Preparation of alginate hydrogels with carbonated water for wound dressings application

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Introduction

➢ Wound healing with hydrogels

- ✓ The skin is the largest organ in our body and very vulnerable to external damage.
- ✓ Depending on the type of the wound site, wound healing can last several years.
- ✓ Wound healing is promoted by keeping the moist environment of wound site[1].

- ✓ Hydrogels have a three-dimensional polymer network structure that contains numerous water molecules.

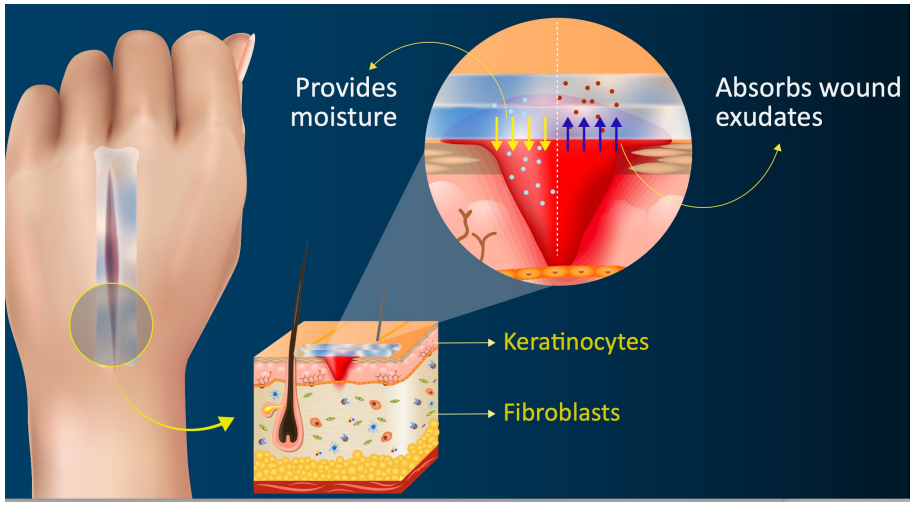


Image citation: https://www.tus.ac.jp/en/mediarelations/archive/20200826_0087.html

Hydrogel has attracted attention as a wound dressing[2,3].

1. K. Nuutila *et al.* Adv in Wound Care, 2021. 10. 685-698.
2. Z. Xu *et al*. Adv. Healthcare Mater. 2020. 9. 1901502.
3. Y. Liang *et al.* ACS Nano. 2021. 15.12687-127222.

Hydrogel provides moist environment for the wound site and can promote wound healing.

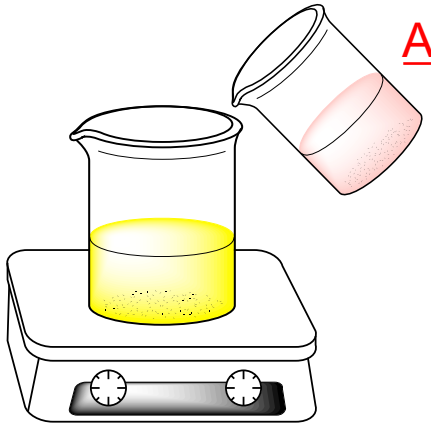
Introduction

➢ Alginate

- ✓ Alginate is an anionic polysaccharide extracted from brown algae.
- ✓ Alginate has high biocompatibility and reacts with Ca^{2+} to form hydrogels.

Alginate hydrogels are widely applied as biomaterials[4].

➢ Preparation method of alginate hydrogel[5]



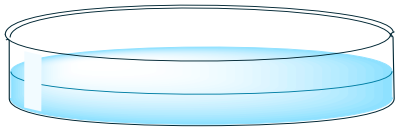
Alginate/$CaCO_3$ solution

Acid Solution

$CaCO_3 + 2H^+ \rightarrow Ca^{2+} + H_2O + CO_2$

Alginate + Ca^{2+} → Hydrogel formation

Gelation



"Hydrogel"

