Test Result Report
Dr. Nano Mini for Dental Performance Evaluation Tests at Extremely Low Water Volumes

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I. Background

Performance evaluation tests on the newly designed Dr. Nano Mini for Dental (hereafter referred to as "Mini") were conducted.
While the water flow rate of a typical faucet is 8-12 liters per minute, the total water flow rate of a dental unit is 2 liters per minute. The water flow rate of each handpiece is extremely low: 100 mL per minute for syringes and 20 mL per minute for turbines and engines. Therefore, we employed 20 mL per minute.

II. Control Test

In evaluating the performance of the Mini, a control test with tap water was conducted under the following conditions:

1. Test conditions:

Day and time:	January 21, 2022 10am-noon
Location:	Our research laboratory (Itabashi-ku, Tokyo)
Test environment:	Air temperature 21°C, humidity 54%, water temperature 15°C, no wind
Test sample:	The pseudo-biofilm was prepared by mixing 45% natto paste with 55% heated-glutinous starch[1] which was colored red using food coloring for easy observation. Two grams were evenly applied to each 10mm-long vinyl tube (inner diameter of 8mm, Fig. 1) and dried for 24 hours (Fig. 2).
Test method:	Two circuits of flow paths were made, one with a Mini attached and the other with nothing attached. The nanobubble water produced by the Mini (hereafter referred to as "Nanobubble water") was tested with tap water as a control. Both with the water flow adjusted to 20 mL per minute (Fig. 3).

[1] In order to increase the viscosity of starch and make biofilm strongly adhesive and bonding.

Fig. 1: 2 g of paste applied evenly

Fig. 2: Sample after 24 hours drying

Fig. 3: Adjustment to 20 mL per minute for both circuits (Slight amount of water water leakage)

Fig. 4: Test environment
Top: the circuit of tap water (control)
Bottom: the circuit with Mini set up

2. Observations:

Nanobubble water began to break biofilms into small pieces 1 minute after water injection, and steadily broke the accumulated biofilms into small pieces, reaching the removal of all biofilms in about 13 minutes. On the other hand, with tap water, about 60-70% of the biofilm remained after 13 minutes, and even at 20 minutes, the remaining biofilm was still about 50%. All the biofilm could not be removed (Table 1).

Table 1: Time-series observation results

Time	Nanobubble water (Bottom)	Tap Water (Top)	Photo
After 3 min.	Biofilm is cut from the center.	No movement observed.	
After 5 min.	Appx. 40% of bottom portion biofilm is detached.	Fine fracturing of biofilm is observed.	
After 6 min.	The upper part of the biofilm is also detached. The entire biofilm was removed from its original position. This clearly shows that nanobubbles are effective to detach and float biofilm from the tube	No change is observed.	

Table 1: Time-series observation results (Continued)

Time	Nanobubble water (Bottom)	Tap Water (Top)	Photo
After 10 min.	All biofilm is crushed into fine fragments.	The biofilm itself begins to detach and float.	
After 13 min.	Removal of all biofilm is complete.	Still 60-70% of biofilm remains.	
After 20 min.		Still 50% remains. Test ends.	

III. Consideration

Control tests between Nanobubble water and tap water have been conducted several times using similar protocols and varying water volumes. In all cases, Nanobubble waster showed superiority. In testing the Mini, we improved the test apparatus and reduced the water volume to an extremely low of 20 mL per minute, in line with the actual situation in dental practice. Mini's biofilm removal ability was clearly confirmed compared to tap water at the extremely low water flow rate.

The principle of Dr. Nano's nanobubble water generation is cavitation. Cavitation is a mechanism whereby when the pressure of a liquid is rapidly reduced below its saturated vapor pressure, the dissolved gas (air) is no longer dissolved and is generated as bubbles.　The higher the flow rate, the greater the pressure difference that occurs when the liquid hits the screw pins, which causes more bubbles to precipitate. In this study, Nanobubble water proved superior biofilm removal performance compared to tap water at a very low water flow rate of 20mL per minute. It could be prudently assumed that the Mini will generate more nanobubble water at 50-100mL per minute and remove biofilm more efficiently.

It is also important in sterilization in the sense that Nanobubble water can remove all biofilms. This is because when a biofilm is partially detached, viable bacteria remaining under the biofilm can regenerate the biofilm in a short period of time. In the tap water used as control, 50% of the biofilm remained after 20 minutes, but if the water stops flowing during clinic closed hours and holidays, the biofilm is likely to be regenerated during this time. In other words, it can be inferred that it is quite difficult to remove biofilm once attached only with tap water flowing during clinic hours.