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RESEARCH ARTICLE

Development of formulation device for periodontal disease

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Abstract

In addition to providing standard surgical treatment that removes the plaque and infected tissues, medications that can regenerate periodontal tissue are also required in the treatment of periodontal disease. As a form of regenerative medication, various growth factors are expected to be used while treating periodontal disease. A protein-like growth factor is often developed as a lyophilized product with dissolution liquid, considering its instability in the solution state. We have clarified that the formulation for periodontal disease needs to be viscous. When the lyophilized product was dissolved using a sticky solution, various problems were encountered, difficulty in dissolving and air bubbles, for example, and some efforts were needed to prepare the formulation. In this research, to identify the problem of preparing a viscous formulation, a lyophilized product (placebo) and sticky liquid were prepared by using vial and ampoule as the conventional containers. Based on these problems, a prototype administration device was developed, and its functionality was confirmed. As a result, it was suggested that the device with a useful mixing system that could shorten the preparation time was developed.

Keywords: Periodontal disease, formulation device, regenerative medicine, viscous formulation, growth factor

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Introduction

Periodontal disease is the most common infectious disease in the dental field and leads to loss of affected teeth[1,2]. A flap operation is performed as a standard surgical treatment for the disease. In this treatment, infected tissues and plaque can be removed, but the lost tissues do not regenerate. For this reason, treatment that can regenerate the removed dental tissues is required and growth factors are expected to be applied for regenerative treatment. Basic fibroblast growth factor (bFGF), a typical growth factor, has been reported to have various bioactivities to stimulate the growth and migration of biological cells, including dental tissue[3–5]. We conducted various evaluations to apply proteins, such as growth factors, to the formulations used for treating periodontal disease.

In our previous study, the requirements for drug formulations for periodontal disease were revealed: the viscosity, about 1×10^4 mPa ▪ s, was found to be one of the most important physical properties[6]. A formulation should be designed so that it can be used in surgical treatment (flap operation), will not run out of the application site, can be administered to the upper lateral disease area, and has adequate resistance when administered in a small amount[6,7]. In addition, because protein such as bFGF is easily denatured by moisture and oxygen, it is often developed as a lyophilized product and dissolved in liquid at the point of use. In that situation, many processes to prepare a formulation are needed. Furthermore, if the dissolution liquid is viscous, it might involve longer preparation time, because of worsening handling. Here is the dilemma between the desirable physical properties and the poor handling. Therefore, the development of an innovative device system to prepare a sticky formulation quickly and easily is necessary for resolving this dilemma.

In this study, we tried to identify the problems of preparing a viscous formulation, by using a mix of lyophilized product and 3% hydroxypropyl cellulose (HPC) solution as a dissolution liquid whose physical properties have already been proved to be useful in a previous report[7]. At the start of this study, conventional containers were used, for example, a vial and ampoule.

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Then we began to address the challenges identified in the first evaluation and designed a new prototype administration device. The usefulness of the new device was evaluated by performing actual preparing operations in two ways. In this article, we describe the issues involved in the preparation of a viscous formulation and the usefulness of a newly developed device.

Methods

Materials

Sucrose (Ensuiko Sugar Refining Co., Ltd.) was used as an excipient of the lyophilized product. HPC (H grade; Nippon Soda Co., Ltd.) was used as a thickener for a viscous dissolution liquid.

Identification of issues with vial and ampoule formulation

Manufacture of lyophilized product and dissolution liquid

Lyophilized product was manufactured as follows: 1 mL of 9% sucrose solution was added to a commercially available vial and lyophilized by a lyophilizer (SF-08; Okawara Mfg Co., Ltd.). Viscous dissolution liquid was manufactured as follows: 2 mL of 3% HPC solution, whose viscosity was approximately 1×10^4 mPa ▪ s, was added to a commercially available ampoule. A dissolution liquid manufactured in the same way using water was used as a control.