In these methods…

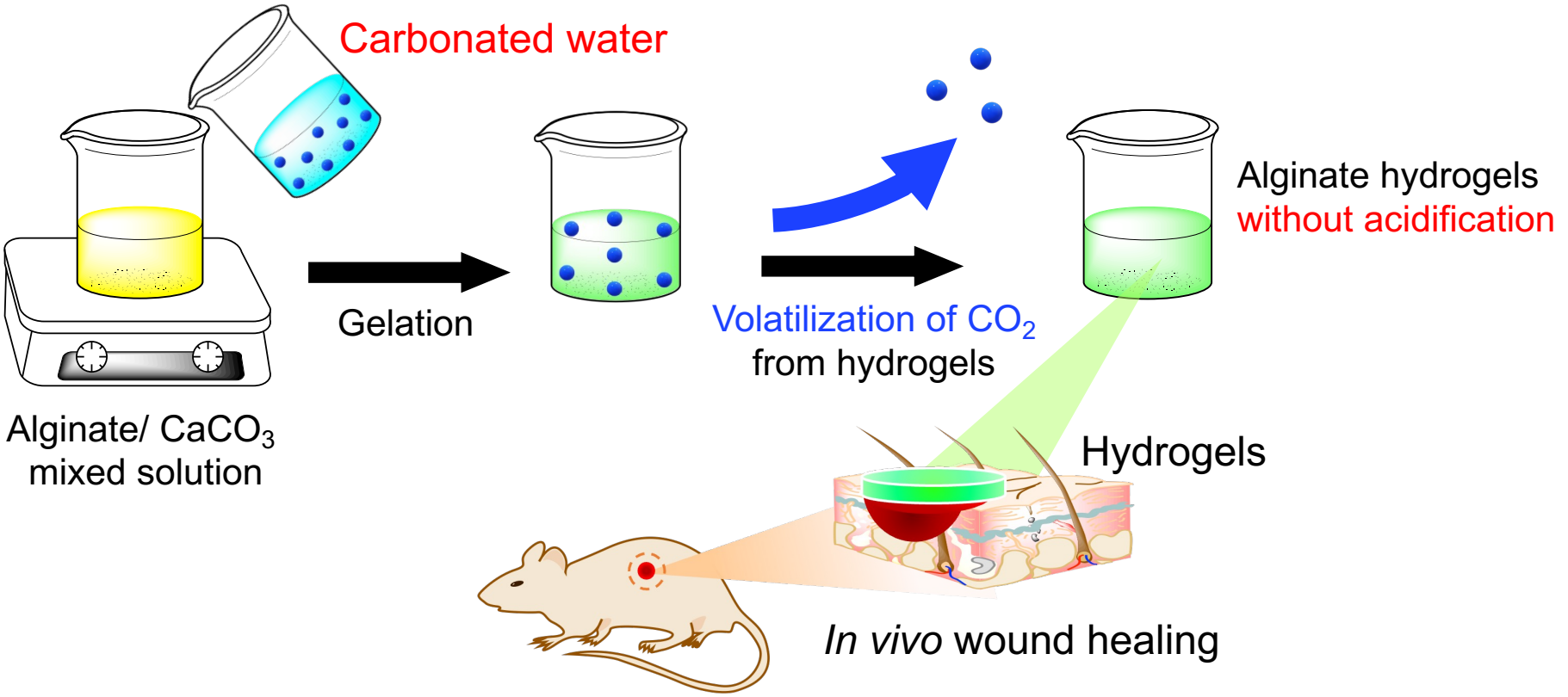
Residual acidic agent in the hydrogel

Acidification of the hydrogel

4. C. J. Maxwell *et al*. J Biomed Mater Res Part A. 2022. 110. 1621-1635.
5. M.C. Straccia *et al*. *Mar. Drugs.* 2015. 13. 2890-2908.

The low pH of hydrogel can inhibit cell proliferation and limit the applicability for wound dressings.

Purpose of this study



CO_2 in carbonated water induces gelation by dissolving $CaCO_3$. with temporary pH decrease and volatilizes from the hydrogel[6].

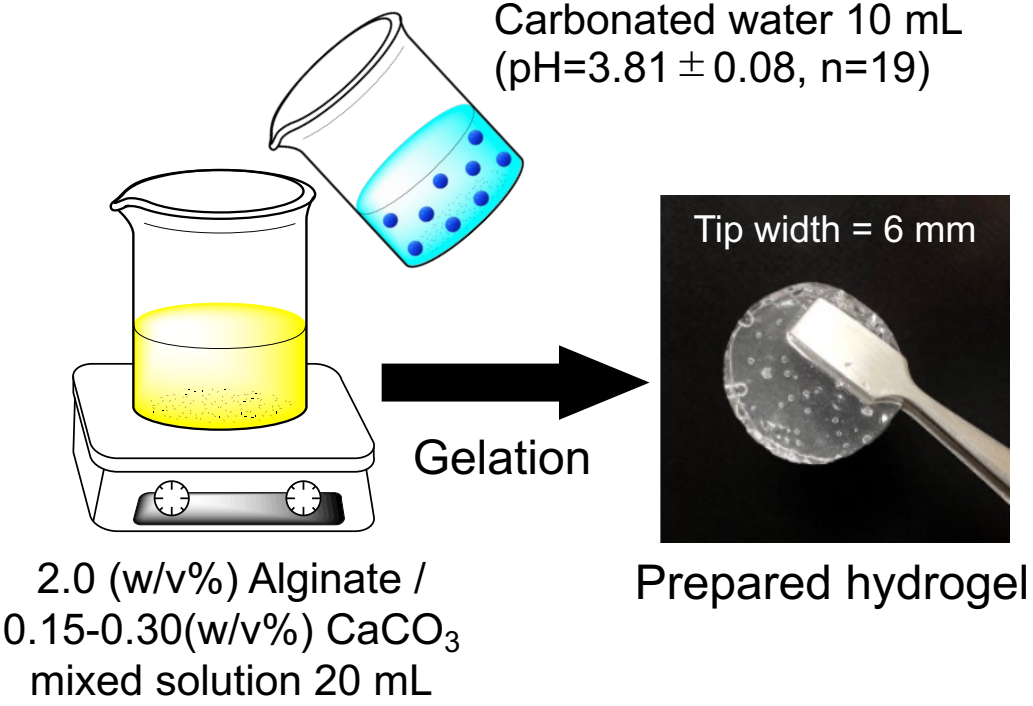
→This methods accelerate gelation without acidification of final hydrogel.

