

Table 1. Composition of the Drug Product Unit Dosage Form

Component	Reference to Quality Standard	Function	Target Concentration	Quantity per Vial ¹
Autologous Fibroblasts	Drug Product-Injection Certificate of Analysis	Drug Substance	1.0 – 2.0 × 10 ⁷ cells/mL	1.2 – 2.4 × 10 ⁷ cells
Phosphate buffered saline (PBS)	In-house Standard (Manufacturer’s Certificate of Analysis plus additional in-house testing)	(b)(4)	N/A	Minimal residual trace ²
DMEM without phenol red	In-house Standard (Manufacturer’s Certificate of Analysis plus additional in-house testing)	(b)(4)	N/A	(b)(4)

¹ Vial contains 1.2 mL of IT

² PBS diluted with DMEM without phenol red such that trace amounts of PBS are possible but not quantifiable.

Abbreviations:

BLA = Biologic License Application

DMEM = Dulbecco’s Modified Eagle’s Medium

----- (b)(4) -----

----- (b)(4) -----

FBS = Fetal bovine serum

----- (b)(4) -----

HA = Hyaluronic acid

----- (b)(4) -----

----- (b)(4) -----

ICH = International Conference on Harmonization

i.d. = Intradermal

----- (b)(4) -----

IND = Investigational New Drug application

IT = Isologen Therapy™

PBS = Phosphate-Buffered Saline

s.c. = Subcutaneous

----- (b)(4) -----

Application History:

- Complete BLA submitted on 6-March-2009 (Paper CTD)

Cross-Reference:

- IND (b)(4) ACTIVE. Autologous fibroblasts (Human, dermal, Isologen Technologies, Inc.) expanded ex vivo, administered intradermally. Treatment for rhytids. Sponsor: Isologen Technologies, Inc. The IND was submitted to FDA on 12-October-1999.

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INTRODUCTION

Proposed Mechanism of Action:

Per the sponsor, the autologous human fibroblasts present in IT are hypothesized to increase the synthesis of extracellular matrix components, including collagen, thus reducing the severity of nasolabial fold wrinkles.

Preclinical Studies:

Module 2: Common Technical Document Summaries

Section 2.4: Nonclinical Overview

Section 2.6: Nonclinical Written and Tabulated Summary

No preclinical studies were conducted with this product. Prior to the 1999 IND submission Isolagen manufactured autologous fibroblasts at a site in New Jersey and administered these cells to many subjects in a non-IND setting. The BLA states that due to this previous commercial experience in humans, and due to the lack of a 'suitable' preclinical animal model for rhytids, no preclinical studies using IT were performed. The pharmacology/toxicology review of the original IND submission is provided at the end of this review.

Isolagen Therapy™ (IT) was sold commercially in the United States (US) as a cosmetic treatment from December 1995 to February 1999. The BLA states that approximately 1100 subjects received IT at 110 clinics in the US within a 4-year period (December 1995 to February 1999) prior to the IND submission in October, 1999. According to a retrospective study report dated March 10, 2003 that was authored by Isolagen Inc., no serious adverse events were observed in 354 subjects evaluated ('Study Report for Retrospective Study of Isolagen Injection for the Treatment of Contour Deformities'; March 10, 2003). The data were collected retrospectively on 397 subjects at 23 clinics. Of the 397 subjects included in the summary, only 354 received the proposed clinical dose ($1.0-2.0 \times 10^7$ cells/ml) of IT.

The BLA states that there were two Phase II and five Phase III studies completed under IND (b)(4) (Table 1).

Table 1. Studies Completed Under IND (b)(4)

Study Protocol	No. of Study Centers	Study Start Study Stop	Study Objectives
Phase II Studies			
IT-R-001	2	3 Jan 03 Completed: Feb 04	Safety and Proof of Concept
IT-R-007	5	22 Mar 07 Completed: Jun 08	Safety and Efficacy
Phase III Studies			
IT-R-002	10	19 May 03 Completed: Jun 05	Safety, Efficacy and Proof of Concept
IT-R-003A	3	20 Jul 04 Completed: May 05	Safety and Efficacy
IT-R-003B	3	21 Jul 04 Completed: May 05	Safety and Efficacy
IT-R-005	7	23 Oct 06 Completed: Jun 08	Safety, Efficacy, Schedule and Dose Confirmation
IT-R-006	6	1 Nov 06 Completed: Jun 08	Safety, Efficacy, Schedule and Dose Confirmation

Safety data from a total of 508 subjects who received at least one administration of IT from the above seven studies are shown in Table 7 below. Adverse events associated with the injection site ranged from mild to moderate and included transient redness, swelling, rash, splotching pruritis at the injection site, bruising, acne, pain, and discomfort at the injection site (Table 7). Per the BLA, no serious adverse events related to IT have been reported in any controlled clinical trial conducted.

Table 7. Treatment-Emergent Adverse Events Occurring in at Least 1% of Subjects in Either Treatment Group for the Integrated Safety Population

System Organ Class Preferred Term	IT, n (%) (N=508)	Placebo, n (%) (N=354)
General Disorders and Administration Site Conditions	343 (68%)	144 (40%)
Injection Site Erythema	81 (16%)	33 (9%)
Injection Site Bruising	54 (11%)	48 (14%)
Injection Site Swelling	69 (14%)	15 (4%)
Injection Site Pain	31 (6%)	6 (2%)
Injection Site Haemorrhage	13 (3%)	16 (5%)
Injection Site Oedema	22 (4%)	0
Injection Site Nodule	20 (4%)	3 (<1%)
Application Site Papules	8 (2%)	3 (<1%)
Injection Site Irritation	6 (1%)	1 (<1%)
Injection Site Dermatitis	5 (1%)	2 (<1%)
Injection Site Pruritus	5 (1%)	3 (<1%)
Injection Site Reaction	5 (1%)	2 (<1%)
Infections and Infestations	80 (16%)	81 (23%)
Sinusitis	8 (2%)	15 (4%)
Upper Respiratory Tract Infection	17 (3%)	11 (3%)
Nasopharyngitis	12 (2%)	7 (2%)
Influenza	8 (2%)	8 (2%)
Urinary Tract Infection	5 (1%)	4 (1%)
Bronchitis	5 (1%)	4 (1%)

Table 7. Treatment-Emergent Adverse Events Occurring in at Least 1% of Subjects in Either Treatment Group for the Integrated Safety Population

System Organ Class Preferred Term	IT, n (%) (N=508)	Placebo, n (%) (N=354)
Herpes Simplex	3 (<1)	4 (1%)
Skin and Subcutaneous Tissue Disorders	47 (9%)	26 (7%)
Acne	8 (2%)	1 (<1%)
Dermatitis Contact	4 (1%)	5 (1%)
Rash	5 (1%)	2 (<1%)
Musculoskeletal and Connective Tissue Disorders	33 (6%)	27 (8%)
Arthralgia	6 (1%)	4 (1%)
Back Pain	5 (1%)	6 (2%)
Injury, Poisoning and Procedural Complications	24 (5%)	31 (9%)
Foot Fracture	2 (<1%)	4 (1%)
Tooth Injury	1 (<1%)	4 (1%)
Nervous System Disorders	24 (5%)	23 (6%)
Headache	15 (3%)	9 (2%)
Respiratory, Thoracic And Mediastinal Disorders	19 (4%)	15 (4%)
Cough	5 (1%)	3 (<1%)
Nasal Congestion	4 (1%)	4 (1%)
Vascular Disorders	12 (2%)	6 (2%)
Hypertension	8 (2%)	6 (2%)

Source: CTD Section 5.3.5.3. 2 – Integrated Summary of Safety Appendix 6 Table 1.9.

Module 4: Nonclinical

Section 4.2: Study Reports

No preclinical study reports were provided in this section.

Section 4.3: Literature References

Per the BLA, laboratory research with intradermal injection of fibroblasts has been documented in the published literature. The following published articles were provided in this section. The sponsor states that these articles are relevant to the use of IT for the treatment of nasolabial fold wrinkles; however, a comprehensive review of these publications by the sponsor was not provided.

