Electronic Supplementary Material (ESI) for Analyst.

Modulation of saliva pattern and accurate detection of ovulation using an electrolyte pre-deposition-based method: a pilot study

Eunji Lee[a], Iljeok Kim[a], Hyoryung Nam[b], Hyungkook Jeon[a,] and Geunbae Lim[a,]**

[a]Department of Mechanical Engineering, Pohang University of Science and Technology (POSTECH), 77 Cheongam-Ro, Nam-Gu, Pohang 790-784, the Republic of Korea

[b]Department of Creative IT Engineering, Pohang University of Science and Technology (POSTECH), 77 Cheongam-Ro, Nam-Gu, Pohang 790-784, the Republic of Korea

Corresponding Author

*Geunbae Lim, E-mail: limmems@postech.ac.kr

*Hyungkook Jeon, E-mail: likeblue@postech.ac.kr

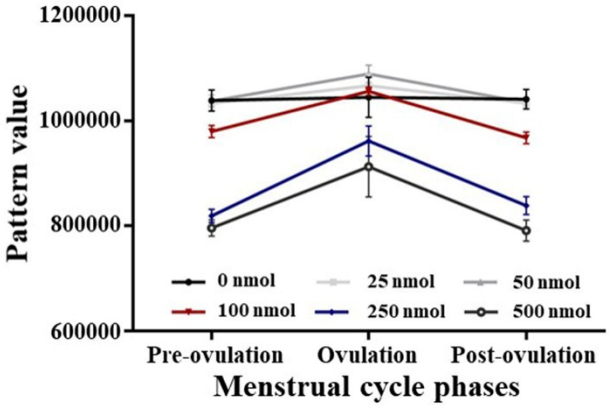


Fig. S1. Pattern value according to the menstrual cycle phases under various concentration of NaCl

As written in Experimental Section, the pattern was quantitatively analyzed based on the amount of light passing through the pattern which decreases as the density of ferning and cubic structures in the saliva patterns. We defined the quantitative value of pattern as the sum of all the pixel values after image conversion process of **Fig.2**. **Fig. S1** shows the pattern value with various concentration of the pre-deposited NaCl according to the menstrual cycle phases. From the results, we observed that the pattern value of ovulation period was highest compared to the pre-ovulation and the post-ovulation period with regardless of concentration of the pre-deposited NaCl; in other words, it is possible to identify the ovulatory phase when pattern value has the highest value. Furthermore, we observed that change of the pattern value according to the increase of concentration of the pre-deposited NaCl shows same tendency, increasing and then decreasing, regardless of menstrual cycle phases.

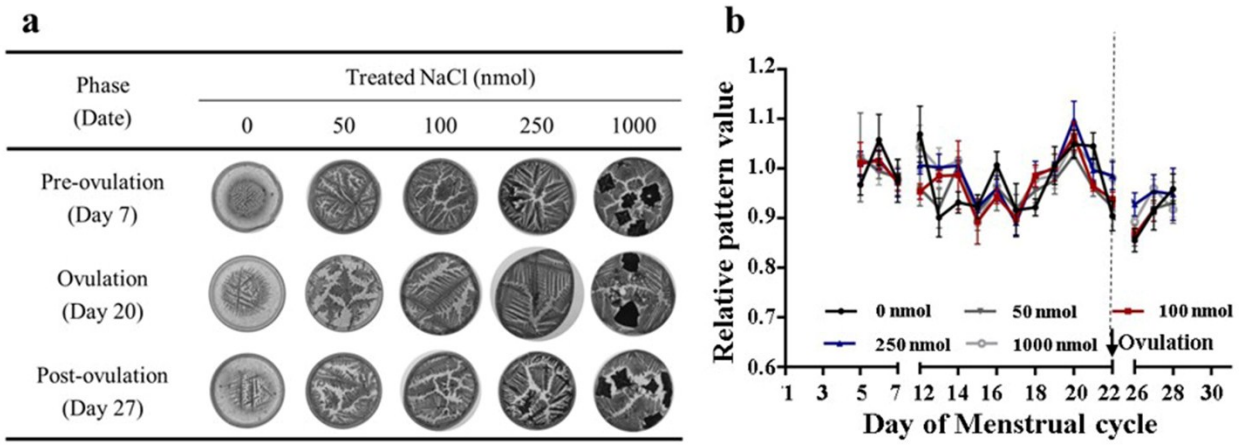
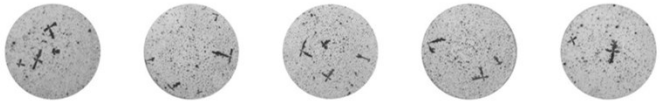
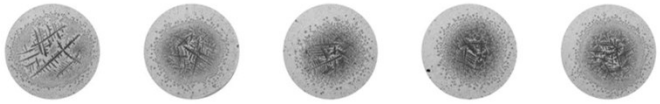


