells exhibited relatively consistent levels of pro-collagen expression (mean at 6 hrs., 57ng /ml/8 x 10^4 cells; range, 42ng – 69ng) in culture supernatants (Fig. 3).

Gene expression analysis was performed using biopsies obtained from 7 subjects of the RCS-01 group at visit 7 (Fig. 1, A), i.e. 1 month after the last injections with RCS-01 and placebo. A single injection of RCS-01 caused a significant increase in mRNA expression of all genes, as compared with mRNA expression of these genes in placebo treated skin (Supplemental Table SIV). Notably, although we did not assess the impact on clinical signs of aging such as wrinkle formation, some of the strongest responses were observed for TGFß1, and also for genes involved in *de novo* collagen synthesis such as COL1A1, COL1A2 and COL3A1 (Fig. 4). Increased gene expression was also observed when RCS-01 was injected 3 times, but overall the response was weaker than that observed after single treatments and mainly included COL1A1, COL1A2 and COL3A1.

Discussion/Conclusion

In this study we show for the first time that single and repeated injections of NBDS-derived autologous cells into the dermis of human buttock skin are well tolerated and do not cause systemic or local adverse reactions for a period of up to 1 year after the first injection. We also provide evidence that this cell-therapeutic approach alters the transcriptional expression profile of genes, which are involved in ECM homeostasis. Specifically, we have found that in comparison to placebo treated skin areas, injection of NBDS-derived fibroblasts induces the expression of TGFß1, CTGF, COL1A1, COL1A2, COL3A1 and lumican.

These genes encode for proteins which are important for ECM homeostasis, thought to be of pathogenetic relevance for skin ageing, where levels of types I and III collagen precursors and crosslinks are reduced [14]. Accordingly, TGFß1 is the major profibrotic cytokine and, together with CTGF, synergistically stimulates type 1 procollagen synthesis in adult human fibroblasts [15]. Also, COL1A1 and COL1A2 are crucial for de novo synthesis of type 1 collagen chains, which are markedly decreased in aged human skin [14-16]. Lumican belongs to small leucine-rich proteoglycan family and is involved in collagen fibril formation in skin [17]. Upon UV irradiation, lumican expression is decreased in human dermal fibroblasts, which might contribute to downregulation of procollagen I in UV-irradiated skin [18].

The present observation that injection of NBDS-derived cells is associated with an increased transcriptional expression of these genes indicates the possibility that cell therapy with RCS-01 might be able to ameliorate the clinical signs of skin ageing, such as wrinkles. This assumption is in line with observations that increased expression of collagen type 1 and type 3 is a prerequisite for wrinkle reduction caused by retinol [19]. We therefore believe that our observations warrant corresponding phase -2 studies to directly assess this possibility. Insufficient cohort size for dose ranging evaluation and subgroup analyses of efficacy was the primary limitation of the current study. Future clinical studies will explore dose responses, the possibility of single and multiple injections involving smaller doses or spaced over longer periods.

The present study does not provide any information about the mechanism underlying RCS-01-induced gene expression in human skin. Since RCS-01 treatment increased the expression of genes, which are primarily expressed by dermal fibroblasts, we believe that RCS-01 mainly acts at the level of the dermis. It should be noted, however, that in this study full thickness punch biopsies were analyzed for gene expression without further separation into dermal and epidermal compartments. We can therefore not rule out that RCS-01 can also have effects on epidermal cells. Increased mRNA expression was not observed in skin areas, which were injected with placebo only, or sham injected,

indicating that modulation of gene expression was not due to the presence of the cryomedium or to mechanical effects caused by the injection procedure per se, but instead required the intradermal presence of the injected NBDS-derived fibroblasts. Further studies will have to clarify how these injected cells can modulate the gene expression pattern expressed in the injected skin sites.

Statements

Acknowledgement

The authors would like to thank Gigi Leung, Heidi Brenden, Dr. Hisae Nakamura, Darrell Panich, and Dr. Rainer Marksteiner for their assistance in the clinical trial and associated research presented in this study.

Statement of Ethics

IRB approval status: Reviewed and approved by Ethikkommission of Medical Faculty of Heinrich Heine University Düsseldorf (dated from June 29, 2015) study No: MO-LKP-779; Registration ID: 2015033322. And by Federal Institute of Vaccines and Biomedicines Langen (dated from August 19, 2015). Clinical trials.gov database identifier: NCT02391935 first posted March 18, 2015

Disclosure Statement

Dr Goessens-Rück has received consultancy fees from Replicel Life Sciences Inc. and is a third-party regulatory and clinical consultant to Replicel Life Sciences Inc.