1. Remmler D, Thomas JR, Mazoujian G, Pentland A, Schechtman K, Favors S, Bauer E. Use of injectable cultured human fibroblasts for percutaneous tissue implantation. An experimental study. Arch Otolaryngol Head Neck Surg. 1986; 115(7):837-844.
2. Keller G, Sebastian J, Lacombe U, Toft K, Lask G, Revazova E. Safety of injectable autologous human fibroblasts. Bull Exp Biol Med. 2000; Aug; 130(8):786-9.
3. Yoon E, Han SK, Kim WK. Advantages of the presence of living dermal fibroblasts within Restylane for soft tissue augmentation. Ann Plast Surg. 2003; 51(6):587-592.
4. Solakoglu S, Tiryaki T, Ciloglu, SE. The effect of cultured autologous fibroblasts on longevity of cross – linked hyaluronic acid used as a filler. Aesthetic Surg J. 2008; 28(4):412-426

5. Zhao Y, Wang J, Yan X, Li D, Xu J. Preliminary survival studies on autologous cultured skin fibroblasts transplantation by injection. *Cell Transplant.* 2008; 17(7):775-783.

Summary of Published Studies:

Title:

Use of Injectable Cultured Human Fibroblasts for Percutaneous Tissue Implantation

Authors: Remmler D, Thomas JR, Mazoujian G, Pentland A, Schechtman K, Favors S, and Bauer E

Journal: *Arch Otolaryngol Head Neck Surg.* 1986; 115(7):837-844

Species: Athymic Balb/c nude mice (8-10 weeks old)

Test and control articles:

1. Hank's balanced salt solution (HBSS)
2. Cultured human facial dermal fibroblasts (HFb) (1.6×10^8 cell/0.5 ml) in Hank's balanced salt solution (HBSS)
3. Cultured human dermal fibroblasts dispersed in Zyderm II collagen (HFb + Zyd) (0.8×10^8 cells/0.5 ml)
4. Zyderm II collagen (Zyd)
5. Zyplast collagen (Zyp)

Route of administration: Subcutaneous (s.c.) injection with a 22-gauge needle

Volume injected: 0.5 ml

Study design:

Facial skin biopsies were collected from three healthy human subjects (code-named A, E, and C). Subcutaneous tissue was removed, the skin was minced into 1 mm cubes, covered with sterile coverslips, and expanded (5-6 passages) in DMEM supplemented with 10% fetal calf serum and antibiotics. The cells were washed and resuspended in HBSS.

Five groups of 4-7 nude mice were s.c. injected with one of the above listed articles using a 22-gauge needle. The nodular swellings that resulted from the injections were measured *in situ* (greatest length, width, and height) immediately following injection and at 3, 6, and 9 weeks post-injection. A total of 1-3 mice from each group were sacrificed on day 10 and at 8, 6, and 9 weeks after injection. The injection sites were excised and the tissue was fixed in 10% buffered formaldehyde solution, embedded, vertically sectioned, and stained with hematoxylin-eosin (H&E).

To confirm the presence of human fibroblasts in the HFb and HFb + Zyd 'nodules', immunoperoxidase staining was performed with anti-human vimentin, which identifies human fibroblasts. This was carried out by using an indirect peroxidase + conjugate

method, in which human fibroblasts are immunohistologically identified with a positive brown stain, whereas non-human fibroblasts are negatively stained with a light green or turquoise color. The positive control consisted of human fibroblasts that were cultured directly on standard glass slides and the negative controls consisted of: (1) noninjected nude mouse skin, (2) nude mouse skin injected only with HBSS, and (3) Zyderm II collagen. The average percent of positive staining fibroblasts in the HFb and HFb + Zyd nodule was determined via the counting of all fibroblasts in 10 randomly selected high-power (400x) fields within the nodule.

Results:

Nodule volumes:

The nodule volume indexes recorded for each injected article were averaged by using data from all surviving mice at each examination interval. The rates of change in the mean volume indexes for each group was determined using a statistical model based on repeated measures analysis of variance that also accounted for any initial mean volume index differences among the groups.

Note: Initial mean volume index was obtained immediately following injection of each respective article.

HBSS: Nodules in the mice injected with HBSS alone were not detected by 24 hours after injection (not shown in Fig. 3).

Human Fibroblasts (HFb) in HBSS:

The mean volume index of the nodules in this group decreased during the first 6 weeks at a significantly greater rate compared to mice injected with Zyd and Zyp ($p < 0.05$). Beyond 6 weeks, there was no further significant volume change ($p > 0.05$) compared to the other three groups (Fig. 3). The mean volume index at 9 weeks was 27% of the initial mean volume index.

Human Fibroblasts + Zyderm II Collagen (HFb + Zyd):

The mice displayed results similar to mice injected with HFb (Fig 3).

Zyderm II Collagen (Zyd):

The mean nodule volume index of Zyd decreased at a significantly slower rate compared to mice injected with HFb or HFb + Zyd ($p < 0.05$) but at a significantly greater rate than the Zyplast (Zyp) implants ($p < 0.05$), (Fig. 3). Although the mean volume index rose slightly between 6 and 9 weeks, this was not statistically significant ($p > 0.05$). The mean volume index at 9 weeks was 62% of the initial mean volume index for this group. At 9 weeks, the mean nodule volume index for this group was significantly higher compared to mice injected with HFb ($p < 0.05$) or with HFb + Zyd ($p < 0.05$), yet remained significantly lower than mice injected with Zyp ($p < 0.05$).

Zyplast Collagen (Zyp):

The mean nodule volume index of this group did not change significantly during the study ($p > 0.05$) (Fig. 3). At 9 weeks the mean nodule volume index was significantly greater than those for all other test substances ($p < 0.05$).

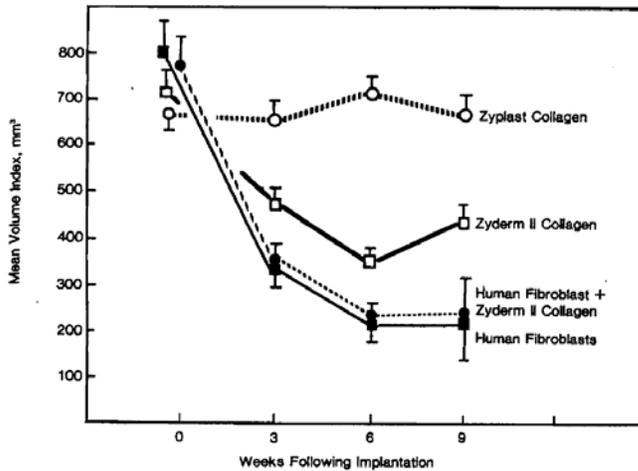


Fig 3.—Course of volume change for injected test implants. Each data point represents mean volume index (error bar indicates SEM). Hanks' balanced salt solution implants were resorbed within 24 hours and therefore are not displayed.

Histology:

HBSS: The mice injected with HBSS alone did not show any aberrations and immunoperoxidase staining of injection site for human cells was negative at each examination interval.

Human Fibroblasts (HFb) in HBSS:

Day 10: 90% of the fibroblasts in the nodule were of human origin. The nodule consisted of solid sheets of viable, polygonal, amphiphilic fibroblasts cells that formed a well-defined mantle around a central core that had undergone coagulative necrosis.

Weeks 3-9: The outer mantle layer of fibroblasts gradually filled the central cavity over time. The population of cells within the nodule became less dense and increasingly dispersed and small numbers of hemosiderin-laden macrophages were present. By 9 weeks, only 25% of the fibroblasts in the nodule were of human origin. A few of the human fibroblasts migrated from the nodule into the surrounding tissue. Invading capillaries were observed within the mantle of human fibroblasts.

Human Fibroblasts + Zyderm II Collagen (HFb + Zyd):

Day 10: 80% of the fibroblasts in the nodule were of human origin. There was no sign of central avascular necrosis.

