

Effect of STAT inhibitor on the murine chronic graft-versus-host disease (GVHD) scleroderma model using an X-ray irradiation device

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Objective

Chronic graft-versus-host disease (cGVHD) is the fatal complication of allogeneic transplantation occurred by immunological response. Scleroderma-like symptoms are major in cGVHD such as progressive sclerosis of skin, peripheral circulatory failure, and multiple organ failure. The current treatment methods for sclerodermatous-GVHD (Scl-GVHD) are only conservative and more effective drugs are waited. The murine GVHD model induced by irradiation and allogeneic cell transplantation is known as one of the models which develops Scl-GVHD-like cutaneous symptoms. The aim of this study is to confirm the pathologic state of the Scl-GVHD model and evaluate the effectiveness of agents on this model. We evaluated Scl-GVHD score, hydroxyproline (HYP) content of the skin and histopathologic examinations. The results indicate that the allogeneic transplantation groups (cell amount A-C) develop cutaneous symptoms compared to the syngeneic group. Effects of Niclosamide were evaluated on this model. Niclosamide is reported for the suppressive effect on Scl-GVHD by inhibiting STAT3 pathway which involved in fibrogenesis.

Materials and Methods

Experiment 1

[Animals]

Donner: 10 week-old B10.D2, female
Recipient: 10 week-old BALB/c, female

[Method]

X-ray irradiation for recipients:

7 Gy, whole body irradiation using MX-160 Labo (mediXtec Japan Corp.).

Donner cell transplantation:

Donner spleen and bone marrow cell suspension was administrated intravenously via tail vein after 6 hours from irradiation.

[Evaluation]

● Scl-GVHD Score:

determined by evaluating dorsal skin alopecia and other cutaneous symptoms twice a week from Day 12 using following criteria.

- Criteria (ranging from 0 to 9)

Dorsal skin:

0: no sign, 0.5: thin hair region < 1 cm², 1: thin hair region > 1 cm², 1.5: alopecia < 1 cm², 2: alopecia > 1 cm²

Other cutaneous symptoms:

add 0.5 for each item with cutaneous symptoms: face, auricle, tail, and/or limbs.

General symptoms:

add 1 for each following item: piloerection, hunched posture or lethargy, vasculitis (one or more purpurial lesions on ears or tail), eyelid sclerosis (blepharophimosis), and/or diarrhea.

● Dorsal connective tissue thickness:

dermal connective tissue thickness was measured on histopathological specimen.

● Histopathological analysis:

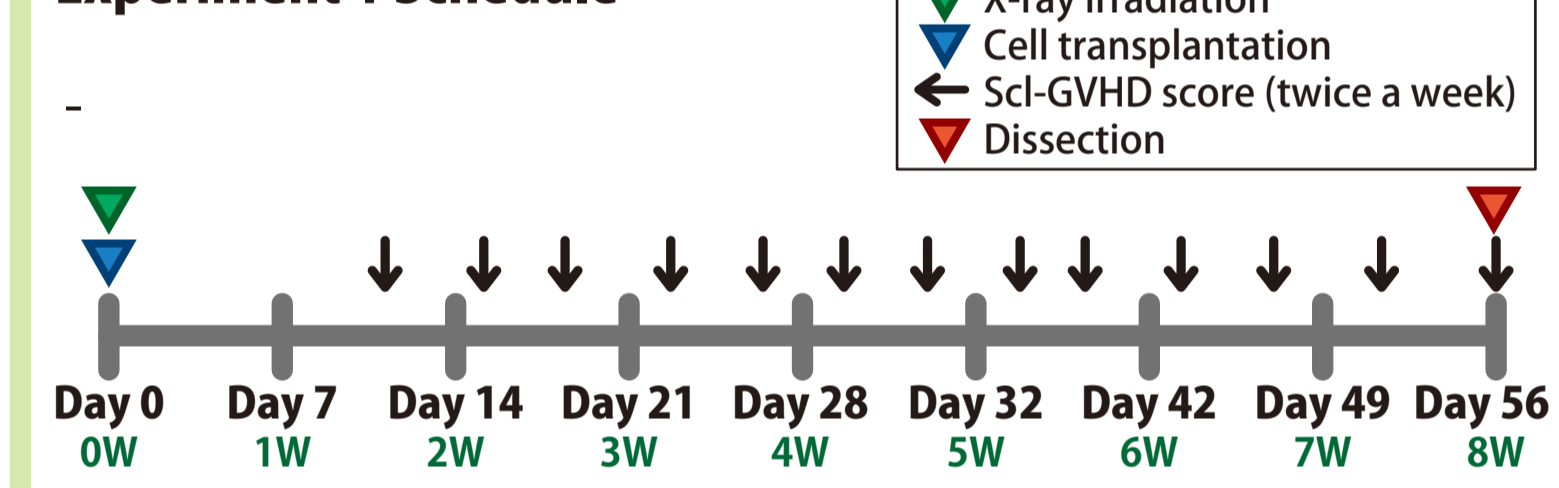
Day 56

Test Groups	X-ray irradiation	Donner (B10.D2) cell amount (cells/body)	n
1 Normal	-	-	3
2 Syngeneic	-	Sp*: 4x10 ⁶ , BM*: 2x10 ⁶	5
3 Cell amount A	7 Gy	Sp: 1x10 ⁶ , BM: 5x10 ⁵	8
4 Cell amount B	7 Gy	Sp: 2x10 ⁶ , BM: 1x10 ⁶	8
5 Cell amount C	7 Gy	Sp: 4x10 ⁶ , BM: 2x10 ⁶	8

Sp: Spleen, BM: bone marrow

*: recipient (BALB/c) cells were transplanted to Syngeneic group

Experiment 1 Schedule



Summary in Japanese

慢性移植片対宿主病 (chronic graft versus host disease, cGVHD) は同種異系間移植の合併症の一つであり、生体内の免疫応答により発症する症状の総称である。強皮症様症状はcGVHDの主要な症状であり、皮膚の硬化、末梢循環障害、多臓器不全を呈する。その治療法は対症療法のみであり、治療薬の開発が待ち望まれる疾患の一つである。

本研究では放射線照射と同種異系細胞移植によるGVHD強皮症モデルの作製を検討し、さらに本モデルを用いて薬効評価検討を行った。評価は病態スコア、皮膚中HYP量測定および病理組織学的検査にて行った。

モデル作製はBALB/cマウスをX線照射後、B10.D2マウス細胞を移植した。細胞移植後Day 14前後で皮膚及び全身症状の発症が始まり、Day 56までは病態の維持が確認された。このモデルに対してニコロサミドを投与し、薬剤の皮膚線維化に対する効果を評価した。ニコロサミドはSTAT3の阻害により線維形成を抑制することが報告されている。

本試験条件下より、ニコロサミド群ではControl群との有意差は認められず、抗線維化効果を捉えることはできなかった。今後、モデルの病態発症の程度や薬効評価方法について引き続き検討を行っていく。

Experiment 2

[Animals]

the same as Experiment 1

[Method]

the same as Experiment 1 except for concentration of administrated cell suspension which was Sp: 1x10⁶ cells/body and BM: 5x10⁵ cells/body.

