Decellularized plants as support-structure for organ formation

Madeleine Fandrich, Julia Hörchner, Cornelia Kreim, Julian Wegner

Summary

Cells require scaffolding for 3-dimensional growth by allowing them to grow. These scaffolds can be obtained from animal organs and plant parts by decellularization. A comparison of different leaf varieties revealed that spinach is the most efficient to decellularize. It was possible to fix cells on a leaf scaffold using a FBS coating and to determine their survival over a few days. In the future, it should be possible to cultivate these cells effortlessly over a longer period of time in a self made bioreactor.

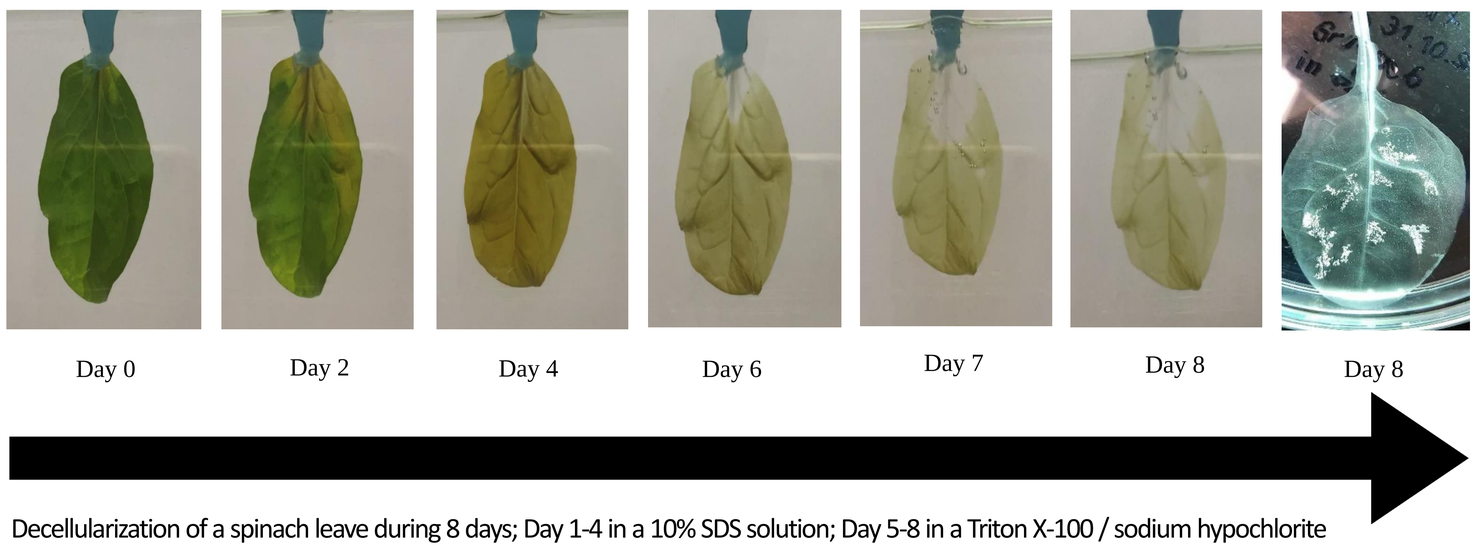
Introduction

It has been shown in the literature that it is possible to remove the extracellular matrix from animal organs by various detergents. After this process, a scaffold (matrix) of high complexity remains, which can not be artificially created today. It has been shown that it is possible to recellularize the scaffold with cells and to prove their viability. For the Department of Tissue Engineering, the techniques of de- and recellularization are a promising perspective. Also, the development of functional organ implants could benefit from this technique. Additionally animal models, or the removal of animal organs is always associated with ethical concerns. Therefore, it is important to advance the innovation of less worrying and ideally, cheap materials for tissue engineering. Like *J.R. Gershlak et al.* described, it is possible to decellularize plant leaves, to sow animal cells on them and to determine their viability.[1][2]

Decellularization

Seeding

Bioreactor



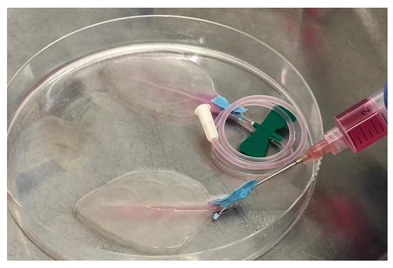
Decellularization of a spinach leave during 8 days; Day 1-4 in a 10% SDS solution; Day 5-8 in a Triton X-100 / sodium hypochlorite solution

Decellularization

Before decellularization, spinach, basil and parsley leaves were cannulated into the petiole of the leaves. The cannulas were glued to the leaves with dental glue. Subsequently, various plant leaves were fixed on a syringe and decellularized over 7 days using 10% SDS solution. To improve the decellularization, the leaves were hung in deionized water to build up a gradient. After treatment with 10% SDS solution, the leaves were placed in Triton X-100 in a sodium hypochlorite solution for 5 days to bleach the leaf structures. Thereafter, the leaves were washed several times and stored in PBS.