Weeks 3-9: By 3 weeks, capillaries and small blood vessels were observed within the nodule. Gradually, the cellularity of the nodule decreased and the Zyderm II collagen material became less dense, allowing for migration of the human fibroblasts a short distance from the implant. By 9 weeks, only 25% of the fibroblasts were of human origin. A few invading small capillaries were observed in the implant.

Zyderm II Collagen (Zyd)

Day 10 to Week 9: The injected article appeared as a distinct white cohesive mass, consisting of a fissured eosinophilic nodule that was clearly separated from the surrounding tissue. An occasional capillary or small blood vessel penetrated the

peripheral regions of the nodule. The surrounding host tissue contained a few inflammatory cells and hemosiderin-laden macrophages.

Zyplast Collagen (Zyp)

Day 10 to Week 9: The injected article appeared as a distinct white cohesive mass, consisting of eosinophilic amorphous collagen. Following an initial neutrophil response, a scant number of mouse fibroblasts remained as the primary inhabitants of the nodule, principally in the peripheral regions. Capillaries and blood vessels were rarely detected.

Publication Conclusion:

The authors concluded that while cultured human fibroblasts can be successfully injected as living grafts, the decreased number of human cells over the time interval studied (out to 9 weeks) indicates that long-term retention of the injected human cells is limited. The administration of the human fibroblasts in combination with a collagen matrix did not enhance the persistence of the cells *in vivo*.

Comments:

- The preparation of the human cells used in this study did not duplicate the procedure that is used for IT preparation; for example, the human cells that were used were separated from the skin biopsy tissue by mincing; however, digestive enzymes are used to prepare IT.
- The source of the human fibroblasts used in this study was human female donors of 54-69 years of age (race unknown).
- The human fibroblasts were expanded for 5-6 passages prior to injection into the mice. For IT, human fibroblasts are expanded for ----(b)(4)----.
- The human fibroblasts were formulated in HBSS and/or a collagen gel suspension, which is not the IT clinical formulation.
- A total of 1-3 mice from each group were sacrificed on day 10 and at 6, 8, and 9 weeks after cell injection. The authors did not specify the exact number of animal sacrificed/group/time point. Given the progressively small number of mice/group with each study measurement interval, the statistical analyses and any potential interpretation from these analyses, is questionable.
- While substantial migration of the fibroblasts from the site of injection was not observed for fibroblasts mixed with collagen, some fibroblasts were detected in the tissue immediately surrounding the nodule due to dispersion upon injection.
- Injection of human fibroblasts in combination with collagen gel did not improve cell survival or the rate of reduction in nodule volume, compared to injection of human fibroblasts alone.

*Title:***Safety of Injectable Autologous Human Fibroblasts***Authors:* Keller G, Sebastian J, Lacombe U, Toft K, Lask G, and Revazova E*Journal:* Bull Exp Biol Med. 2000; Aug; 130(8):786-789*Species:* Athymic Balb/c nude mice (1.5 months old; n=2)*Test article:* Cultured human facial dermal fibroblasts*Route of administration:* s.c. injection*Cell dose:* 4×10^7 fibroblasts*Volume injected:* Not provided*Study design:*

Biopsy specimens (4 mm) were taken from the retroauricular area of patients aged 37-61 years with facial rhytids and atrophic facial scars and placed in 10 ml EMEM supplemented with 10% FBS, 0.1 g/liter sodium pyruvate, and various antibiotic and antimicrobial agents. The specimens were transferred to 60 mm culture dishes and incubated at 37°C in a 5% CO₂ incubator for 2 weeks, followed by trypsinization and seeding in 25 cm² flasks, then re-cultured for 5-7 weeks (passages 4-6).

For evaluation of collagen production, 5×10^5 fibroblasts (passage 6) were washed with PBS containing 0.1% sodium azide and 2% FBS and stained using murine monoclonal antibodies against human type I collagen and FITC-labeled goat polyclonal antibodies against mouse IgG/IgM. The unstained cells incubated only with FITC-labeled goat antimouse antibodies served as the study controls. The stained cells were resuspended in 50 µl EMEM for 30 min on ice. The various cell preps ($>10^5$ cells) were then analyzed via flow cytometry for collagen production.

For evaluation of fibroblast contraction potency, the fibroblasts (passage 6, 10^5 cells/ml) dispersed with 0.05% trypsin and 0.02% EDTA in PBS, were mixed 1:1 with 0.2% atelocollagen gel¹, transferred to 35 mm dishes (3 ml/dish), and incubated at 37°C and 5% CO₂. The diameter of the gel was measured every 4 hrs for a total of 24 hrs. Cultured human fibroblasts obtained from a biopsy from a hypertrophic scar (passage 6) were used as the control.

For evaluation of oncogenic potential, 4×10^7 cells (passage 7) were s.c. injected into two mice; these mice were followed for 2 months.

¹ Preparation method 0.3% pepsin-processed type I atelocollagen (pH 7.3) was mixed with 6-fold Minimum Essential Medium and 10% embryo calf serum (4:1:1).

Results:**Production of collagen:**

The cultured fibroblasts actively produced type I collagen (fluorescence intensity 8.49 for experimental vs. 0.09 arbitrary units for controls; Fig. 3).

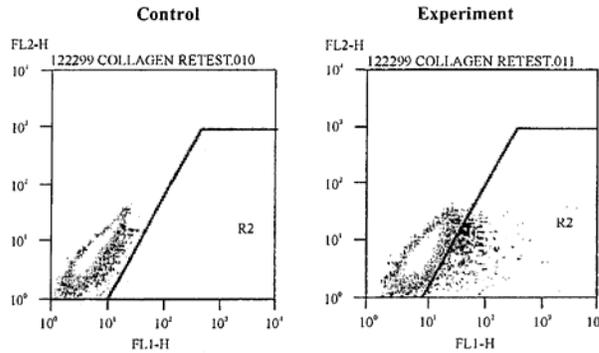


Fig. 3. Expression of type I collagen in fibroblasts after 6 passages in culture.

Fibroblast contraction:

The diameter of the collagen gel containing the human fibroblasts was 31.8 ± 0.9 mm vs. 20.9 ± 0.7 mm for the control at 24 hrs post-incubation (Fig. 4).

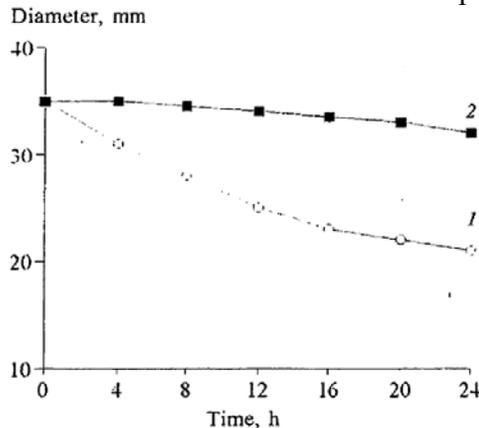


Fig. 4. Contraction of collagen gel after 24-h incubation with fibroblasts isolated from hypertrophic scar (1) and normal skin (2). Differences are significant ($p < 0.05$) in each time point.

Oncogenic potential:

No tumors were observed in the nude mice at 2 months.

Publication Conclusion:

The authors concluded that the cultured human fibroblasts: 1) were functionally active and produced a higher quantity of type I collagen compared to control and 2) had a collagen gel contraction capacity that was comparable to control. The cultured human fibroblasts exhibited no oncogenic properties following administration into nude mice.

Comments:

- Human donors of 37-61 years of age were used in this study. The race and gender of these donors were not provided in the publication.

- The isolation procedure for the fibroblasts was not provided in the publication.
- The human fibroblasts were expanded for 5-7 passages prior to *in vitro* and *in vivo* testing. For IT, human fibroblasts are expanded for ----(b)(4)----.
- The human fibroblasts were formulated in EMEM, while DMEM is used to formulate IT.
- Regarding the *in vivo* assessment of tumorigenic potential: 1) only two animals were used and 2) the study duration was only 2 months, therefore no conclusions can be made about this safety endpoint.