[Administration]

Niclosamide:

1 mg/mL, 10 mL/kg, administrated intraperitoneally 3 times a week (every Monday, Wednesday and Friday) from Day 14 (total 12 times).

[Evaluation]

the same as Experiment 1 except for following:

● Scl-GVHD Score:

determined by evaluating dorsal skin alopecia and other cutaneous symptoms twice a week from Day 14 using following criteria.

- Criteria (ranging from 0 to 4.5)

Dorsal skin:

0: no sign, 0.5: thin hair region < 1 cm², 1: thin hair region > 1 cm², 1.5: alopecia < 1 cm², 2: alopecia 1-2 cm², 2.5: alopecia 2-3 cm², 3: alopecia > 3 cm²

Other cutaneous symptoms:

add 0.3 for each item with cutaneous symptoms: snout, periocular, auricle, tail, and/or limbs.

● Histopathological analysis:

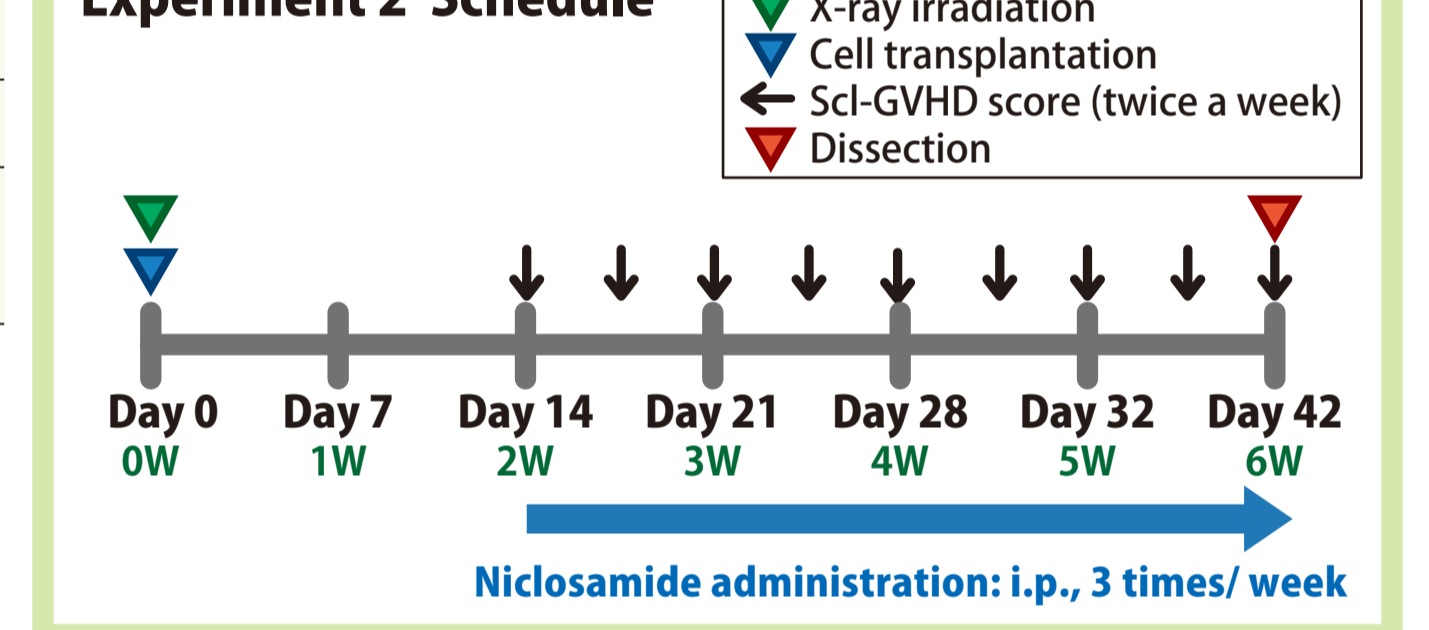
Day 42

Test groups	Administration	X-ray irradiation	Donner (B10.D2) cell amount (cells/body)	n
1 Syngeneic	i.p.	7 Gy	Sp*: 1x10 ⁶ , BM*: 5x10 ⁵	5
2 Control	10 mL/kg		Sp: 1x10 ⁶ , BM: 5x10 ⁵	8
3 Niclosamide			Sp: 1x10 ⁶ , BM: 5x10 ⁵	8