6. R. Teshima *et al*. Polym Adv Technol. 2020. 31. 3032-3038.

In this study, the physicochemical properties and wound-healing effects of hydrogels were evaluated.

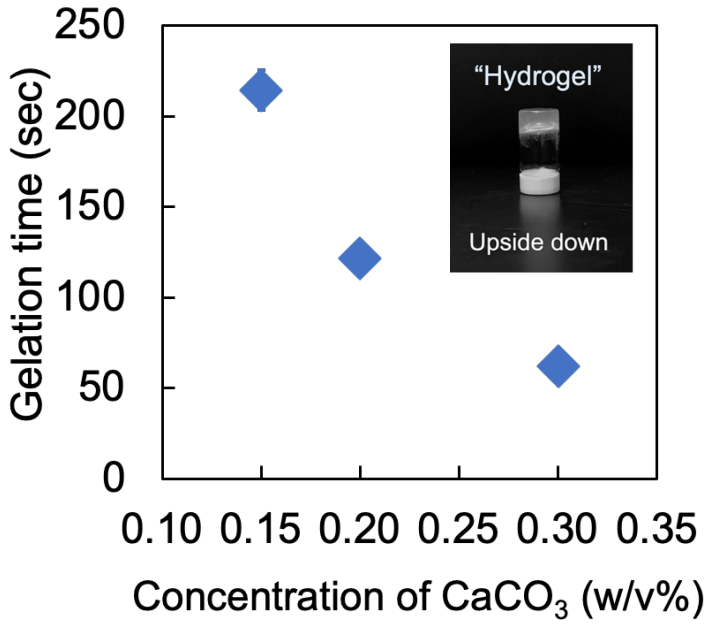
Results and Discussion

➢ Hydrogel preparation



➢ Gelation time

Gel volume: 5 mL, Temperature: 23-25℃, Values are expressed as the mean ± SD; n=3 (Error bars were smaller than the plot size).



The prepared gel was transparent and could be handled with tweezers.

The gelation time can be controlled with $CaCO_3$ concentration.

The mixed solution transited to a hydrogel within 4 min.

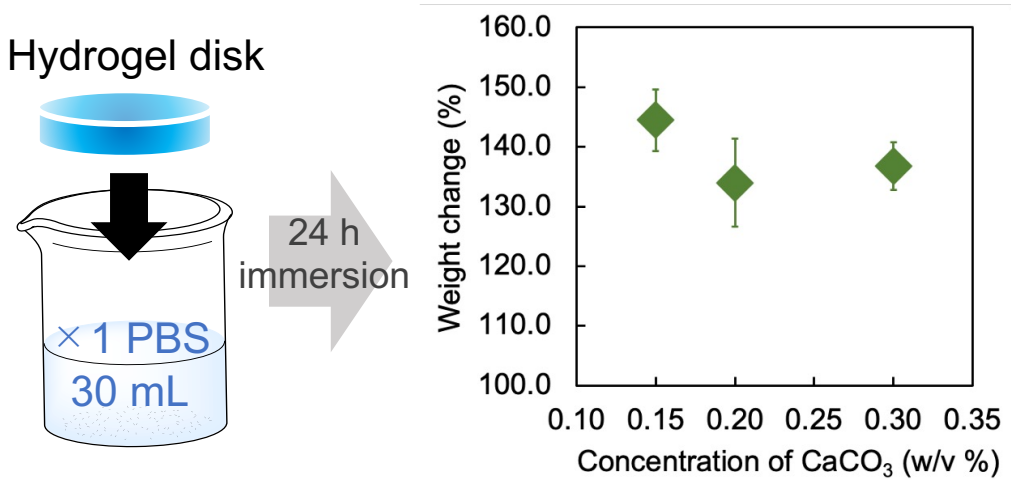
Results and Discussion

➢ Water absorption test

Hydrogel disk: φ17.5 mm × 3.5 mm, Temperature: 23-25℃, Values are expressed as the mean ± SD;n=3.

$$Weight\ change\ (\%) = \frac{W_s}{W_p} \times 100$$

W_p= the weight of prepared gel, W_s= the weight of the swollen gel



All hydrogels absorbed × 1 PBS solution.