*Title:***Advantages of the Presence of Living Dermal Fibroblasts within Restylane for Soft Tissue Augmentation***Authors:* Yoon E, Han SK, and Kim WK*Journal:* Ann Plast Surg. 2003; 51(6):587-592*Species:* Athymic nude mice (n=12) [age was not provided]*Test article:* Cultured human facial dermal fibroblasts*Route of administration:* s.c injection*Cell dose:* 5×10^5 cells in DPBS + Restylane (Hyaluronic acid)²*Volume injected:* 400 μ l*Study design:*

Skin tissue from three randomly selected healthy adult subjects was collected, de-epithelialized, and minced into 2 x 1 mm samples. The samples were evenly spread over the surface of a 100 mm tissue culture plate precoated with 3 ml of DMEM/Ham's F-12 containing 50% FBS, and incubated at 37°C for 4 hours to facilitate tissue adhesion. After incubation, 12 ml of DMEM/F-12 containing 10% FBS and 25 μ g/ml gentamycin was added and the plates were returned to the incubator. The confluent cultured fibroblasts were then trypsinized, the dissociated cells were diluted 2.7-fold with DPBS without Mg²⁺ and Ca²⁺ (DPBS), and collected by centrifugation. The cells were washed twice in 40 ml DPBS, resuspended in 5 ml of DPBS, and filtered through a nylon mesh. Cell density was determined using hemocytometer, and viability was assessed by the Trypan blue exclusion test. Cultures from passage 3 were used for all experiments.

A total of 5×10^7 cultured human fibroblasts were suspended in 200 μ l of DPBS and then dispersed in 200 μ l of Restylane. Human fibroblasts were combined with Restylane in an attempt to enhance the longevity (survival) of injected cells. The controls consisted of

² Restylane is commercially prepared from nonanimal source of stabilized hyaluronic acid

200 µl of DPBS + 200 µl of Restylane. The control and test articles were s.c injected using a 1-ml syringe into the back of the mice at six sites: the control article was injected into three sites on the left side and test article was injected into three sites on the right side. The nodular swellings that resulted from the injections were excised at 1, 2, 4, 8, 12, and 16 weeks (assumed 2 animals/sacrifice time point). Excision of the nodules involved 5 mm punch biopsies that included skin surrounding the nodule down to the panniculus carnosus layer. The weight of each nodule collected from every animal was recorded and the averaged values for the test article were compared to the control article. Immunohistochemical staining was performed in an attempt to confirm the presence of human collagen type I in the nodules injected with fibroblasts + Restylane. Human skin was used as a positive control for anti-collagen type I. Collagen type I immunoreactivity was evaluated in the spindled stromal cells and in the stromal components. The presence of immunoreactivity was recorded as positive or negative.

Results:

One mouse died during the study (cause of death not provided), thus 11 mice were evaluated.

Nodule weights:

The injected sites remained as well-defined nodules that were similar in appearance for the first 2 weeks after injection for both cell-injected and control-injected sites. Thereafter, the appearance of the nodules became increasingly varied, which was reflected in the weights of the nodules (Table 1). The mean control nodule weights were 100%, 94%, 84%, 69%, 65%, and 60% for weeks 1, 2, 4, 8, 12, and 16, respectively. The mean cell-injected nodule weights were 100%, 95%, 92%, 90%, 90%, and 91% for weeks 1, 2, 4, 8, 12, and 16, respectively.

TABLE 1. Weights of test and control specimens at different time points (mg)

Treatment	Week 1	Week 2	Week 4	Week 8	Week 12	Week 16
Experimental (Restylane + fibroblast)	408	425	424	372	407	411
	446	497	375	406	425	381
	459	443	398	395	403	426
	472	440	405	409	385	
	455	401	411	441	396	
	451	345	456	405	401	
Mean ± SD	448.7 ± 21.8	425.2 ± 50.4	411.5 ± 27.2	404.7 ± 22.4	402.8 ± 13.2	406.0 ± 22.9
Control (Restylane only)	421	415	389	305	364	241
	426	435	336	276	305	284
	445	428	348	288	296	267
	461	411	403	326	257	
	459	420	385	307	269	
	450	397	388	331	248	
Mean ± SD	443.6 ± 16.8	417.7 ± 13.4	374.5 ± 27.2	305.5 ± 21.2	289.8 ± 42.5	264 ± 21.6
Difference	5.0 ± 2.82a*	7.5 ± 30.4ab	36.7 ± 6.59abc	99.2 ± 3.06bc	113.0 ± 32.5c	
<i>P</i> value of week effect [†]	0.0079					

*Differences of mean weights between the 2 groups were compared by repeated measures of analysis of variance with 3 difference values. *P* value per nodule mouse of week effect is 0.0079. *P* values of repeated and week × repeated are 0.1751 and 0.3380, which are Greenhouse-Geisser adjusted. Week and repeated interaction equal 0.3380.

[†]a, b, c indicate the results of multiple comparisons based on Scheffe's method.

Histology:

Histological examination of each control nodule injected showed only negative immunohistochemical staining for human collagen. There was also no evidence of immunoreactivity for weeks 1 and 2 for the cell-injected nodules; however, positive staining for human collagen occurred in the nodules examined after week 2. The positive staining was primarily visible at the periphery of the nodule.

Publication conclusion:

The authors concluded that Restylane mixed with cultured human dermal fibroblasts can survive and produce humane dermal matrices, and also stimulates the production of new collagen matrices, and did not produce local tissue reaction.

Comments:

- The source of the human fibroblasts (donor race, gender, and age) was not provided.
- The human fibroblasts were formulated in DPBS, which is not the IT clinical formulation.
- The number of animals sacrificed per time point was not provided in the article.

Title:

The Effect of Cultured Autologous Fibroblasts on Longevity of Cross-Linked Hyaluronic Acid Used as A Filler

Authors: Solakoglu S, Tiryaki T, and Ciloglu, SE

Journal: Aesthetic Surg J. 2008; 28(4):412-426

Species: Sprague Dawley rats (n=30) [age not provided]

Test article: Cultured autologous rat dermal fibroblasts

Route of administration: i.d. injection

Cell dose: 30 x 10⁶/ml

Volume injected: 1 ml

Study design:

Skin biopsies (size not provided) were obtained from the backs of Sprague Dawley rats and the dermal connective tissue was separated from the epidermis, minced into pieces, and treated with collagenase B and DNAase (1 mg/ml and 0.1 mg/ml, respectively) for one hour. The filtrates were suspended with DMEM-F12 and 10% FCS and cultured in 25 cm² flasks at 37°C humidified 5% CO₂ and air mixture for 3 weeks (2-3 passages). The cells were then detached from the surface of the culture plate with trypsin (0.5%),

followed by washing and dispersion of the cells in hyaluronic acid (HA)³. The aim of the study was to compare the longevity of the filling effect, in terms of resorption time, of cultured fibroblast suspension, HA matrix alone, cultured fibroblasts in HA matrix.

The autologous fibroblasts ($3 \times 10^7/\text{ml}$) alone, fibroblasts mixed with HA, culture medium alone and HA alone were i.d. injected into each rat as follows:

- Culture medium alone - left front leg, Site 1
- HA matrix alone - right front leg, Site 2
- Fibroblasts + culture medium - left back leg, Site 3
- Fibroblasts + HA - right back leg, Site 4

At 4 and 8 months post-injection the rats were sacrificed (assumed 15 rats/sacrifice time point) and skin biopsies from each injection site and from non-injected skin were collected. The samples were stained with H&E and Masson's trichrome and examined. The thickness of the dermis and the number of visible vascular structures in the dermal connective tissue were evaluated via light microscopy, while the thickness of the collagen fibers (ultrastructural features of connective tissue constituents) was evaluated with a Jeol 1011 electron microscope. Measurements were performed on digital images captured with a Mega View II digital camera attached to the electron microscope.