Dr McElwee has received contract research support for his role as an investigator from Replicel Life Sciences Inc. He has received shares in Replicel Life Sciences Inc as a company co-founder and for consultation and participation in advisory boards. He has received payment for his role as Chief Scientific Officer of Replicel Life Sciences Inc.

Dr. Rolf Hoffmann has received shares in Replicel Life Sciences Inc as a company co-founder. He has received payment for his participation in advisory boards and for his role as Chief Medical Officer of Replicel Life Sciences Inc.

Dr Jean Krutmann has received consultancy fees from Replicel Life Sciences Inc.

IUF has received contract research support from Replicel Life Sciences Inc. for conducting the study.

Funding Sources

The study was supported by Replicel Life Sciences Inc, Vancouver, Canada. Replicel Life Sciences Inc had a role in the design and conduct of the study; and no role in collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Author Contributions

Author Contributions: Drs Marini, Grether-Beck, Jaenicke and Krutmann had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Drs Grether-Beck and Marini are co-first authors; Profs Krutmann and Hoffmann are co-last authors.

Study concept and design: All authors.

Acquisition, analysis, or interpretation of data: Grether-Beck, Marini, Jaenicke, McElwee, Goessens-Rück.

Drafting of the manuscript: Krutmann, Grether-Beck, Marini.

Critical revision of the manuscript for important intellectual content: McElwee, Goessens-Rück, Hoffmann.

Statistical analysis: Grether-Beck, Marini.

Obtained funding: Krutmann.

Administrative, technical, or material support: All authors.

Supervision: Krutmann.

References

1. Dimri, G.P., et al., *A biomarker that identifies senescent human cells in culture and in aging skin in vivo.* Proc Natl Acad Sci U S A, 1995. **92**(20): p. 9363-7.
2. Ressler, S., et al., *p16INK4A is a robust in vivo biomarker of cellular aging in human skin.* Aging Cell, 2006. **5**(5): p. 379-89.
3. Waldera Lupa, D.M., et al., *Characterization of Skin Aging-Associated Secreted Proteins (SAASP) Produced by Dermal Fibroblasts Isolated from Intrinsically Aged Human Skin.* J Invest Dermatol, 2015. **135**(8): p. 1954-1968.
4. Yaar M, *Clinicial and histological features of intrinsic versus extrinsic skin aging*, in *Skin Aging*, Krutmann J and Gilchrest BA, Editors. 2006, Springer-Verlag,: Berlin, Heidelberg. p. 9-21.
5. Rigotti, G., et al., *Expanded Stem Cells, Stromal-Vascular Fraction, and Platelet-Rich Plasma Enriched Fat: Comparing Results of Different Facial Rejuvenation Approaches in a Clinical Trial.* Aesthet Surg J, 2016. **36**(3): p. 261-70.
6. Ito, M. and Y. Sato, *Dynamic ultrastructural changes of the connective tissue sheath of human hair follicles during hair cycle.* Arch Dermatol Res, 1990. **282**(7): p. 434-41.
7. Oh, J.K., et al., *Connective tissue sheath of hair follicle is a major source of dermal type I procollagen in human scalp.* J Dermatol Sci, 2012. **68**(3): p. 194-7.
8. Heitz, J., et al., *Bone-forming cells with pronounced spread into the third dimension in polymer scaffolds fabricated by two-photon polymerization.* J Biomed Mater Res A, 2017. **105**(3): p. 891-899.
9. Grether-Beck, S., et al., *Molecular evidence that oral supplementation with lycopene or lutein protects human skin against ultraviolet radiation: results from a double-blinded, placebo-controlled, crossover study.* Br J Dermatol, 2017. **176**(5): p. 1231-1240.
10. Marini, A., et al., *Prevention of polymorphic light eruption by oral administration of a nutritional supplement containing lycopene, beta-carotene, and Lactobacillus johnsonii: results from a randomized, placebo-controlled, double-blinded study.* Photodermatol Photoimmunol Photomed, 2014. **30**(4): p. 189-94.
11. Marini, A., et al., *Pycnogenol(R) effects on skin elasticity and hydration coincide with increased gene expressions of collagen type I and hyaluronic acid synthase in women.* Skin Pharmacol Physiol, 2012. **25**(2): p. 86-92.
12. Grether-Beck, S., et al., *Effective photoprotection of human skin against infrared A radiation by topically applied antioxidants: results from a vehicle controlled, double-blind, randomized study.* Photochem Photobiol, 2015. **91**(1): p. 248-50.
13. Livak, K.J. and T.D. Schmittgen, *Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method.* Methods, 2001. **25**(4): p. 402-8.
14. Talwar, H.S., et al., *Reduced type I and type III procollagens in photodamaged adult human skin.* J Invest Dermatol, 1995. **105**(2): p. 285-90.
15. Quan, T., et al., *Reduced expression of connective tissue growth factor (CTGF/CCN2) mediates collagen loss in chronologically aged human skin.* J Invest Dermatol, 2010. **130**(2): p. 415-24.
16. Griffiths, C.E., et al., *Restoration of collagen formation in photodamaged human skin by tretinoin (retinoic acid).* N Engl J Med, 1993. **329**(8): p. 530-5.
17. Chakravarti, S., et al., *Lumican regulates collagen fibril assembly: skin fragility and corneal opacity in the absence of lumican.* J Cell Biol, 1998. **141**(5): p. 1277-86.
18. Shin, J.E., et al., *Transcriptional regulation of proteoglycans and glycosaminoglycan chain-synthesizing glycosyltransferases by UV irradiation in cultured human dermal fibroblasts.* J Korean Med Sci, 2011. **26**(3): p. 417-24.
19. Kong, R., et al., *A comparative study of the effects of retinol and retinoic acid on histological, molecular, and clinical properties of human skin.* J Cosmet Dermatol, 2016. **15**(1): p. 49-57.

