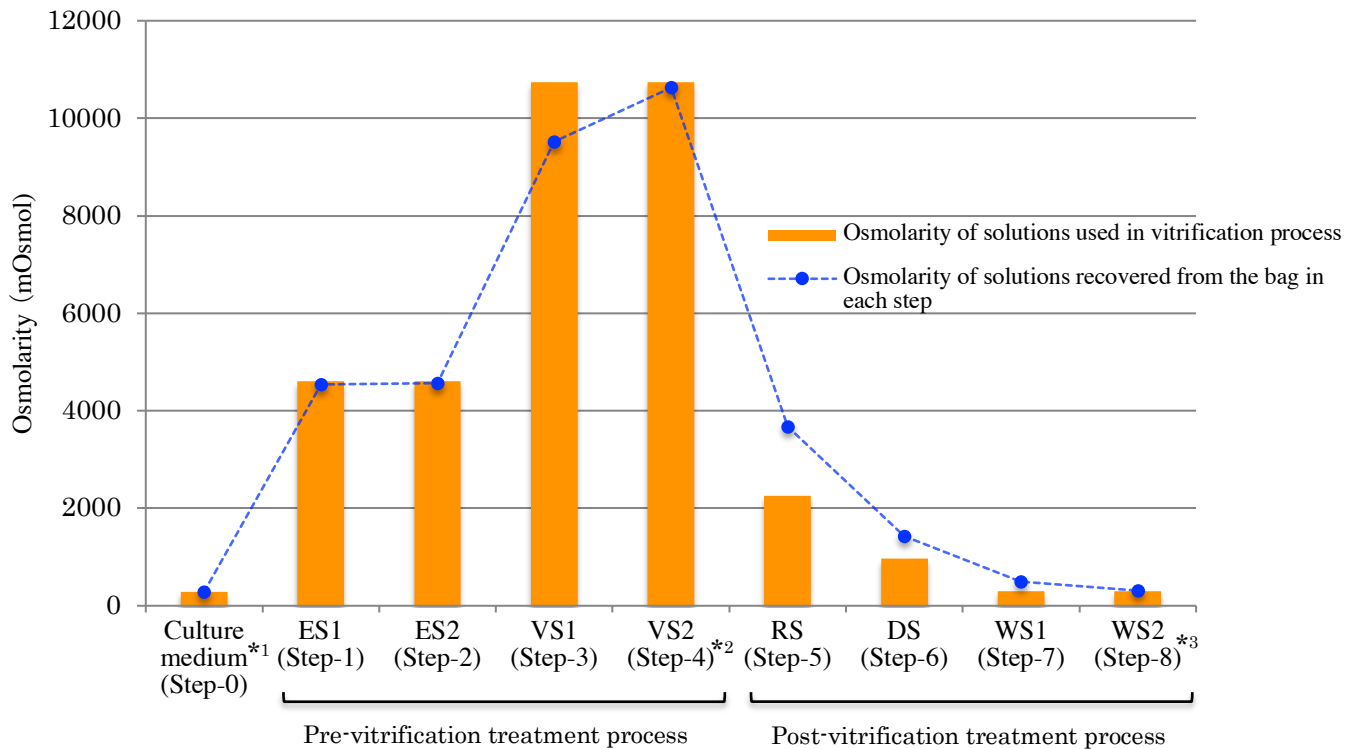


Supplemental Figure



Supplemental Fig. 1. Solution replacement efficiency of the cell sheet treatment procedure using the circulating vitrification bag. In the circulating vitrification bag method, a total of 8 solutions required for pre-vitrification treatment and post-vitrification treatment are circulated successively in the bag. In this experiment, we checked whether the solution in the bag was replaced properly by the solution for each treatment step. The orange bar represents the osmolarity of the solution used in each step. The blue dot represents the osmolarity of the solution recovered from the bag in each step. For the osmolarity measurement, an OSMOMAT® 030 (Gonotec GmbH, Berlin, Germany) was used. The average measured values of 4 trials are shown.

*¹ When placing the cell sheet in the circulating vitrification bag (Step 0), only a very small amount of culture medium originally attached to the sheet is brought into the bag. As a result, in Step 1, when injecting ES1, the dilution of ES-1 due to the presence of culture medium in the bag was interpreted to be minimal.

*² Pretreatment with VS1 (Step 3) led to almost complete replacement of the solution in the bag with VS-2 in Step 4.

*³ In the post-vitrification treatment process, the residual solution from the previous step can be observed. In the final step (Step 8), however, the solution in the bag was judged to be almost entirely replaced by WS. ES: equilibration solution, VS: vitrification solution, RS: rewarming solution, DS: dilution solution, WS: washing solution.