Results:

The dense and typical irregular arrangement of collagen bundles and areas containing amorphous substances were seen in all injection sites with light microscopy. No difference was seen in Site 1 compared to non-injected area, with observation of inactive fibrocytes in fusiform shape (Fig. 1). The presence of HA was observed in Sites 2 and 4 at both 4 and 8 months. The amount of ground substance containing HA in Site 4 was relatively abundant compared to Site 2 at 8 months.

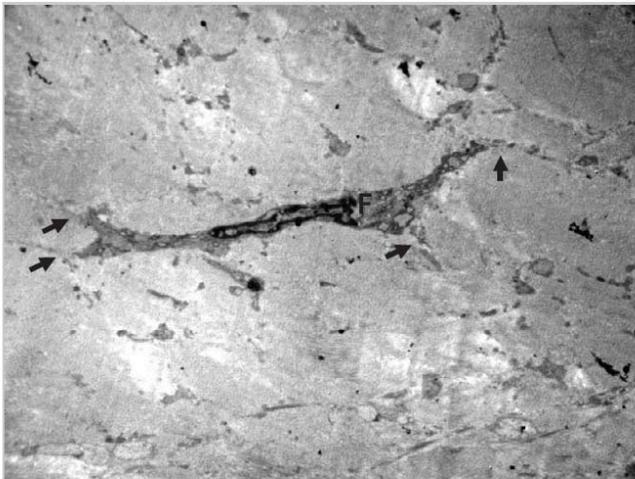


Figure 1. Electron micrograph of an inactive fibrocyte (F) in the culture media-injected site at the fourth month (original magnification, $\times 5000$).

³ A large polymer made up of disaccharides repeats of N-acetylglucosamine and glucuronic acid.

The diameter of the collagen fibrils located in Site 4 was thinner compared to Sites 1, 2, and 3 at 8 months (Table 1). The other major components of connective tissue (elastin and microfibrils) were intact for all sites, and elaunin⁴ and oxytalan⁵ components were abundant in Site 4 (Fig. 2).

Table 1. Thickness of collagen fibrils in 4 administration sites

Site no.	Thickness (μm) (mean \pm SD)
1 (medium)	48.3 \pm 4.4
2 (hyaluronic acid alone)	53.2 \pm 5.2
3 (fibroblast alone)	50.1 \pm 4.7
4 (hyaluronic acid plus fibroblast)	45.3 \pm 5.1

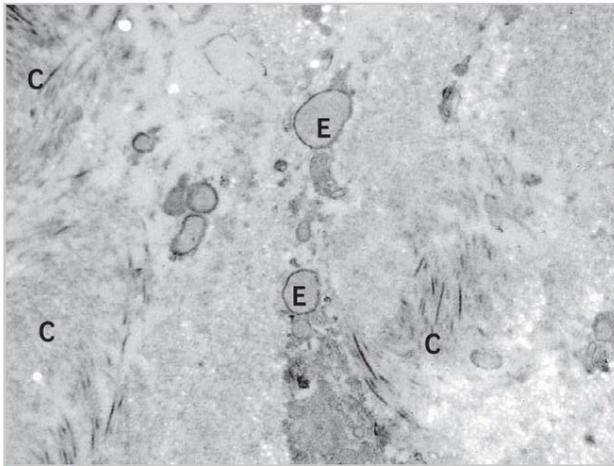


Figure 2. Elastin material (E) amongst collagen fibrils (C) in the hyaluronic acid matrix and fibroblast mixture-injected site at the eighth month (original magnification, $\times 8000$).

The average thickness of the dermis layer in Site 4 was significantly increased compared to the other sites (Table 2 and Fig. 3).

Comment:

- Although the biological significance of the thicker dermis layer injected with cells + HA was not discussed, given the intended clinical use of IT, this reviewer presumes that the addition of HA to the IT formulation would be undesirable.

⁴ Elaunin; it is Elaunin fiber in dermal tissue, a component of elastic fibers formed from a deposition of elastin

⁵ Oxytalan; it is Oxytalan fiber in dermal tissue, a type of connective tissue fiber distinct from collagen or elastic fibers

Table 2. Average thickness of dermis layer in 4 administration sites

Site no.	Thickness (mm) (mean \pm SD)
1 (medium)	2.63 \pm 0.21
2 (hyaluronic acid alone)	2.56 \pm 0.51
3 (fibroblast alone)	2.61 \pm 0.24
4 (hyaluronic acid plus fibroblast)	3.23 \pm 0.82 ^a

^aSignificant difference compared to the results of the control (medium) group ($P < .05$).

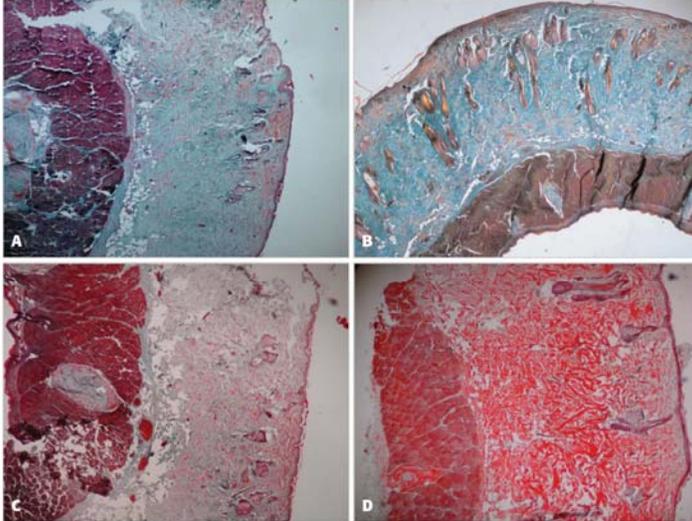


Figure 3. A. Micrograph from cross-sectioned skin of the medium-injected site at the eighth month. B. Micrograph from cross-sectioned skin of hyaluronic acid-injected site at the eighth month. C. Micrograph from cross-sectioned skin of fibroblast mixture-injected site at the eighth month. D. Micrograph from cross-sectioned skin of hyaluronic acid matrix and fibroblast mixture-injected site at the eighth month (original magnification, $\times 5000$).

The distribution of vascular structures per every 0.25 mm² was significantly increased in Sites 2 and 4 compared to the other sites (Table 3).

Table 3. Numbers of vascular structures (arterioles, capillaries, and venules) in administration sites (per 0.25 mm²)

Site no.	No. of vascular structures (mean \pm SD)
1 (medium)	24.0 \pm 1.7
2 (hyaluronic acid alone)	32.2 \pm 2.3 ^a
3 (fibroblast alone)	20.4 \pm 1.4
4 (hyaluronic acid plus fibroblast)	28.3 \pm 2.6 ^b

^aSignificant difference compared to the results of the control (medium) group ($P < .01$).

^bSignificant difference compared to the results of the control (medium) group ($P < .05$).

Comment:

- The number of blood vessels in Site 3 was decreased compared to Sites 1, 2, and 4 (Table 3), potentially showing that the fibroblasts did not significantly contribute to the formation of vascular structures.

Colonization of macrophages associated with capillaries was observed in Sites 2 and 4 using electron microscopy (Fig. 4). Normal fusiform-shaped fibroblasts were observed in sections from all sites. Enlarged, oval-shaped fibroblasts containing abundant granular endoplasmic reticulum and euchromatic nuclei were prevalent at both 4 and 8 months for Sites 2 and 4; these cells were in close proximity to ground substance and collagen fibers (Figs. 4 and 5).

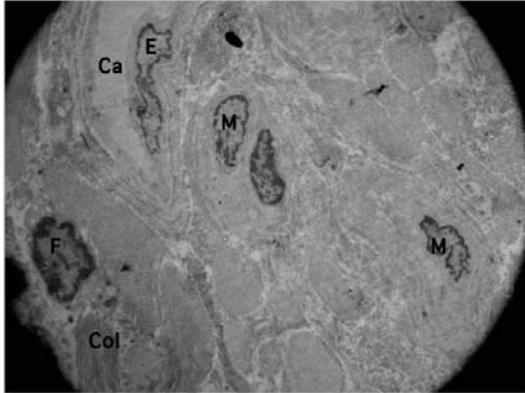


Figure 4. Colonization (Col) of blood vessel-forming macrophages (M) in hyaluronic acid matrix-injected site at the fourth month (original magnification, $\times 5000$). Ca, capillary; E, endothelial cell; F, fibroblast.