Figures

Figure 1
(a)

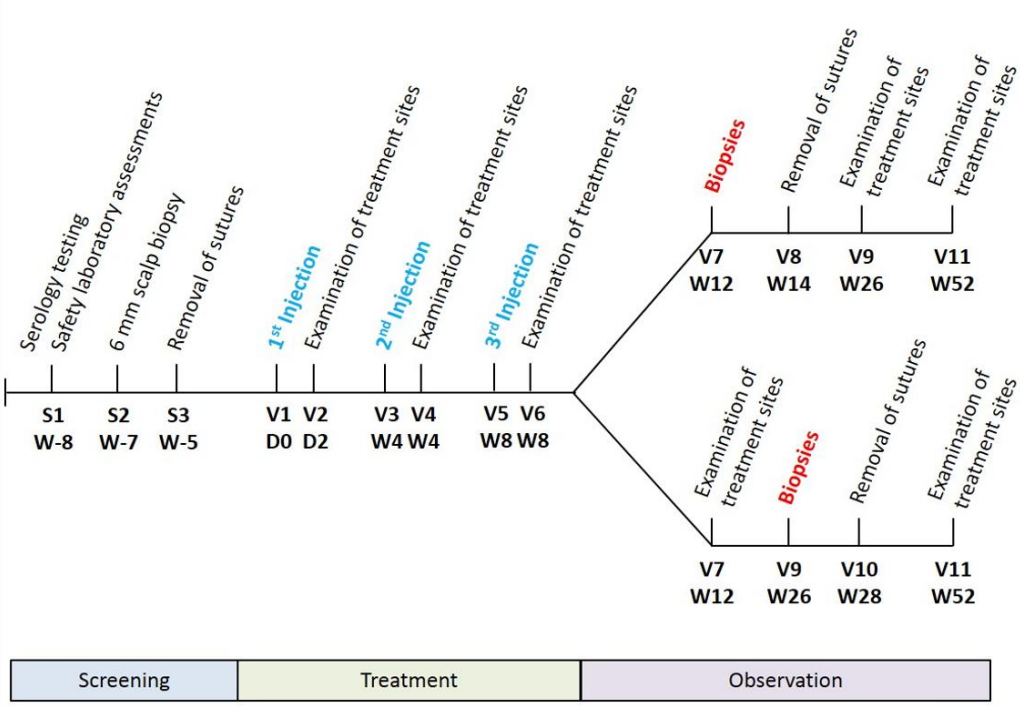

Figure 1
(b)

Example treatment pattern for RCS-01 and Placebo Groups:

Time point of intradermal injections	RCS-01 Group			
	e.g. Left buttock		e.g. Right buttock	
	(lateral) [a]	(medial) [b]	(medial) [c]	(lateral) [d]
Visit 1 (Day 0)	Placebo	RCS-01	Sham	Sham
Visit 2 (Week 4)	Placebo	RCS-01	Sham	Sham
Visit 3 (Week 8)	Placebo	RCS-01	Placebo	RCS-01
	Placebo Group			
	e.g. Left buttock		e.g. Right buttock	
	(lateral) [a]	(medial) [a]	(lateral) [c]	(medial) [c]
Visit 1 (Day 0)	Placebo	Placebo	Sham	Sham
Visit 2 (Week 4)	Placebo	Placebo	Sham	Sham
Visit 3 (Week 8)	Placebo	Placebo	Placebo	Placebo