Sp: Spleen, BM: bone marrow

*: recipient (BALB/c) cells were transplanted to Syngeneic group

Experiment 2 Schedule



Results

Experiment 1

Figure 1 Scl-GVHD score

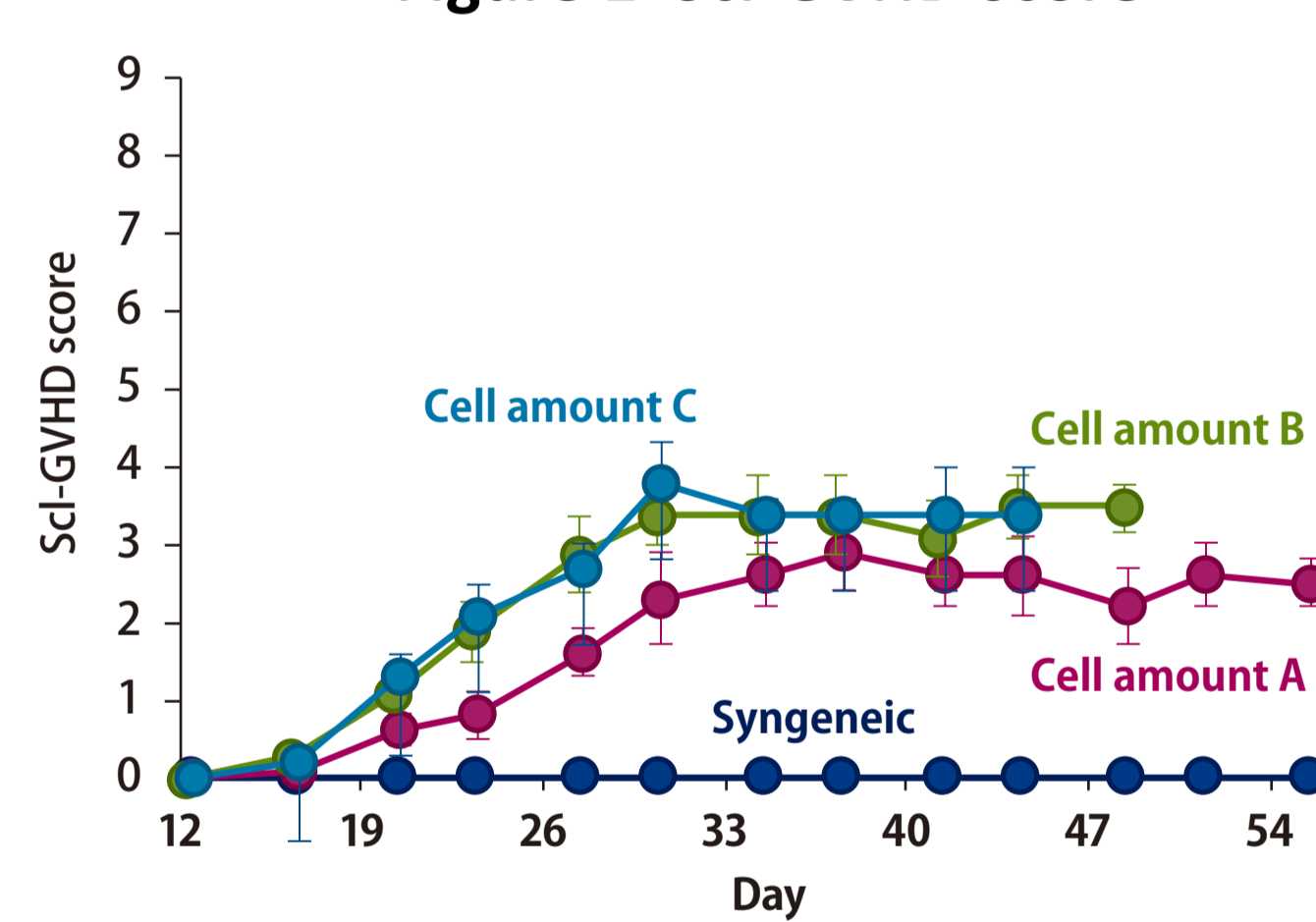
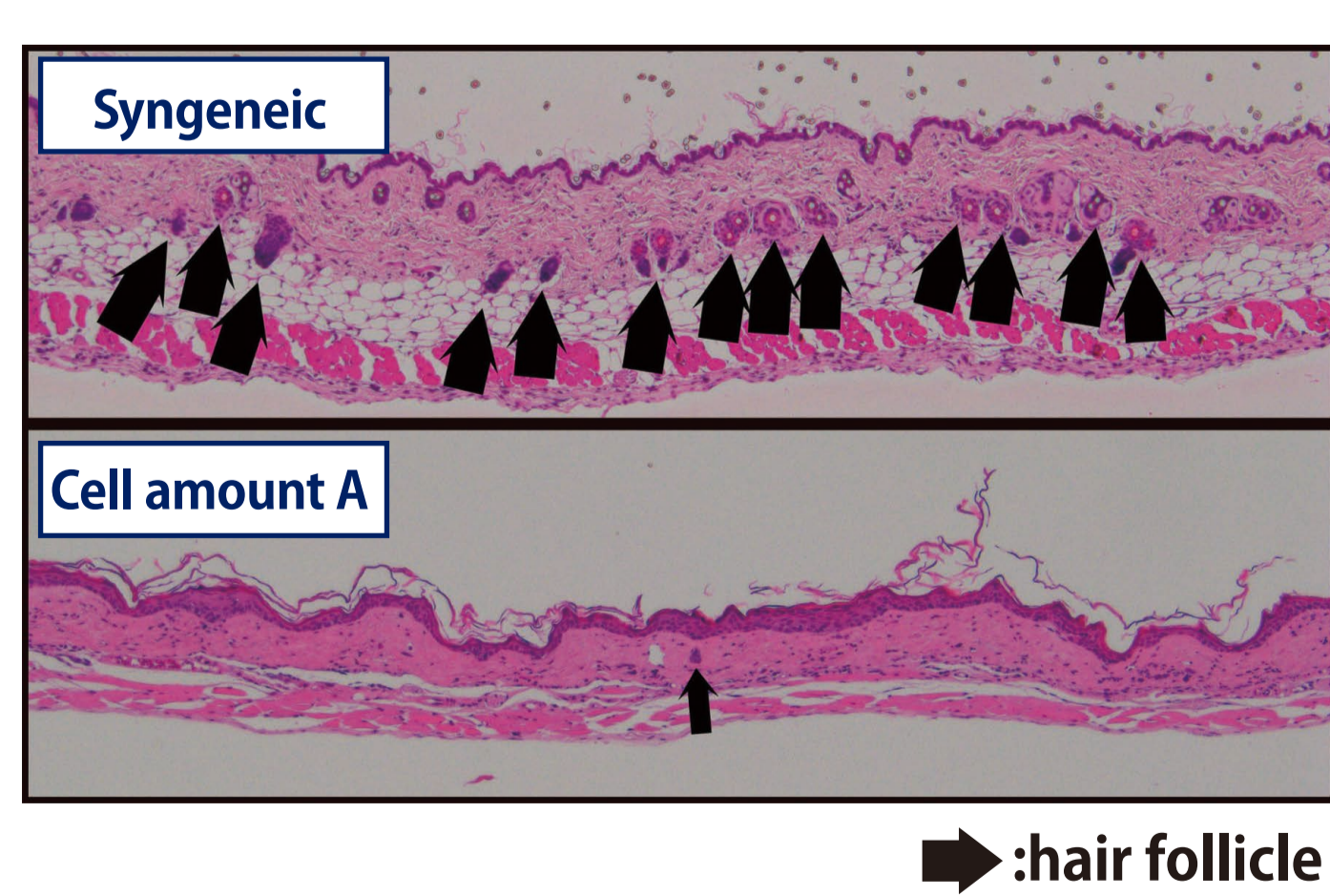
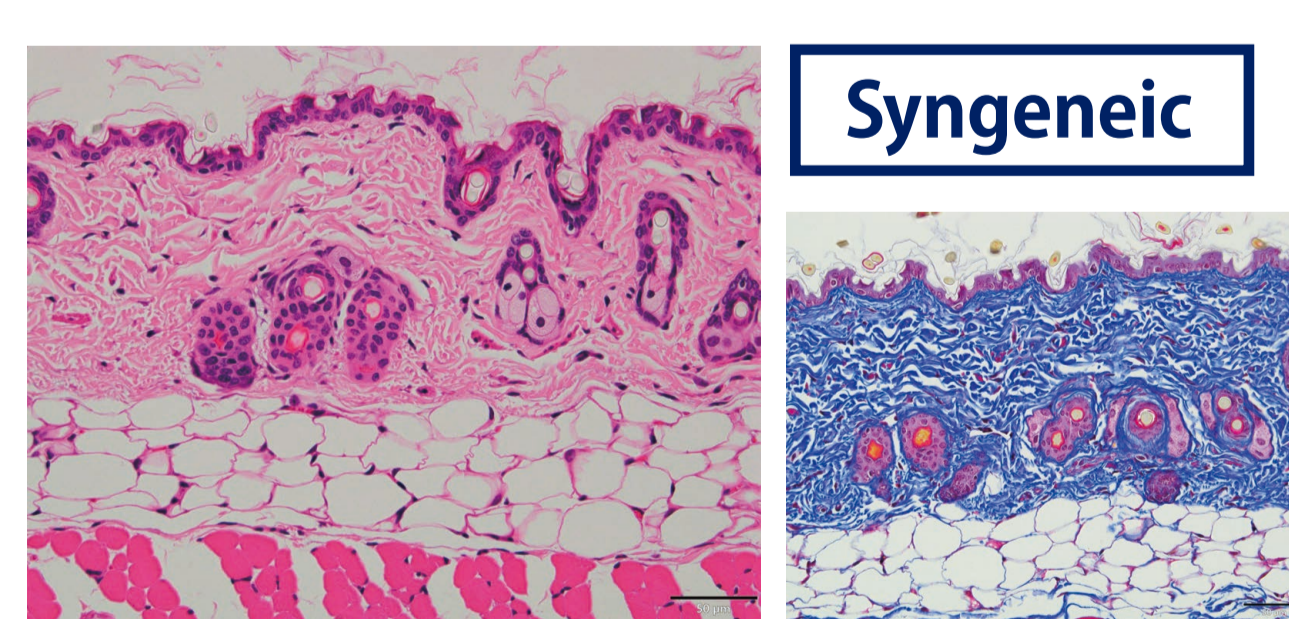