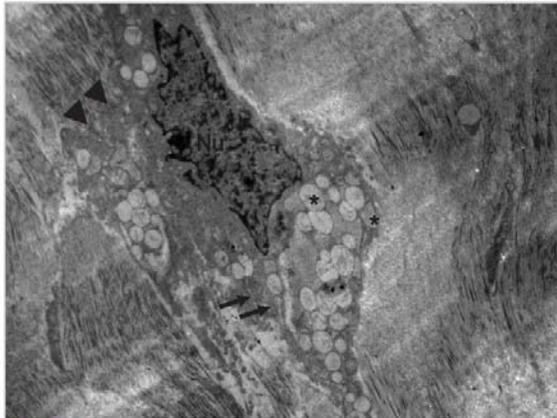


Figure 5. Electron micrograph of an enlarged, oval-shaped fibroblast containing granular endoplasmic reticulum cisterns (*arrows*) and cytoplasmic procollagen vesicles (*asterisks*) with tropocollagen-containing coves (*arrowheads*) in the hyaluronic acid matrix and fibroblast mixture-injected site at the eighth month. (*Nu*, nucleus; original magnification, $\times 5000$).

No findings related to inflammation or granulation was observed in any group.

Publication Conclusion:

The authors conclude that: 1) the cells were tolerated and no adverse findings (i.e., apoptosis, inflammation or necrosis) were observed and 2) cultured rat dermal fibroblasts combined with HA can provide a suitable, biocompatible, and long-lasting material. Similarly, cultured autologous human dermal fibroblast may have similar effects.

Comments:

- The preparation of the rat analog cells used in this study did not duplicate the procedure that is used for IT preparation; for example, the rat cells were separated from the skin biopsy by mincing and treating with collagenase B and DNAase; however, only digestive enzymes are used to prepare IT.
- The rat fibroblasts were formulated in DMEM-F12 and HA, which is not the IT clinical formulation.
- The role and importance of enlarged, oval-shaped fibroblasts containing abundant granular endoplasmic reticulum and euchromatic nuclei were not discussed in the article. It may represent the fibroblast are live and active in synthesis of the matrices.

*Title:***Preliminary Survival Studies on Autologous Cultured Skin Fibroblasts Transplantation by Injection**

Authors: Zhao Y, Wang J, Yan X, Li D, and Xu J

Journal: Cell Transplant. 2008; 17(7):775- 783

Species: New Zealand White rabbits (2 months old, 1.4-1.6 kg; n = 10)

Test article: Cultured autologous rabbit skin fibroblasts

Route of administration: Injection into the superficial and deep dermal junction of the dorsal aspect of the ears

Cell Dose: 8×10^7 fibroblasts/injection; administered a total of three times, at 2-week intervals

Volume injected: 1 ml/injection

Study Design:

Skin biopsies (size not specified) were taken from rabbits, cut into small pieces, and digested with dispase (Type II) at 37°C for 1 h. After digestion, the epidermis was separated from the dermis, and the dermis was cut into small pieces and incubated in DMEM supplemented with 10% heat-inactivated FBS, penicillin, streptomycin, and glutamine. The skin segments were left undisturbed for seven days until the medium was changed. When the cultures reached confluence in the dish, the adherent cells were treated with 0.25% trypsin at 37°C for 3-5 min. The detached fibroblasts were collected by centrifugation, resuspended in DMEM medium containing 10% FBS, and cultured in 175-cm² tissue culture flasks at 37°C under 5% CO₂/95% O₂ conditions. At confluency, the fibroblasts from the 4th generation were trypsinized and the cell pellet was suspended in 500 µl of DMEM medium containing 10% FBS. A 10 µl suspension of cells diluted with 0.05% Evan's blue dye in a 1:9 ratio was counted using a hemocytometer under a

phase-contrast microscope. The cell suspension was then centrifuged and resuspended in an appropriate volume of PBS to yield a final cell concentration of 8×10^7 cells/ml. The fibroblasts were labeled with [^3H]TdR, which is incorporated nearly exclusively into DNA.

The 10 rabbits were divided into two groups: the [^3H]TdR-labeled autologous fibroblast transplantation group (2 animals) and the unlabeled autologous fibroblast transplantation group (8 animals). The right ears were injected with autologous fibroblasts as the experimental subgroup, while the left ears were injected with PBS as the control subgroup. Injections into the same site were repeated at 2-week intervals for a total of three injections. The animals were sacrificed at 5 months post-last injection and the injection sites were excised. The tissue sections were stained with H&E and Sirius Red, and the samples were evaluated independently by three blinded investigators using both light and polarization microscopes. The dermal depth from the epithelium to the cartilage was calculated and the collagen content using the slides stained with Sirius Red were measured (total absolute area of the red thick type I collagen fibers and the green thin type III collagen fibers calculated with MIAS software by an automatic image analyzer).

Results:

Gross Morphology

The skin at the injected sites was normal; there was no erythema or swelling by 5 days following each injection in both groups. At 5 months the skin overlying the injected sites was soft, pliable, congruent with the surrounding skin, with no evidence of nodules in any animal.

Survival of the [^3H]TdR-Labeled Cells

The [^3H]TdR-labeled fibroblasts were detected out at least to 5 months post-injection in the experimental subgroup (Figs. 1A and 1B). No [^3H]TdR-labeled fibroblasts were found in the control subgroup. A large amount of extracellular matrix was observed around the [^3H]TdR-labeled cells. No lymphocytes, macrophages, or fibrous membrane was observed around the [^3H]TdR-labeled cells.

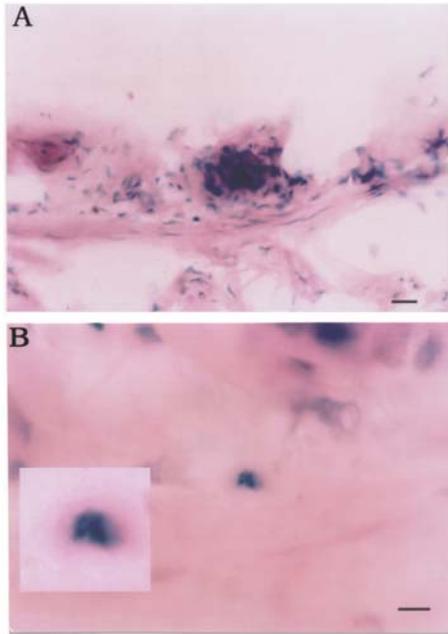


Figure 1. Autoradiography stained with methylgreen & pyronin in the skin 5 months after [³H]TdR-labeled fibroblast transplantation. (A) The [³H]TdR-labeled cells survived near the ear cartilage, distributed around the injected sites (100 \times). Scale bar: 20 μ m. (B) The four black grains on the nucleus shows the labeled fibroblast, which survives for 5 months posttransplantation (500 \times). Scale bar: 5 μ m. The same scale bar represents 2.5 μ m in the inset.

Histology (H&E stain) of the Unlabeled Cells

Numerous embryo fibroblasts were found and converged at the injected sites, with no macrophages or lymphocytes present. The fibroblasts contained a spherical nucleus and a limited amount of cytoplasm, and were surrounded by a thin layer of extracellular matrix (collagen; Fig. 2A). In the control subgroup, fibroblasts were attached to individual collagen bundles and elongated longitudinally, with a cell density that was lower than the experimental subgroup (Fig. 2B).