➢ Water contents

Hydrogel disk: φ17.5 mm × 3.5 mm, Values are expressed as the mean ± SD; n=3.

$$Water\ content\ (\%) = \frac{W_w - W_d}{W_w} \times 100$$

W_w= the weight of prepared gel, W_d= the weight of dry gel.

$CaCO_3$ conc.(w/v%)	Water content(%)
0.15	98.6 ± 0.2
0.20	98.7 ± 0.1
0.30	98.5 ± 0.1

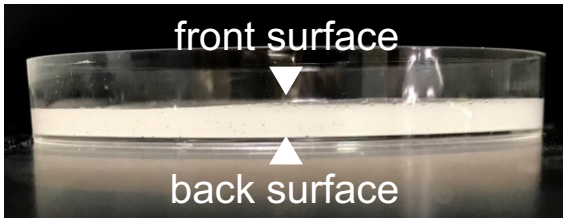
All hydrogels had high water content (~99%).

The hydrogel provides an adequate moist environment to the wound site and can maintain exudate.

Results and Discussion

➢ Surfaces pH measurements of hydrogels

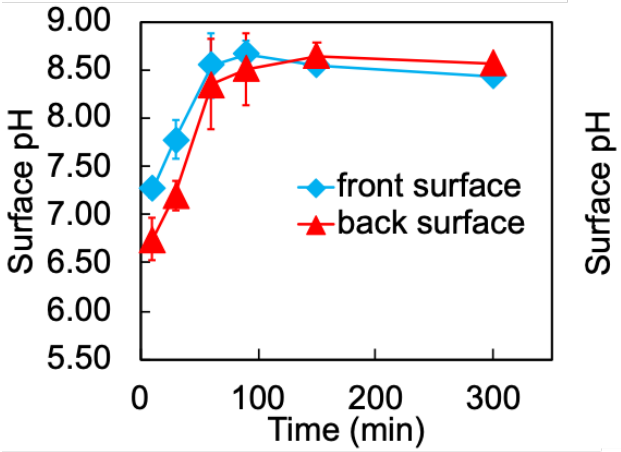
Hydrogel disk: φ17.5 mm × 3.5 mm, $CaCO_3$ concentration: 0.20 (w/v%), Temperature: 23-25℃, Values are expressed as the mean ± SD; n=3-6.



The surfaces pH was measured by flat electrode over time.

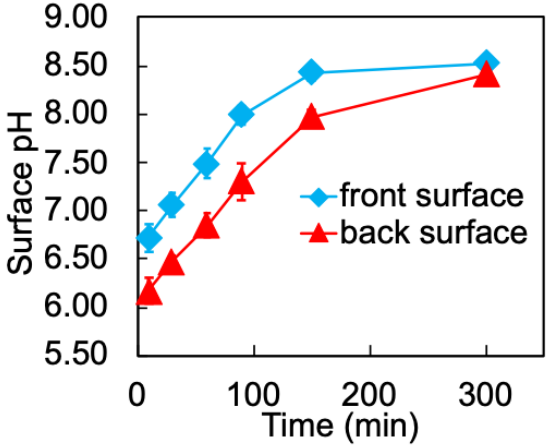
Gel thickness= 1.23 ± 0.38 mm, 3.46 ± 0.32 mm, 5.89 ± 0.29 mm

Thickness = 1.23 ± 0.38 mm



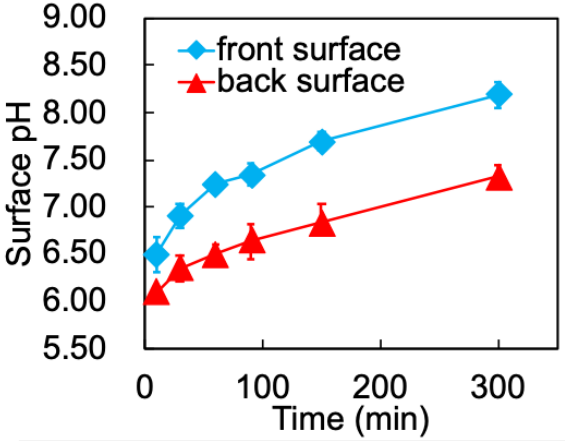
Convergent pH =~8.5

Thickness = 3.46 ± 0.32 mm



Convergent pH =~8.5

Thickness = 5.89 ± 0.29 mm



No convergence

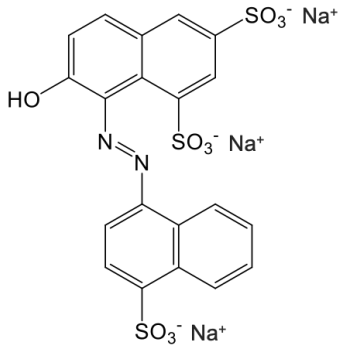
CO_2 volatilizes through the front surface of hydrogels and final pH is suitable for fibroblast and epidermal cell growth.