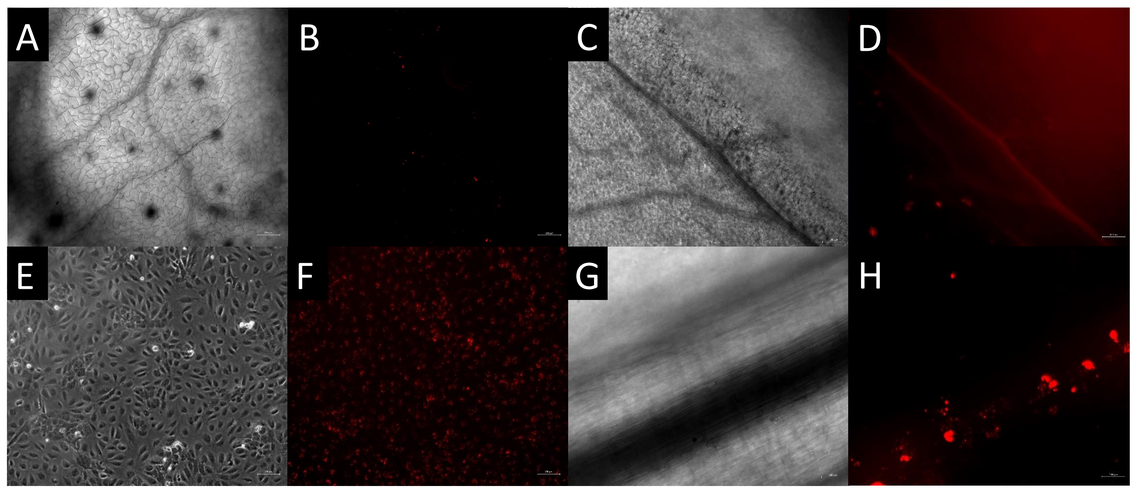
Seeding

For our experiment, we use L929 cells (mouse fibroblasts) and HUVECS (human endothelial cells). The HUVECS were applied through the attached cannula into the leaf veins. With the L929 cells, we tried to re-cellularize the leaves. In order to improve the adhesion of the cells, we have to apply the leaves before recellularization with a 1:1 FCS/PBS solution for 60 minutes at 37 ° C. On the leaf top, a cell count of 1.5 × 10^6 L929 cells was applied. 1 ml of HUVEC cell suspension with a cell count of 1 × 10^6 cells was injected via the cannula. Both leaves were incubated at 37 °C for several days.



Two decellularized spinach leaves seeded through a cannula with 1 ml of HUVECs with a cell count of 1 x 10^6 cells

Results



HUVECs (human umbilical vein endothelial cells) can be used to recellularize the decellularized spinach leaves. (A) spinach, phase contrast. **(B)** spinach, Cy3 (549/562), negative control. **(C)** spinach, seeding by injection with HUVECs, phase contrast. **(D)** spinach, seeding by injection with HUVECs, Cy3 (549/562). **(E)** HUVECs seeded on PS-well-plate, phase contrast. **(F)** HUVECs seeded on PS-well-plate, Cy3 (549/562), positive control. **(G)** HUVECs seeded at the top of spinach, phase contrast. **(H)** HUVECs seeded at the top of spinach, Cy3 (549/562)

Conclusion

The decellularization of the leaves was successful. Furthermore, the decellularized leaves could be recellularized with L929 cells. Recellularization with HUVECs was equally successful. This was initiated via a cannula in the petiole. Besides, experiments with bioreactor were performed. To validate these, further laboratory days would have been necessary after completion.

[1] Gershlak, Joshua R.; Hernandez, Sarah; Fontana, Gianluca; Perreault, Luke R.; Hansen, Katrina J.; Larson, Sara A. et al. (2017): Crossing kingdoms: Using decellularized plants as perfusable tissue engineering scaffolds. In: *Biomaterials* 125, S. 13–22. DOI: 10.1016/j.biomaterials.2017.02.011.

[2] Fontana, Gianluca; Gershlak, Joshua; Adamski, Michal; Lee, Jae-Sung; Matsumoto, Shion; Le, Hau D. et al. (2017): Biofunctionalized Plants as Diverse Biomaterials for Human Cell Culture. In: *Advanced healthcare materials* 6 (8). DOI: 10.1002/adhm.201601225.