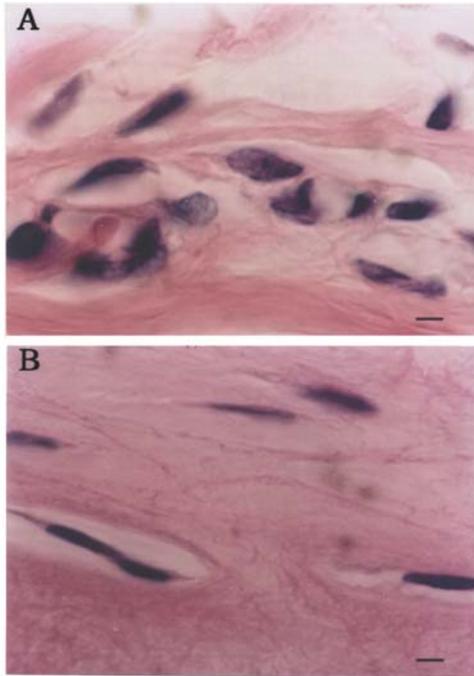


Figure 2. Histological staining with hematoxyline & eosin sections of the experimental subgroup (A) and the control subgroup (B) of the nonlabeled autologous fibroblasts group (200x). (A) A lot of embryo fibroblasts with a spherical nucleus and some amounts of cytoplasm in the skin 5 months after fibroblasts transplantation. The eosin surroundings of the extracellular matrix are more colorful. (B) The normal skin usually shows deployment along bundles of collagen fiber, with elongated nucleus. Scale bar: 10 μ m.

Histology (Sirius Red Stain) of the Unlabeled Cells

Using polarization microscope morphometry, collagen I was observed as thick red fibers and collagen III was observed as thin green fibers in the same area. In the experimental subgroup, collagen III appeared as more discontinuous green fragments that were arranged irregularly (Fig. 3A). The control subgroup section showed red bundles with minimal green collagen III staining, suggesting that collagen I was the main fiber (Fig. 3B).

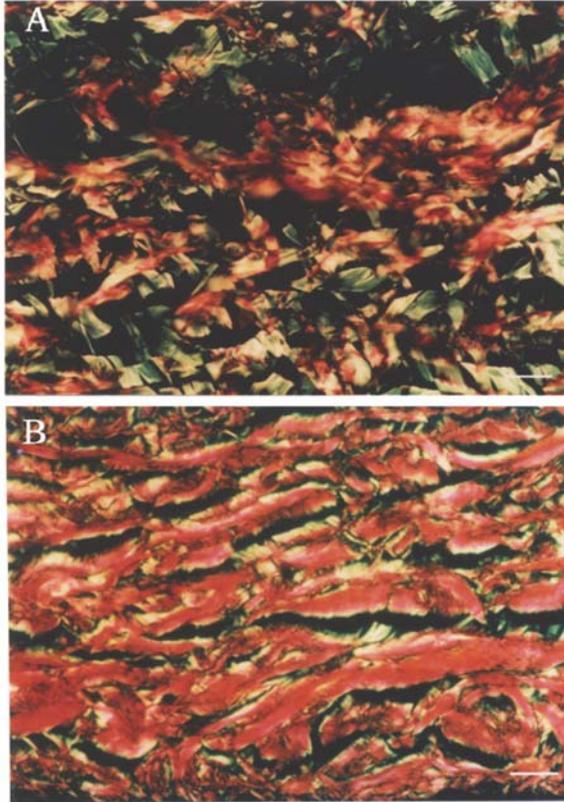


Figure 3. Histological staining with sirius red under the polarization microscope morphometry sections of the experimental subgroup (A) and the control subgroup (B) of the unlabeled autologous fibroblasts group. (A) Collagen III appears as more discontinuous green fragments, which are arranged irregularly in the skin 5 months after fibroblast transplantation (300 \times). (B) Collagen I appears as largely continuous red streaks with minimal green collagen III in normal skin (300 \times). Scale bar: 10 μ m.

Dermal Depth

The dermal depth of the experimental subgroup (701.3 \pm 31.5 μ m) was significantly thicker than the dermal depth of the control subgroup (638.3 \pm 23.9 μ m) ($p < 0.01$, paired t-test).

Skin Collagen

The absolute area of the collagen I fibers in the experimental subgroup (55.41 \pm 16.59 cm^2) was lower than in the control subgroup (56.25 \pm 14.41 cm^2) ($p > 0.05$, paired t-test). The absolute area of collagen III in the experimental subgroup (2.63 \pm 1.41 cm^2) was significantly higher than the control subgroup (1.05 \pm 0.90 cm^2) ($p < 0.05$, paired t-test) (Table 1).

Table 1. The Absolute Area of Collagen I and Collagen III

Group	Collagen I (cm ²)	Collagen III (cm ²)
Control subgroup (n = 8)	56.25 ± 14.41	1.05 ± 0.90
Experimental subgroup (n = 8)	55.41 ± 16.59*	2.63 ± 1.41†

p* = 0.91 > 0.05.†*p* = 0.02 < 0.05.Publication Conclusion:**

The authors stated that: 1) the autologous cultured fibroblasts could survive *in vivo* for more than 5 months and 2) the fibroblasts increased collagen formation, accompanied by a concomitant increase in the thickness and density of dermal collagen. Thus, the authors conclude that dermal injection of autologous cultured fibroblasts could potentially be used to treat dermal and subcutaneous facial defects.

Comments:

- The preparation of the rabbit analog cells used in this study did not duplicate the procedure that is used for IT preparation; for example, the rabbit cells were separated from the skin biopsy by mincing and treating with dispase; however, only digestive enzymes are used to prepare IT.
- The rabbit fibroblasts were formulated in PBS, and some cells were labeled with [³H]TdR, which is not the IT clinical formulation.
- A significantly higher amount of collagen III fibers was observed in the sites injected with fibroblasts compared to the PBS-injected sites. The biological implications of this finding were not discussed. Both collagen I and III fibers are responsible for skin strength and elasticity; collagen I is mainly found in tendons, skin, artery walls and collagen III is mainly found in granulation tissue and reticular fiber.

Toxicology Studies:

Toxicology studies as described in the International Conference on Harmonisation (ICH) Safety ('S') guidelines, consisting of pharmacokinetics, acute toxicology, chronic toxicology, genotoxicity, carcinogenicity, reproductive and developmental toxicity, safety pharmacology, and immunotoxicity (<http://www.ich.org/cache/compo/276-254-1.html>) were not conducted by the sponsor due to the autologous nature of IT and the previous human experience. The pharmacology/toxicology review of the original IND submission supported the sponsor's decision, noting that these cells are autologous, minimally manipulated, highly unlikely to change in phenotype, dedifferentiate, migrate or become tumorigenic (see Attachment I).

Comments:

- Gaps identified in the preclinical information provided included the following:
 - Evaluation of cell survival and migration; cell output (i.e., production of collagen, elastin, etc...) following *in vivo* administration of IT.
 - Cell ‘integration’ into the dermis and the potential for tissue remodeling abnormalities (i.e., scarring, keloids, etc..) that adversely effects normal dermal architecture following *in vivo* administration of IT.
 - The potential for tumorigenicity and/or other abnormal growth following *in vivo* administration of IT.
 - The potential toxicity of the DMEM injection medium and/or any potential variation in each batch/lot of DMEM.
- These gaps could potentially be addressed via limited studies in animals:
 - Human facial skin xenograft model in immunodeficient mice (dorsal skin flap). This study would use the intended clinical product, with the collection and processing of the cells identical to the clinical manufacturing process. The cell dose and formulation administered would need to mimic clinical, as feasible. The study duration would be the lifetime of the mouse (usually about 9 months) in order to evaluate the potential for tumor growth. **Note** that study would not provide any insight regarding tumor promotion/enhancement potential.
 - An acute *in vivo* toxicity study of the final product formulation without the cells in rabbits. This study could potentially provide a basic toxicity profile of several lots of the DMEM formulation. The intended clinical route of administration, dosing volume (2 ml) and the clinical dosing schedule would be duplicated. Basic toxicology data (i.e., clinical signs, body weights, appetite, Draize scoring, histopathology, etc..) could be collected.