Results and Discussion

➢ Release behavior of New Coccine from hydrogel

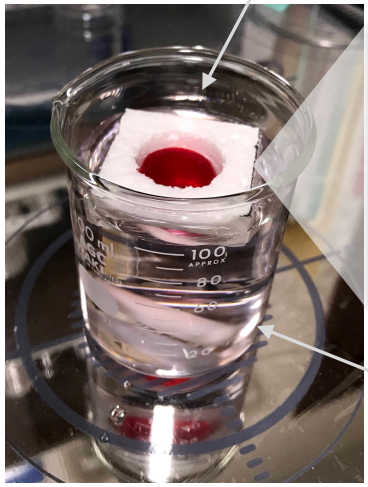
Hydrogel disk: φ17.5 mm × 3.5 mm, New Coccine (NC) concentration in gels: 1.98 mg/g-gel, Temperature: 23-25℃, Physiological saline volume: 100 mL, Stirring speed: 200 rpm, Values are expressed as the mean ± SD; n=2.

New Coccine (NC)



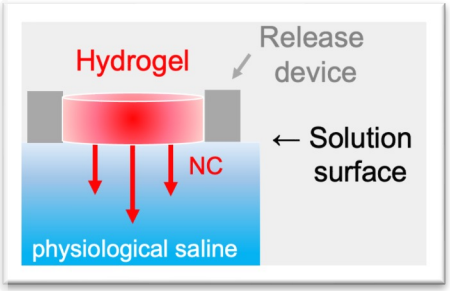
Molecular Weight = 604.5

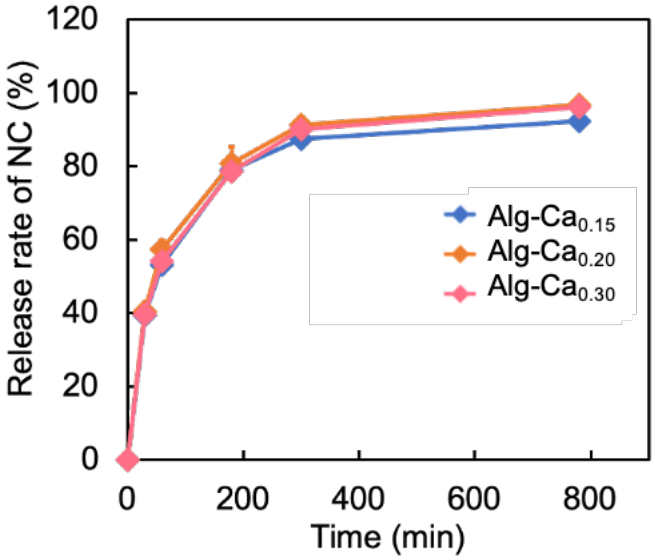
The hydrogel with NC (1.98 mg/g-gel)



Physiological saline 100 mL

Release devise





Alg-Ca_x : x = $CaCO_3$ concentration

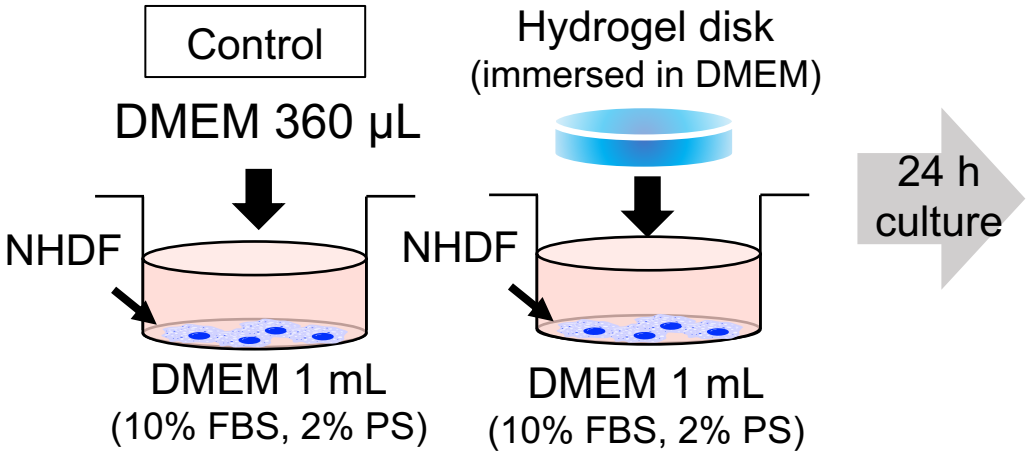
The hydrogel released ~90% of NC from hydrogel surface within 300 min.