RCS-01 = human autologous cultured hair follicle NBDS cells in 1.0 mL cryomedium
Placebo = cryomedium
Sham = needle penetration of the skin without injection of fluid
[a] Triple treatment placebo (placebo / placebo / placebo)
[b] Triple treatment verum (RCS-01 / RCS-01 / RCS-01)
[c] Single treatment placebo (sham / sham / placebo)
[d] Single treatment verum (sham / sham / RCS-01)

Fig. 1. A summarised schedule of assessments (A) and an example treatment pattern for the RCS-01 and Placebo treatment group (B): Each subject had 4 treatment sites on the buttock (two on each buttock), which were identified by a tattoo. On visit 1, subjects from the RCS treatment group

received either placebo (cryomedium), RCS-01, or sham injections (needle penetration without injection of any fluid) according to a randomisation schedule. After 4 weeks, subjects returned and received the same treatment in the same pattern as before. Treatment evaluation sites treated with two doses of placebo or RCS-01 at visits 1 and 3 received a third injection of RCS-01 or placebo. The other two treatment evaluation sites previously receiving sham injections randomly received injections of either RCS-01 or placebo. For subjects in the Placebo group, the treatment pattern was similar except that RCS-01 was replaced by placebo.

Figure 2

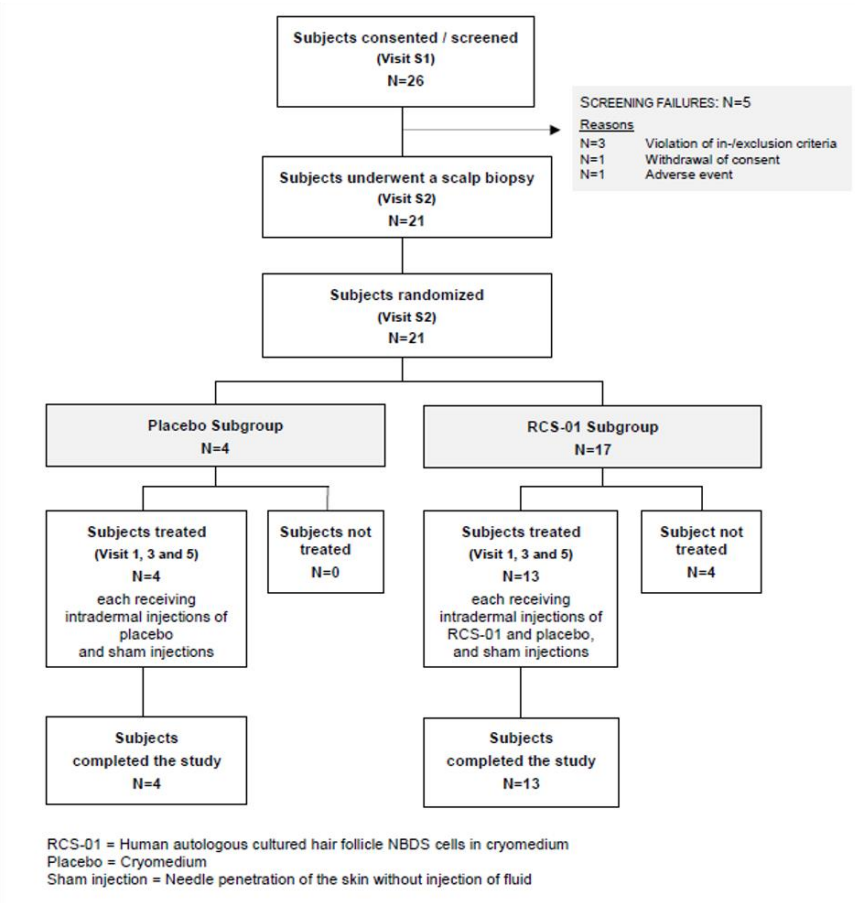

Fig. 2. Disposition of trial subjects.