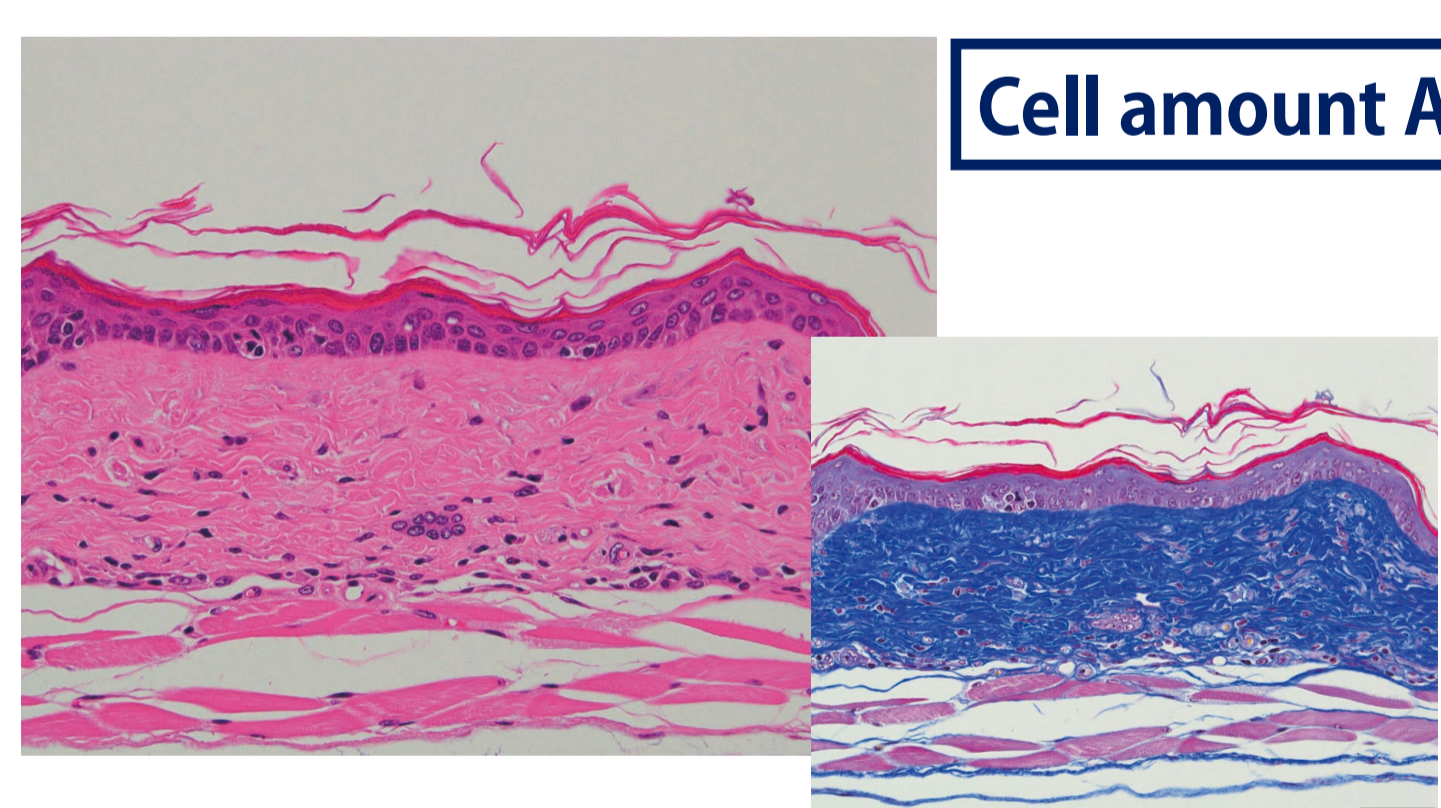
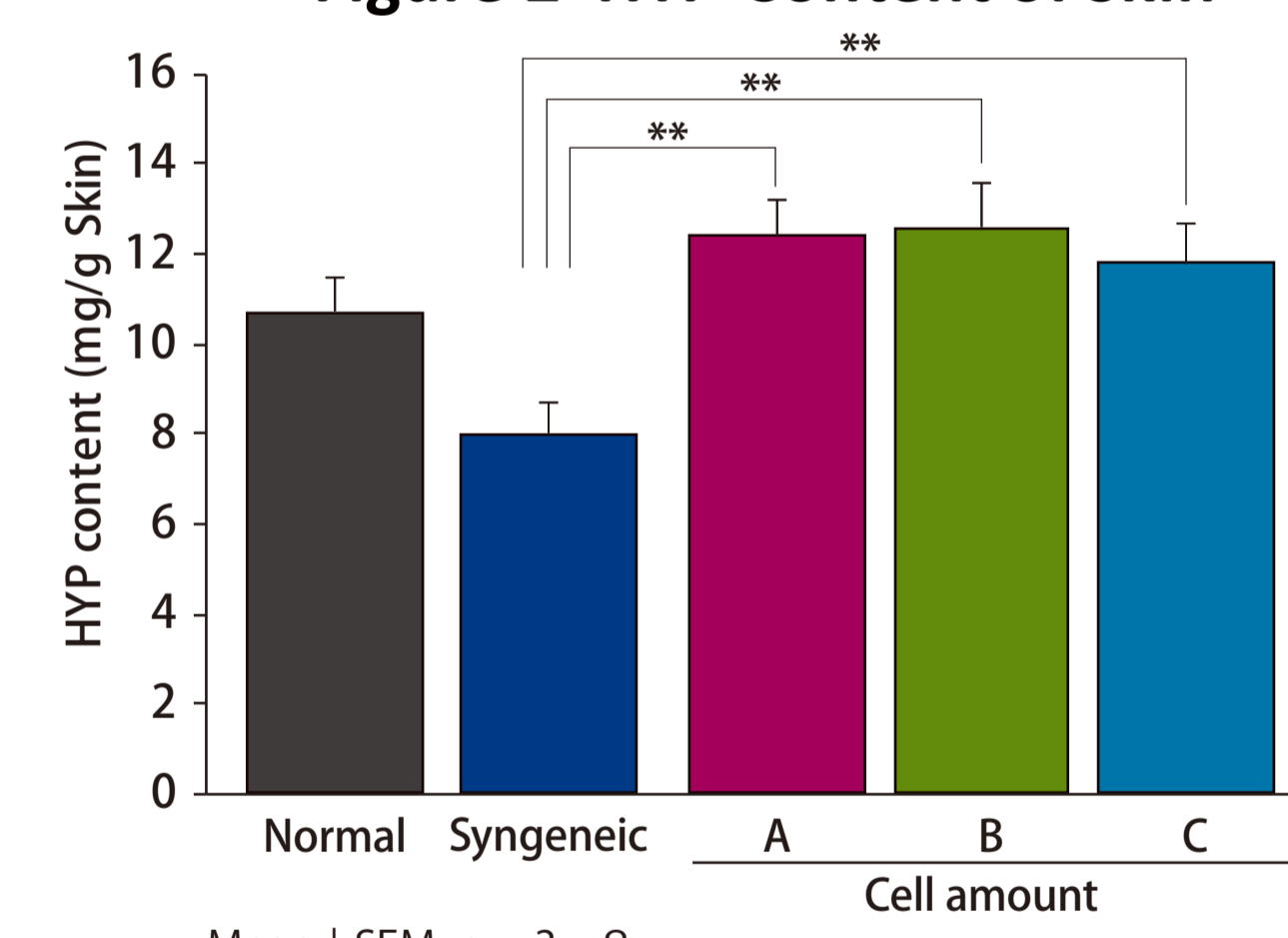
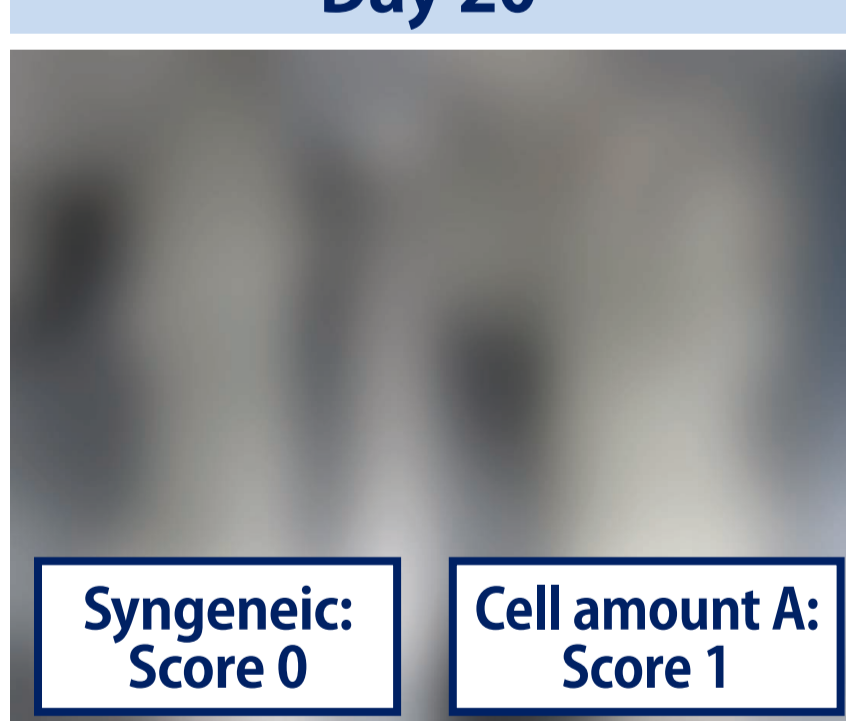