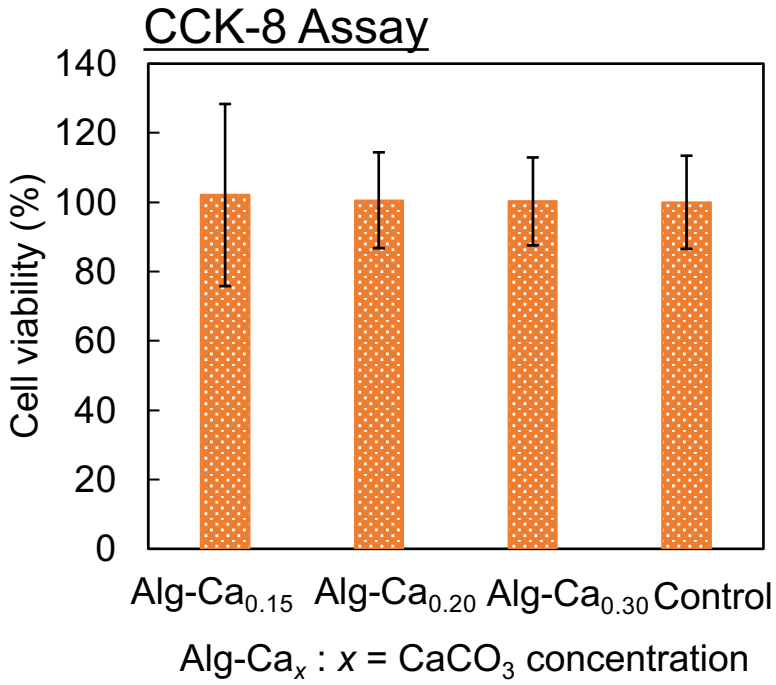
The hydrogels have potential application for drug delivery material on wound site.

Results and Discussion

➢ Biocompatibility of hydrogels

Hydrogel disk: φ11.5 mm × 3.5 mm, $CaCO_3$ concentration: 0.20 (w/v%), Cell: Normal Human Dermal Fibroblasts (NHDF), Passage: p15 ,Cell density: 2.5×10^4 cells/well, pre-Incubation time: 12 h (37℃, 5% CO_2), Incubation time: 24 h (37℃, 5% CO_2), Values are expressed as the mean ± SD ;n=4.





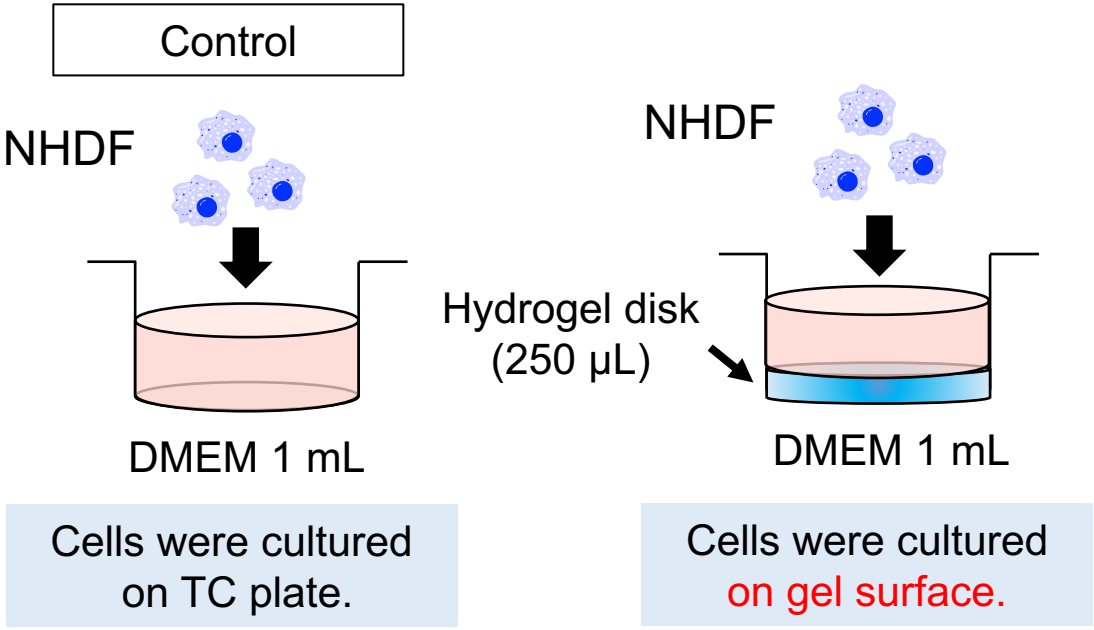
Hydrogels prepared in this study do not inhibit NHDF cells proliferation.

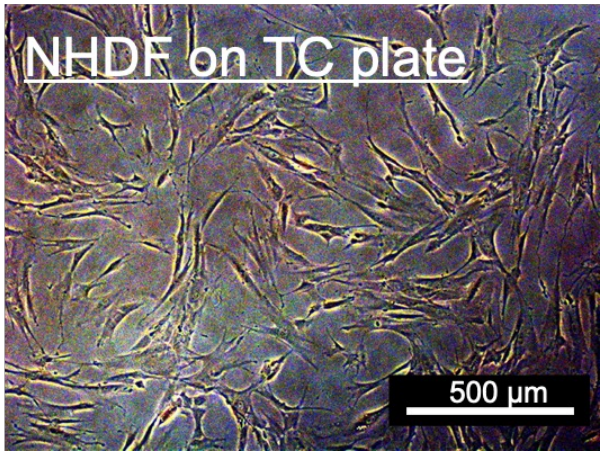
The hydrogels have biocompatibility.