However, there are multiple uncertainties associated with these studies, such as: 1) whether the graft itself would survive on the backs of the mice; 2) the ability of the graft to reflect any potential immunological reaction to the formulation buffer; 3) the utility of animal data versus obtaining biopsies from humans following IT injection (i.e., biopsy) to asses many of the above parameters; 4) the existing extensive data showing that DMEM is a well-defined media and is characterized/qualified by the certificate of analysis that is required for every lot/batch used; and 5) if a change in the DMEM occurs in the future, the sponsor is required to submit an amendment to any IND and a supplement to any existing BLA, as the DMEM is considered a critical reagent in the final formulation of IT.

This reviewer's recommendations are as follows: 1) assessment of any changes in the dermal architecture of the injected wrinkle should be obtained via biopsies in the human subjects; however, if additional questions arise based on the biopsy data, the conduct of a murine xenograft study could be considered to potentially investigate these concerns and 2) if the composition of the DMEM significantly changes with future lots, the acute safety of the DMEM should be tested in animals before incorporating into the cell formulation, with subsequent injection into humans.

CONCLUSION

No preclinical studies were conducted by the sponsor with the clinical product, Isolagen Therapy™, or with an animal cellular analog in support of early phase to later phase clinical trials. The commercial manufacturing experience and subject administration experience in Europe were considered sufficient to support initial clinical studies conducted under US IND. The sponsor instead provided five published articles of various *in vitro* and *in vivo* preclinical studies that they consider applicable to the administration of Isolagen Therapy™ for the treatment of nasolabial fold wrinkles. The fibroblasts used in these articles were of human and animal origin. The cell isolation procedure, culture condition, passage number, and formulation for each experiment described in the various publications were different from what is used for IT. The fibroblasts were administered to immune competent animals (animal analog cells) and to immunodeficient mice (human cells). General conclusions that can be made from the publications include the following:

1. Doses of $3-8 \times 10^7$ fibroblasts subcutaneously injected in mice, rats, and rabbits were functional, as evidenced by synthesis of type I collagen and elastin.
2. The long-term *in vivo* survival of the injected fibroblasts in the different animal models varied, with autologous rat fibroblasts surviving up to 8 months post administration, autologous rabbit fibroblasts surviving at least 5 months, and xenogeneic human fibroblasts surviving to at least two months in nude mice.
3. Following injection of human fibroblasts in combination with collagen in nude mice, 80-90% human cells were present in the injection site on day 10 and 25% were present by week 9.
4. Although not explicitly evaluated, no apparent adverse findings in the animals were cited.

This reviewer's recommendations are as follows: 1) assessment of any changes in the dermal architecture of the injected wrinkle should be obtained via biopsies in the human subjects; however, if additional questions arise based on the biopsy data, the conduct of a murine xenograft study could be considered to potentially investigate these concerns and 2) if the composition of the DMEM significantly changes with future lots, the acute safety of the DMEM should be tested in animals before incorporating into the cell formulation, with subsequent injection into humans.

Key Words/Terms: Cell transplantation, IT, dermal fibroblasts, type I collagen, type III collagen, dermis, soft tissue augmentation, contraction, hyaluronic acid, immunodeficient animals, skin biopsy, tumor, pharmacology, toxicology

Attachment I

Pharmacology/Toxicology Review of the Original IND -(b)(4)-:

Name: _____
Date: 1/3/00

Pharmacology Worksheet¹ (use for original INDs)

Background: (previous human experience; drug structure, known hold issues)

These files from _____ and Isologen are to evaluate a mature cell type, autologous fibroblasts, used by injection into skin defects on the face; clinical aspects of these files are nearly identical (see Dr. Marzella's review).

This is a privileged category of cell INDs- the cells are autologous, minimally manipulated, and used in an anatomically homologous location (skin cells to skin implant). In this case, it is highly unlikely the cells would adopt an unpredicted phenotype, or dedifferentiate, migrate, or become tumorigenic. No preclinical studies for safety are required in this case as that for Isologen. Safety issues are generally going to come from product/GMP concerns.

This procedure of implanting autologous fibroblasts (expanded *ex vivo* in injected i.d.) has been conducted as practice of medicine in the plastic surgery community for >5 years; this area is now under regulation by the FDA which should be beneficial in generating more controlled clinical trial data on safety and efficacy.

1. **INDs** (number and title as indicated on the IND routing slip):
Isologen, autologous fibroblasts for facial wrinkles and

number of volumes:	1 each
Related INDs: Held by same sponsor -	0
Held by different sponsor-	0

2. **Indicate the key or principle mechanism(s) of action as presently known:**

Autologous fibroblasts may stay and live where implanted, secrete collagen, and fill a hollow or crease in the skin, or in the case of _____, populate the surface of an unhealed burn from facial laser surgery. There are not good alternative therapies for this latter clinical problem.

- 2.1. **Receptor:** N/A
2.2. **Species Cross Reactivity:** human use, autologous cells

3. **Indicate the principle model used for efficacy:**

Human fibroblasts in a soluble collagen gel were implanted in athymic nude mice. Time courses were performed; within a limited implant field, human cells were present at 90% at day 10 after implant, but after 9 weeks, only 25% of the cells were of human origin, implying in the mice, the cells implanted were slowly being rejected. (Remmler D., et al. Arch Otolaryngol Head Neck Surg 115; 1989 p837).

Were sufficient and appropriate animal studies of activity performed? Y/N

yes

4. **Anticipated risks (list adverse effects, organ system or functional endpoint)**

The autologous cells may not assume a flat, desirable appearance, or may cause inflammation/scarring partly triggered by dying injected cells.

5. **Does this IND contain original studies not previously reported? Y/N**

No.

- 5.1 **If so, indicate the original studies cited in this IND .**

List below by title and study number:

N/A

- 5.2. **Fill in the following and indicate source (by reference to 5.1. above/ another IND, if possible). Identify a study number, study name, or literature citation used as the source of the information:**

Dosage:

PEL².

¹. Abbreviations: NA= not applicable; ND=not determined.

NOAEL³:

Max⁴:

Equivalency factor⁵:

Duration index⁶:

5.3 For the NOAEL indicate species, dose, toxicity, duration and dosing regimen:

6. Indicate:

6.1. Will study be in normal subjects?: Y/N

Yes.

6.2. Initial clinical dose:

6.3. Cite previous or ongoing trial(s) with investigational agent and highest dose used:
This is hard to verify since previous clinical experience was uncontrolled.

6.4. Clinical dose escalation scheme and regimen:

No escalation is planned.

7. Determine tSF⁷: (*tentative safety factor and indicate means of computation*)

N/A

8. Are the potential clinical toxicities adequately known? Y/N

If no indicate why -

Yes.

9. Are the toxicities considered extensions of pharmacological action? Y/N

Yes, the cells are unlikely to migrate or change phenotype.

Inflammation caused by necrosis of injected cells may be expected.

10. Are any additional studies necessary? Y/N - If yes, indicate the nature of the deficiency⁸:

No.

11. Recommendations: clinical hold - Y/N

Justification:

No hold is warranted from a pharmtox perspective.

Comments.

It is hard to ascertain from patients how long the treatment may be effective if the cells have a limited lifespan. Clinical plans should include a plan for tracking retreatment frequency.

². PEL is the lowest dose producing a pharmacological effect which is different from control, but without adverse effect.

³. NOAEL is the immediate dose below that producing an adverse effect.
(a dose could be both the PEL and NOAEL level)

⁴. Maximum dose used in the studies.

⁵. Equivalence factor is that number used to adjust the preclinical data to be more relevant to the clinical situation, eg. adjusts for relative potency, pharmacokinetic parameters or surface area..

⁶. The duration of the longest study in animals which you consider a reasonable approximation to the clinical study divided by the anticipated duration the clinical study.

⁷. tSF (tentative safety factor) is the NOAEL or PEL divided by the initial clinical dose.

⁸. For example, did not address autoimmunity or studies were of insufficient duration