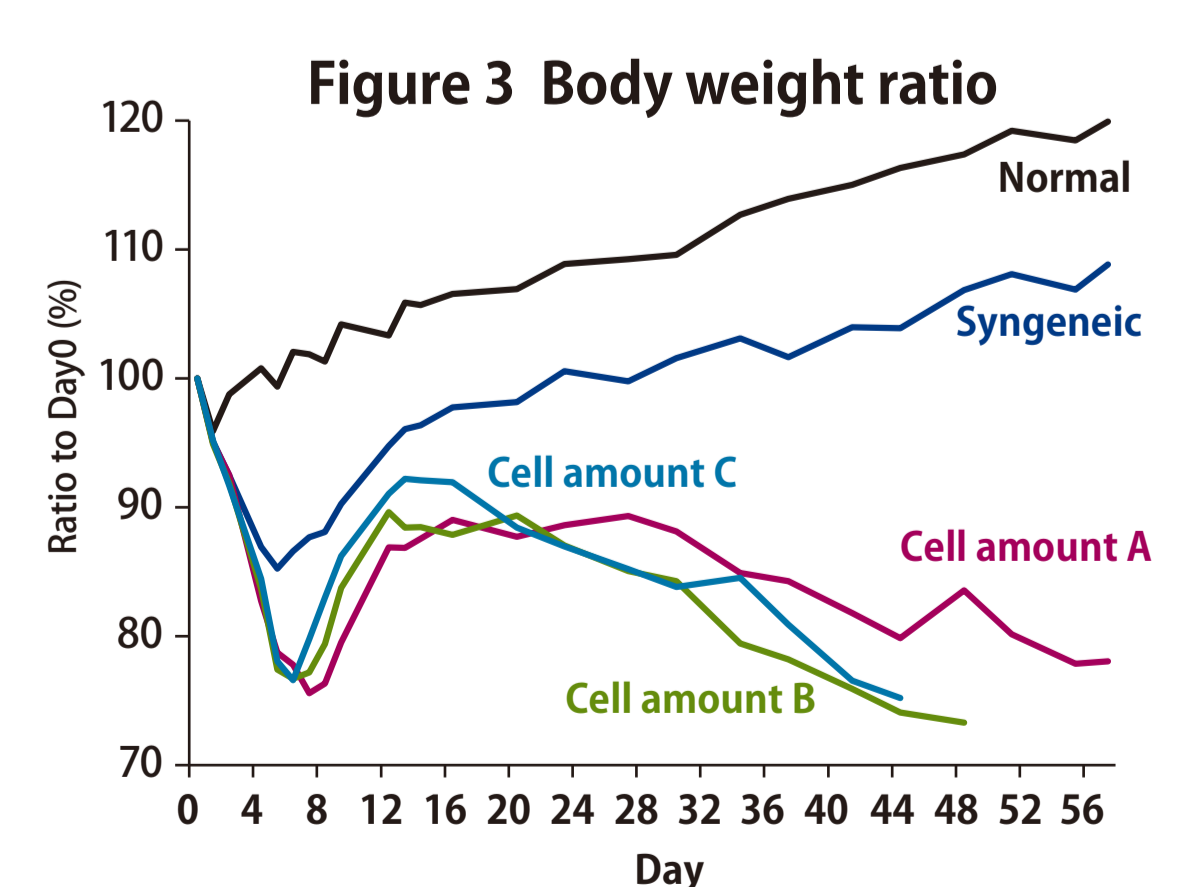
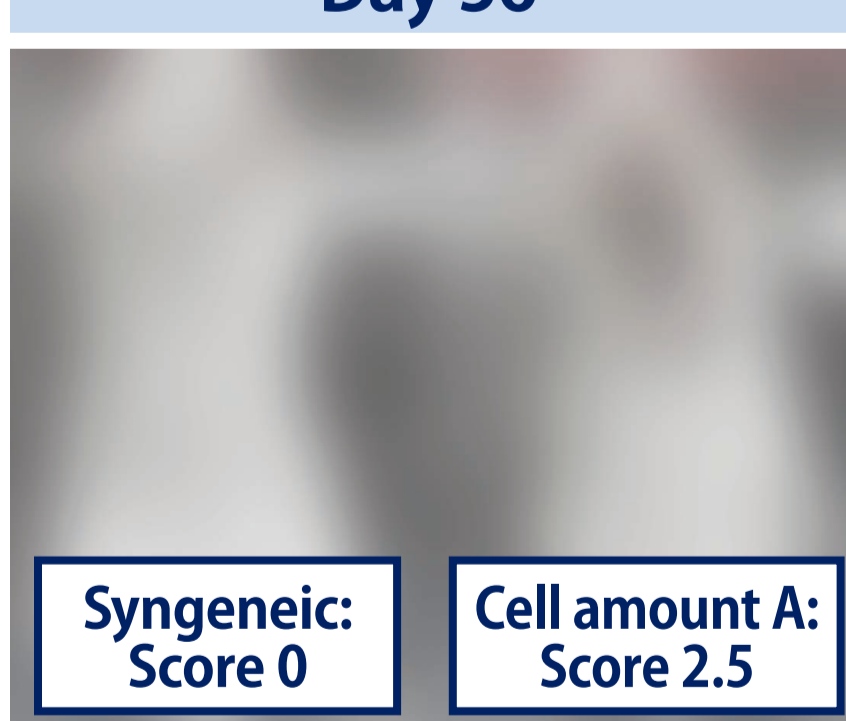
Figure 2 HYP Content of Skin