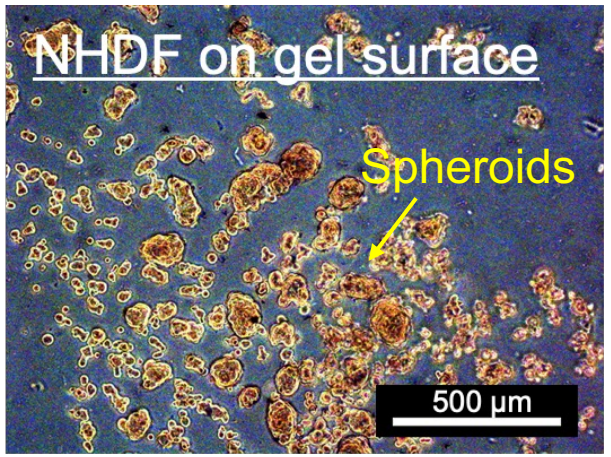
Results and Discussion

➢ Cell adhesion test of hydrogels

Gel volume: 250 μL, $CaCO_3$ concentration: 0.20 (w/v%), Cell: Normal Human Dermal Fibroblasts (NHDF) Passage: p11 ,Cell density: 2.0×10^4 cells/well, Incubation time: 17 h (37℃, 5% CO_2)







NHDF formed aggregation structures (“Spheroids”) on the gel surface.

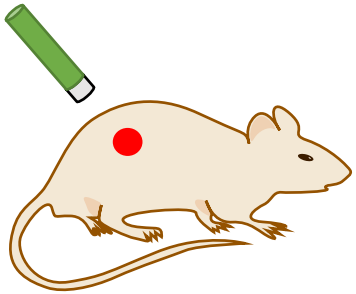
→ NHDF do not adhere to hydrogel surface.

Hydrogel can be exchanged without damaging tissue.

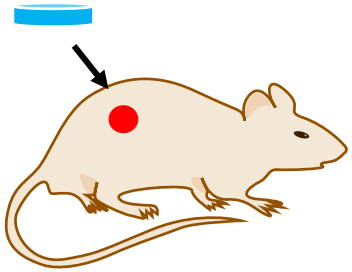
Results and Discussion

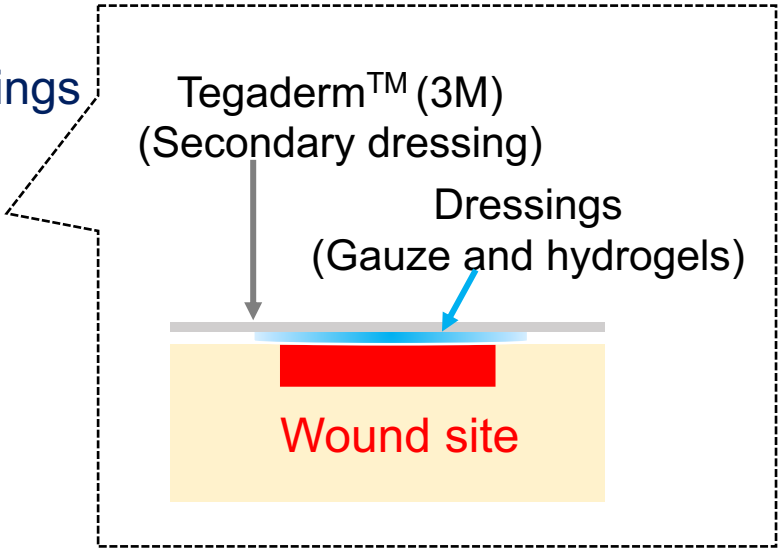
➢ Experimental model with full-thickness skin defects

Excisional wound formation



Attachment of dressings





C57BLKS/Jlar- +Lepr^{db}/ +Lepr^{db} mice
Wound average area = 29.8 mm^2
Weight average = 34.6 g