Evaluation of vial and ampoule formulation

The formulation was prepared by five volunteers. Since the dissolution liquid was viscous, an 18-gauge needle (Nipro Co., Ltd., Japan, φ; 1.2 mm) was used for preparation, and a 27-gauge needle (Nipro Co., Ltd., Japan, φ; 0.4 mm), widely used in the dental field, was used for administration (Figure 1). Dosage volume was set at 0.2 mL, reflecting the area of periodontitis in one tooth. The formulation was prepared with lyophilized formulation and dissolution liquid, 3% HPC solution or water, using a disposable syringe (Nipro Co., Ltd., Japan; 1 mL) and the above needles. The details of preparation are shown in Figure 2. Preparation time, entered air bubbles, and administration quantity of the formulation in conventional containers were evaluated.

The time necessary for preparing the formulation was evaluated. The time to mix the lyophilized product and dissolution liquid was assumed to be T1 (Figures 2A and 2B). T2 represents the time of the dissolution and air-removal process (Figure 2C). T3 is the time to administer the formulation (Figures 2D–2E).

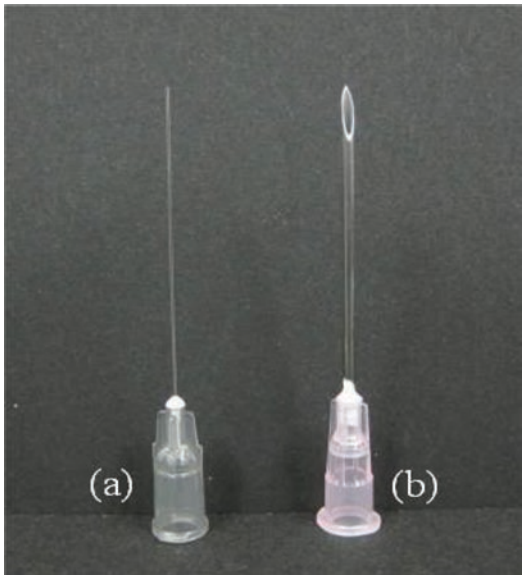

Figure 1. Two types of needle used in this study: (A) 27-gauge needle, and (B) 18-gauge needle.

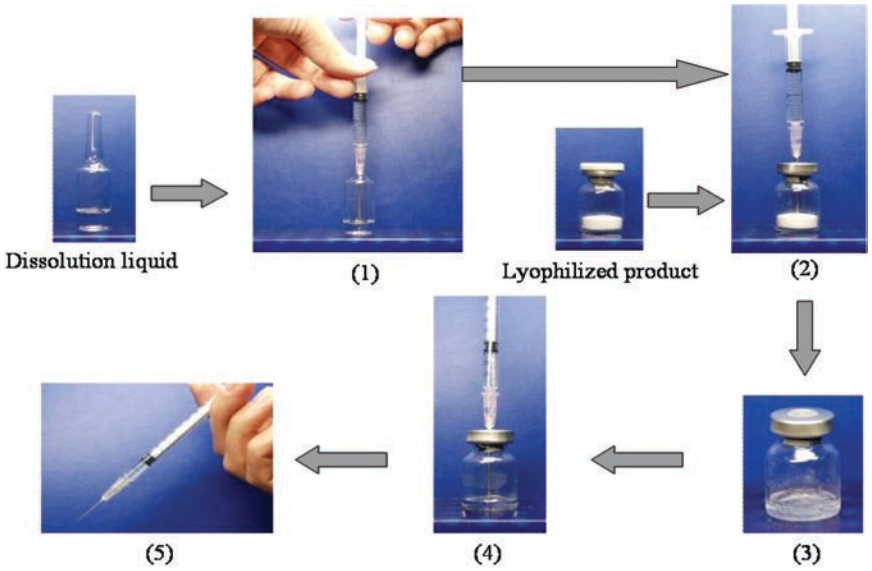

Figure 2. Preparation of formulation using an ampoule and vial as containers: (A) collect 1-mL dissolution liquid, (B) add the collected solution to lyophilized product, (C) dissolve by leaving for several hours, (D) recover 0.2 mL of the solution, and (E) administer the solution.