Day 20



Day 56



Experiment 2

Figure 4 Scl-GVHD score

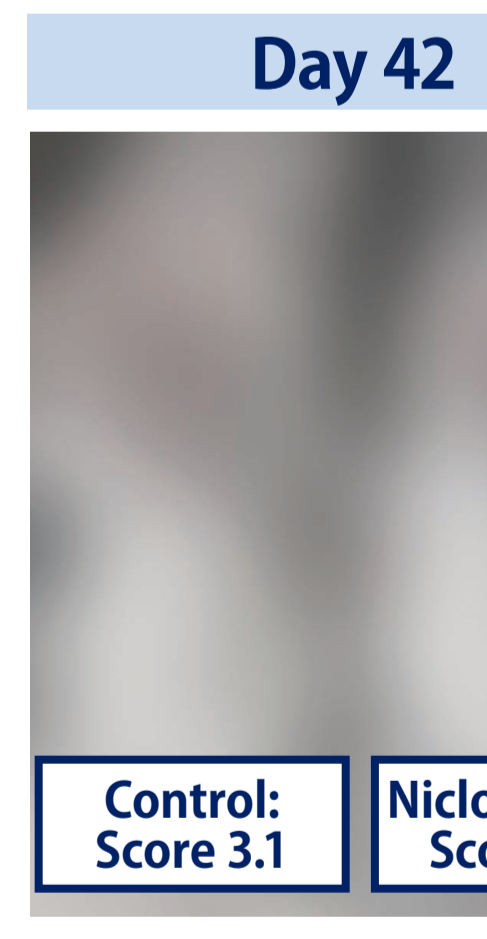
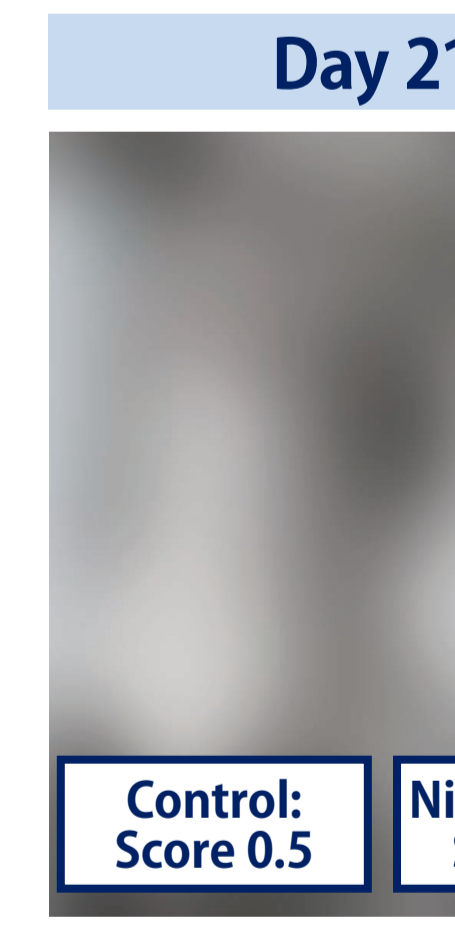
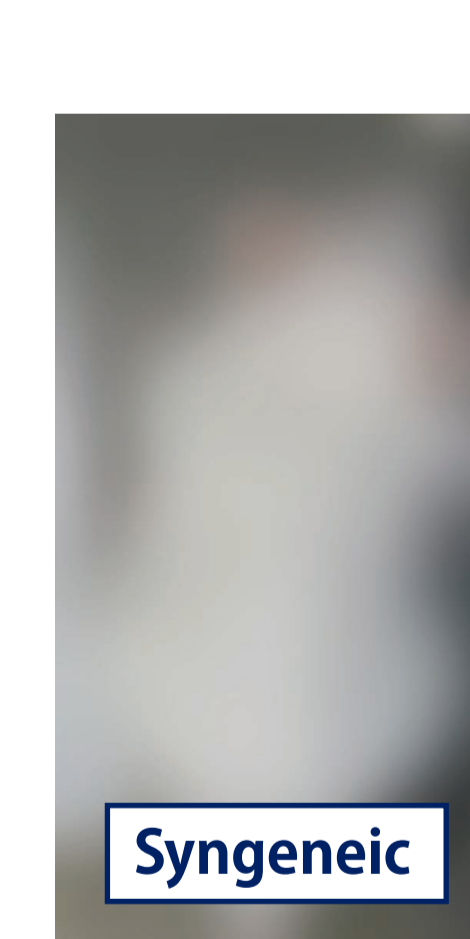
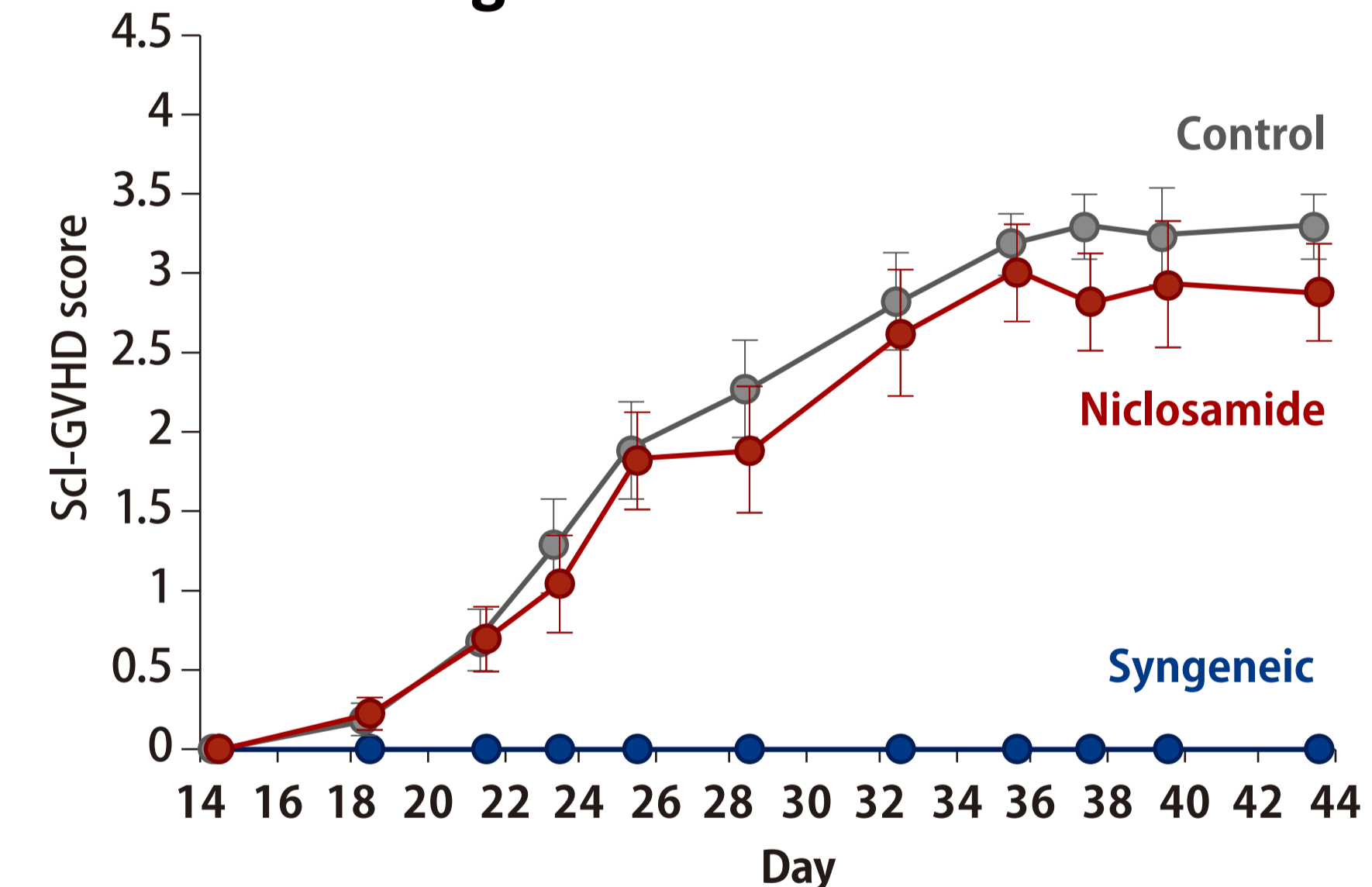
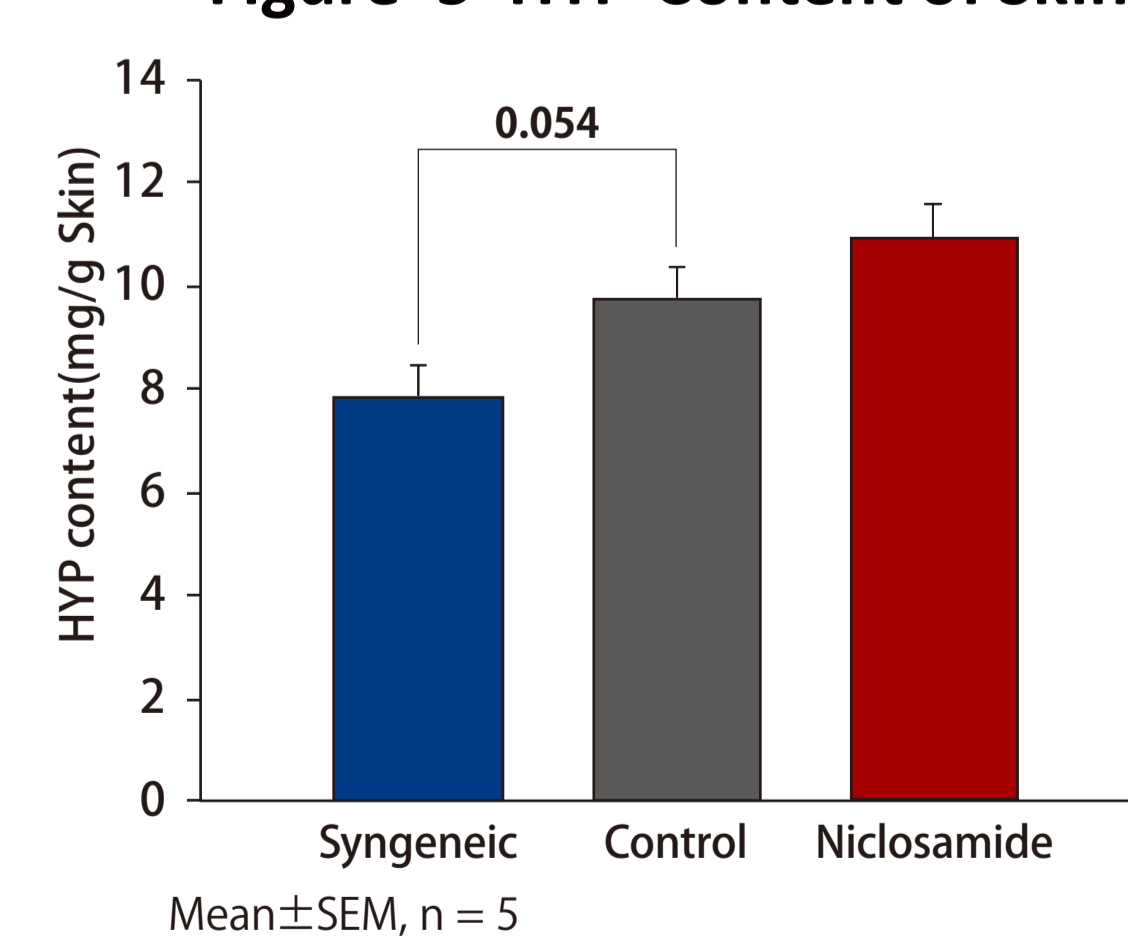


