Protocol to make a cellnest sponge

1. The sponge inner structure and shape can be controlled by the concentration of cellnest in the water solution, freezing conditions and mold.
2. *In vivo* degradability can be controlled by crosslinking temperature and time.

[Preparation]

<Materials>

・cellnest, recombinant peptide based on human collagen type I lyophilized (hereinafter “cellnest”): 100 mg

・Water for injection: 2.4 mL

<Necessary tools>

・Micro tubes (2 ml): 4

・Freezer (-20°C)

・Freeze dryer

・Vacuum oven

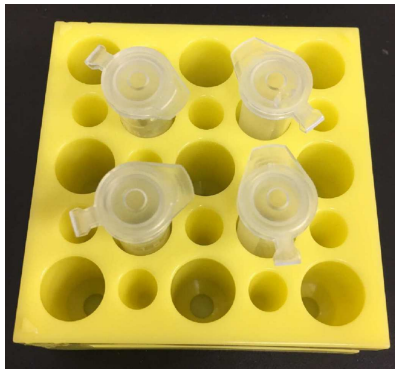
[Protocol]

(1) Add 2.4 mL of injection water to a 100 mg cellnest vial.

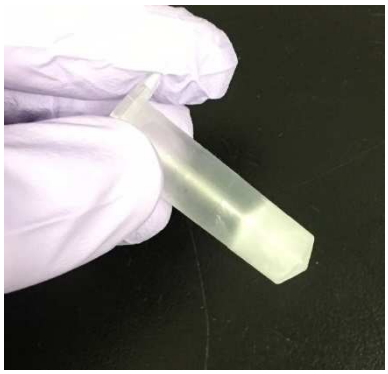
(2) Invert the container so that the injection water reaches all of the cellnest.

(3) Keep it for 1 hour in a 37°C incubator and let the cellnest dissolve.

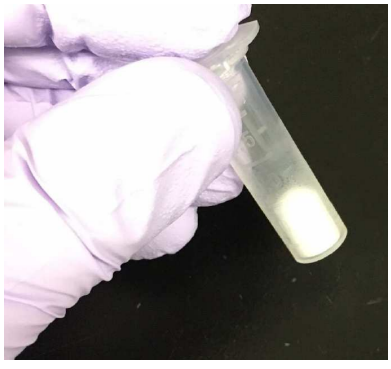
(4) Add the cellnest solution (0.6 mL) in Step (2) into micro tubes (2 mL) and degas with centrifuge.



(5) Keep the micro tubes overnight in a freezer (-20°C) so that the solution freezes.



(6) Freeze-dry it in a pre-cooled freeze dryer to make the cellnest sponge (maybe good to give information on the lyophilisation process settings, e.g. plate temperature and pressure)



(7) Take the cellnest sponge out of the micro tubes and crosslink them for 10 hours in a vacuum oven (160ºC, pressure?).