Wound Dressings

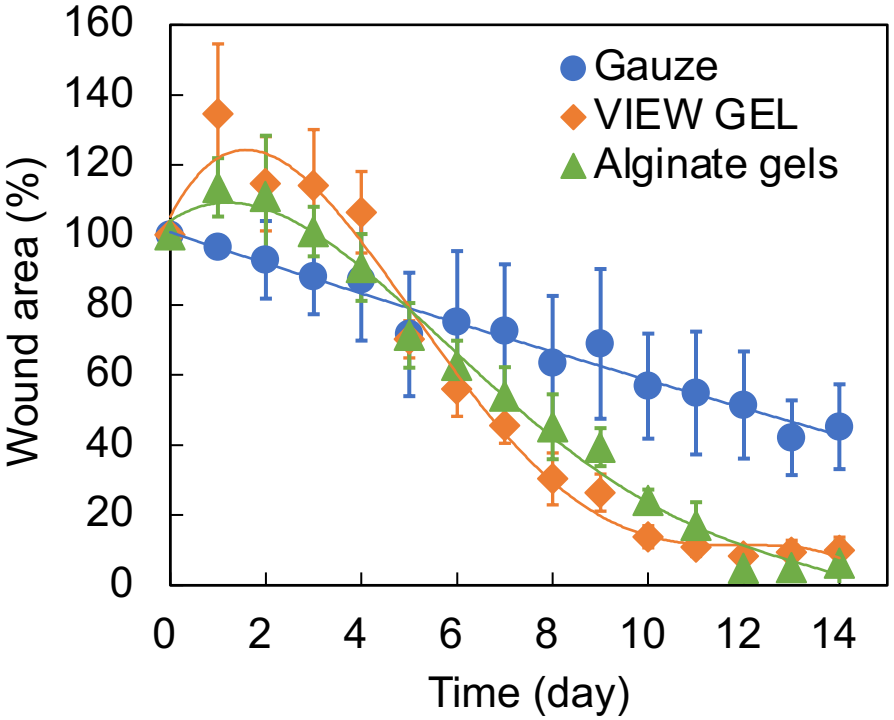
- Dry Gauze (Libatape, Japan)
- VIEW GEL®(clinical hydrogel dressings, Nichiban, Japan)
- Alginate hydrogels prepared in this study
  (Gel thickness = ~1.2 mm, $CaCO_3$ concentration = 0.20 w/v%)

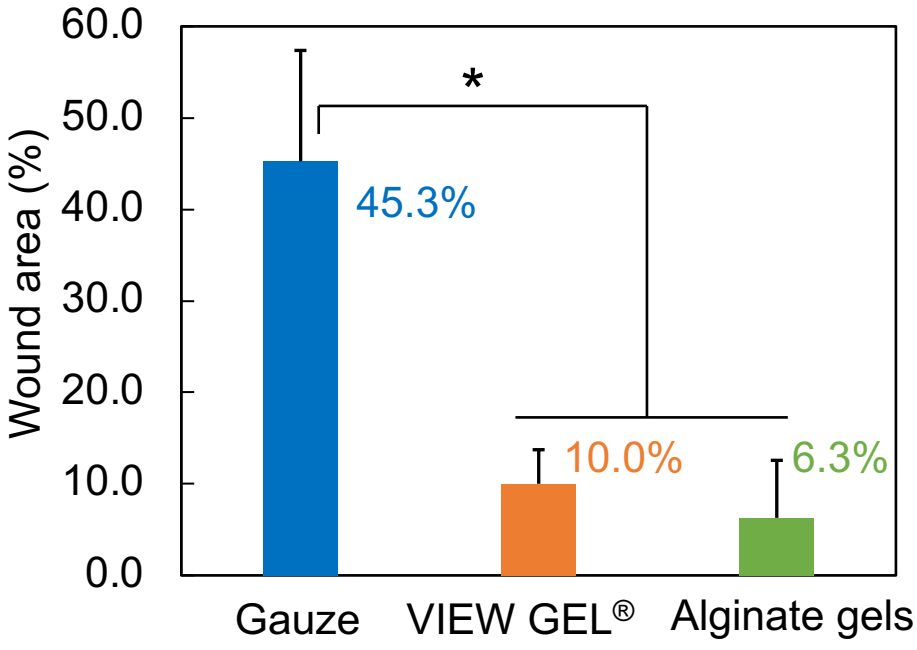
This animal experiment was approved by
Ethical Committee of Tokyo University of Science, Japan (Protocol Y21024).

Results and Discussion

➢ Change of wound area

$$Wound\ area\ (\%) = \frac{Wound\ area\ at\ \mathrm{X}\ day\ (mm^2)}{Wound\ area\ at\ 0\ day\ (mm^2)} \times 100$$





Values are expressed as the mean ± SE ;n=4. *p<0.05

Alginate hydrogel promoted wound healing compared to dry gauze.

The hydrogel prepared in this study showed excellent wound healing effects.

Conclusions

This study thoroughly evaluated the property of alginate hydrogel with carbonated water and its wound healing effects.

- ✓ The addition of carbonated water induced gelation of alginate hydrogel in short time.
- ✓ The prepared hydrogels have high water content (~99%).
- ✓ The surfaces pH of hydrogels are suitable for skin cell growth.
- ✓ The hydrogels have biocompatibility and can be exchanged without damaging tissue.
- ✓ The hydrogels prepared in this study showed excellent wound healing effects.

◆ Acknowledgment

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Prof. Akihiko Kikuchi, Tokyo University of Science, for valuable discussions.
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