Figure 5 HYP Content of Skin

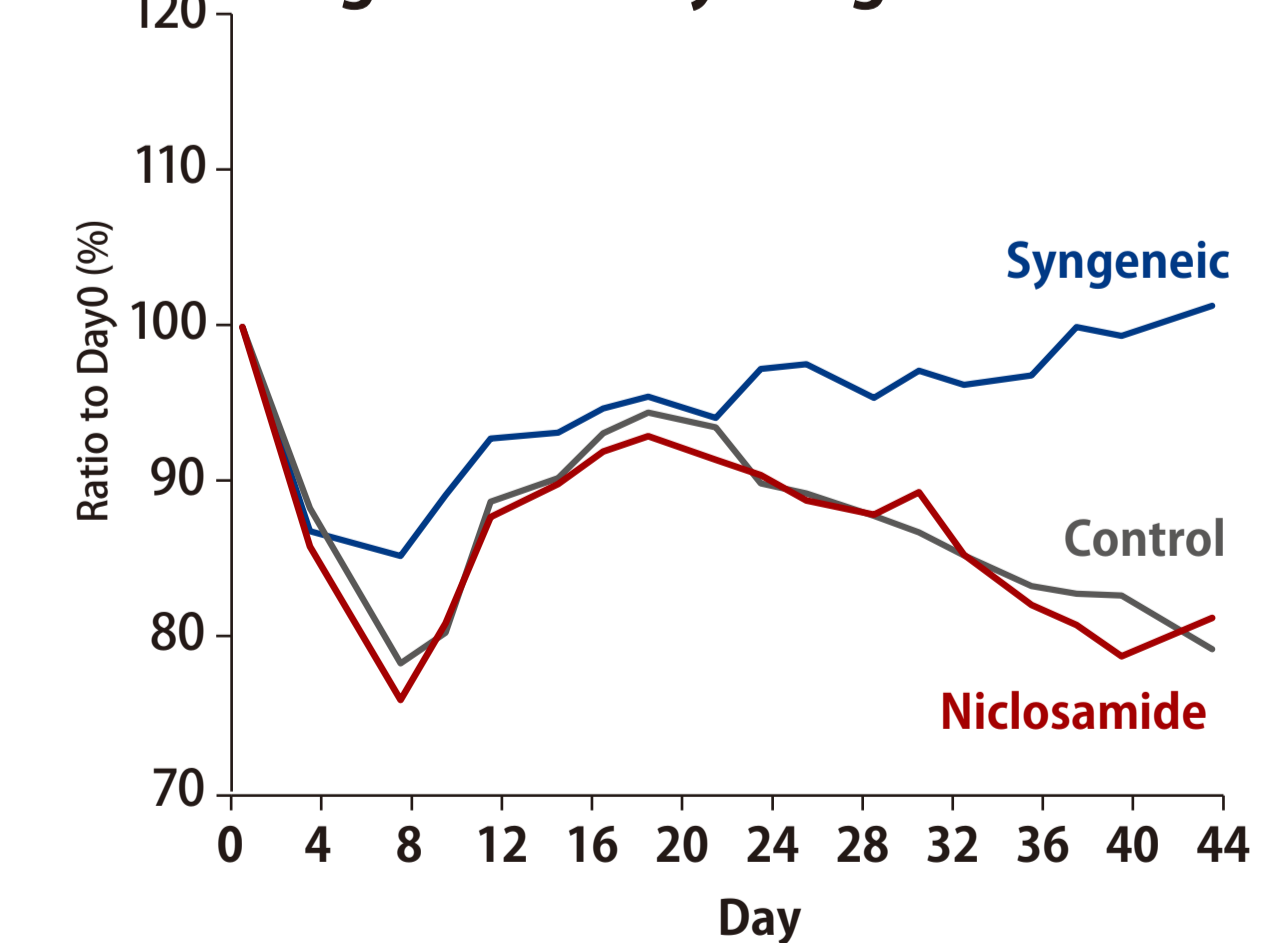


Mean ± SEM, n = 5

no significant difference from syngeneic group. (Student's t-test)

no significant difference from control group. (Student's t-test)

Figure 6 Body weight ratio



Conclusion

- The Scl-GVHD model which develops dermal fibrosis and histopathological changes seen in human was produced. The transplanted cell amount affect the development speed of pathological condition.
- Results of Experiment 2 revealed that Niclosamide (10 mg/kg) showed no significant improving effect on this model (Fig. 4-6).
- We continue to revise the method of induction to achieve stable pathological conditions of dermal fibrosis for a better model, which could be used for the evaluation and development of new anti-fibrosis agents.

- There was significant increase in Scl-GVHD score and HYP content of skin of Scl-GVHD group compared to the Normal group (Fig. 1, 2).
- Histopathological analysis in dorsal skin showed the remarks seen in human patient of Scl-GVHD such as swelling of collagen fibers and decrease of hair follicles in all model groups.
- Transplanted cell amount affect the development speed of pathological condition and the systemic condition of animals (Fig. 1, 3).
- Scl-GVHD score reached plateau on Day 30 for cell amount B and C groups, and Day 37 for cell amount A group while the appearance (alopecia and/or other cutaneous symptoms) of animals change afterwards.
- From the results of Experiment 1, the cell transplantation was conducted with cell suspension prepared with concentration of cell amount A (Sp: 1x10⁶, BM: 5x10⁵) in Experiment 2. For Scl-GVHD score, we changed the criteria to enable the score to evaluate more than 37 days.