Figure 3

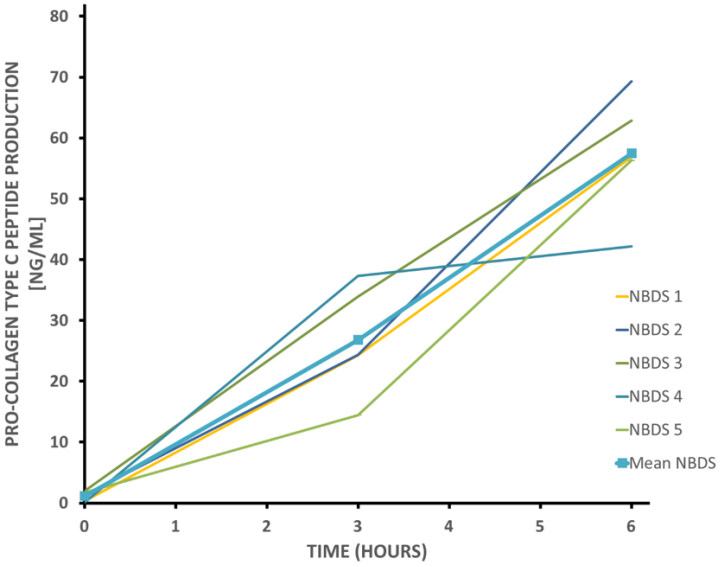

Fig. 3. Concentration of pro-collagen Type I C-peptide in culture supernatants from 8x104 non-bulbar dermal sheath cells (NBDS) processed and cultured from 5 individuals, and mean concentration for NBDS cells.

Figure 4

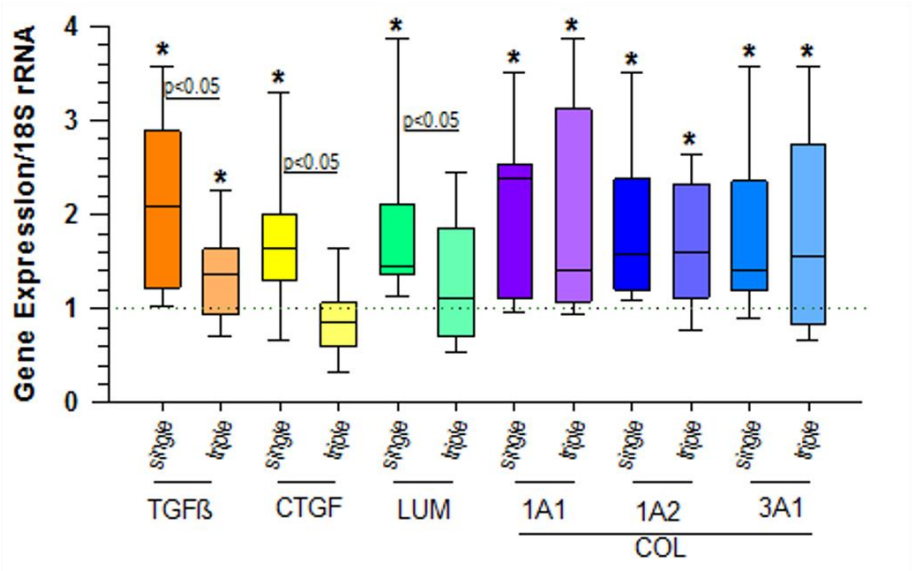

Fig. 4. Upregulation of mRNA levels for dermal markers after single or triple treatment with RCS01 as compared to a single placebo treatment. Given are box plots of n=9, error bars reflect 95% and 5% percentiles. Dashed line indicates placebo treated level set arbitrarily equal to 1. *p<0.05 indicated significant difference for each marker vs first, single placebo treatment (ANOVA on ranks).

Tables

Table 1. Overview of subjects with adverse events

Systemic / local AEs	**RCS-01 Subgroup (N=13, 100%)**	**Placebo Subgroup (N=4, 100%)**	**Total (N=17, 100%)**
Total subjects with AEs	10 (76.9%)	4 (100.0%)	14 (82.4%)
Subjects with at least one local AE	10 (76.9%)	4 (100.0%)	14 (82.4%)
Solicited local AEs	10 (76.9%)	4 (100.0%)	14 (82.4%)
Unsolicited local AE	0	0	0
Subjects with at least one systemic AE	3 (23.1%)	1 (25.0%)	4 (23.5%)
Onset before first treatment (Visit S1 - Visit 1)	2 (15.4%)	0	2 (11.8%)
Onset after first treatment (Visit 1 - Visit 11 / Visit 12)	1 (7.7%)	1 (25.0%)	2 (11.8%)
Subjects with at least one serious AE	0	0	0

Clinical symptoms monitored and if present at the injection sites recorded as solicited local AEs were:

- Erythema
- Bruising/haemorrhage
- Pyoderma/infection
- Eczema
- Granuloma/papules/nodules
- Hypertrichosis/hair density
- Hypopigmentation
- Hyperpigmentation
- Scarring