Fig. S2. Pattern analysis of actual saliva with NaCl pre-deposition **(a)** Final patterns of actual saliva under various concentrations of NaCl. **(b)** Change in relative pattern value to the pattern value at pre-ovulatory phase according to the menstrual cycle phases under various concentrations of NaCl.

As shown **Fig. S2**, in the conventional saliva based ovulation test without NaCl pre-deposition, it is quite difficult to detect the ovulation due to the small difference between patterns of ovulation and non-ovulation phases. Whereas, with the NaCl pre-deposition, the difference in the pattern formation is significantly amplified, resulting in detection of ovulation period. From the results, 100 nmol NaCl was chosen as the optimum condition where the standard deviation of the pattern value (0.027) is about 0.7 times lower than the standard deviation at 250 nmol (0.037), and the relative pattern value of the pattern at ovulatory phase is about 0.065.

a

Date	without NaCl pre-deposition						
	1	2	3	4	5	Relative value	Standard deviation
Day 3						1.032	0.078
Day 12						0.933	0.064
Day 13						1.044	0.073

b

Date	with NaCl pre-deposition of 100 nmol						
	1	2	3	4	5	Relative value	Standard deviation
Day 3						0.975	0.022
Day 12						1.122	0.023
Day 13						1.106	0.044

c

Treated NaCl (nmol)	0	50	100	250	1000
Max Relative value	1.044	1.151	1.122	-	-
Standard Deviation	0.053	0.032	0.023	-	-
SNR (mean/S.D.)	18.49	31.58	45.83	-	-
Detection rate (%)	33.3	44.4	66.6	33.3	33.3

Fig. S3. Statistical pattern analysis of actual saliva **(a)** without NaCl pre-deposition and **(b)** with NaCl pre-deposition of 100 nmol, **(c)** overall performance comparison depending on the pre-deposited NaCl concentration.

As shown in **Fig. S3a** (without NaCl pre-deposition), there was no significant difference at Day 3 and Day 13 in their pattern shapes and relative pattern values, especially for the first sample of Day 13, which makes it difficult to distinguish them. On the other hand, in the case of the NaCl pre-deposition condition (**Fig. S3b**), difference at Day 3 and Day 13 in their pattern shapes and relative pattern values is enough to be differentiated, resulting in more precise ovulation detection. In addition, when using the NaCl pre-deposition method, the NaCl pre-deposited samples shows very consistent pattern shape and relative pattern value for a specific Day #, resulting in lower standard deviation on the relative pattern values compared to the non-pre-deposited ones, which shows higher stability and reliability of the NaCl pre-deposition method (**Fig. 3Sa and b**). Also, we found that the signal-to-noise ratio (SNR=mean/standard deviation, which represents sensitivity) is the largest at 100 nmol (0 nmol [18.49] < 50 nmol [31.58] < 100 nmol [45.83]) (**Fig. S3c**). Due to its effective pattern amplification with higher sensitivity and reliability, we obtained high ovulation detection rate (66%) at the optimal NaCl pre-deposition condition (100 nmol) which is twice as high as the conventional saliva ovulation test without the NaCl pre-deposition (**Fig. S3c**).