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The amount of air entering the formulation was evaluated by checking the appearance. The weight of the formulation extruded as 0.2 mL was measured to evaluate the administration variability.

Development and evaluation of prototype administration device

Composition of prototype administration devices

Devices such as glass cartridge (Shiotani Glass Co., Ltd., Japan; 1 mL), rubber packing, gasket (SRI Hybrid Co., Ltd., Japan; 1 mL) aluminum cap (Ishida Press Industry Co., Ltd., Japan; 8 φ), plunger, holder (Maeda Industry Co., Ltd., Japan), connector (Baxa Co., Ltd., USA, 13901), and the administration needle (Nipro Co., Ltd., Japan, 27 gauge) were adopted for the prototype administration device. Figure 3 shows the device kit.

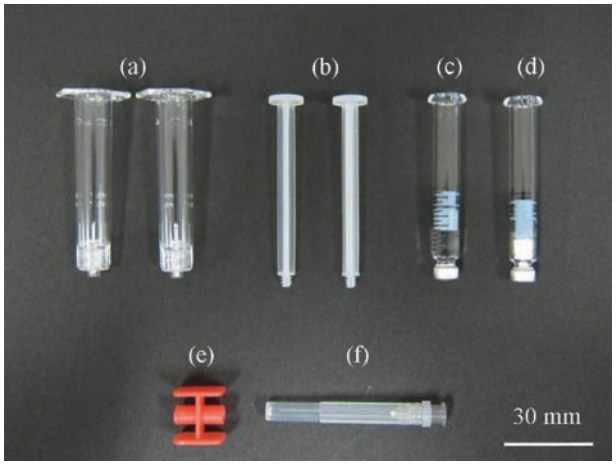

Figure 3. Prototype device kit: (A) holders with double-ended needle (18 gauge), (B) plungers, (C) glass cartridge of dissolution liquid, (D) glass cartridge of lyophilized product, (E) connector, and (F) administration needle (27 gauge).

Manufacture of a lyophilized product and dissolution liquid for prototype administration device

The lyophilized product was manufactured as follows: 0.3 mL of 9% sucrose solution was added to a glass cartridge, freeze-dried by a lyophilizer and then closed with the gasket in a vacuum. The filling volume of the formulation was set based on the capacity of the dead space of the prototype administration device to administer 0.2-mL formulation. Viscous dissolution liquid was manufactured as follows: 0.3 mL of 3% HPC solution was added to a glass cartridge and then closed with the gasket in vacuum to stop air from entering. A dissolution liquid manufactured in the same way, but using water, was used as a control.

Evaluation of prototype administration device

The formulation was prepared by five volunteers using prototype administration device, and two different procedures were performed. Procedure 1 was intended to be a simple and quick preparation, and Procedure 2 aimed to stop air bubbles from entering the formulation. The details of Procedure 1 and 2 were shown in Figures 4 to 7. The device, as well as vial and ampoule formulation, were evaluated.

The time necessary for preparing the formulation using the prototype administration device was evaluated. The time to mix the lyophilized product and dissolution liquid was assumed to be T1 (Procedure 1: Figures 5A–5C, Procedure 2: Figures 7A–7D). T3 is the time to administer the formulation (Procedure 1: Figures 5C and 5D, Procedure 2: Figures 7D and 7E). T2, the time of the dissolution and air-removal process, was assumed as zero because the device was hermetic and bubbles entering the formulation could not be removed by standing.

The amount of air entering the formulation prepared by two different procedures was evaluated by checking the appearance. The full dosing weight of the formulation prepared by the two procedures was measured to evaluate the administration variability.

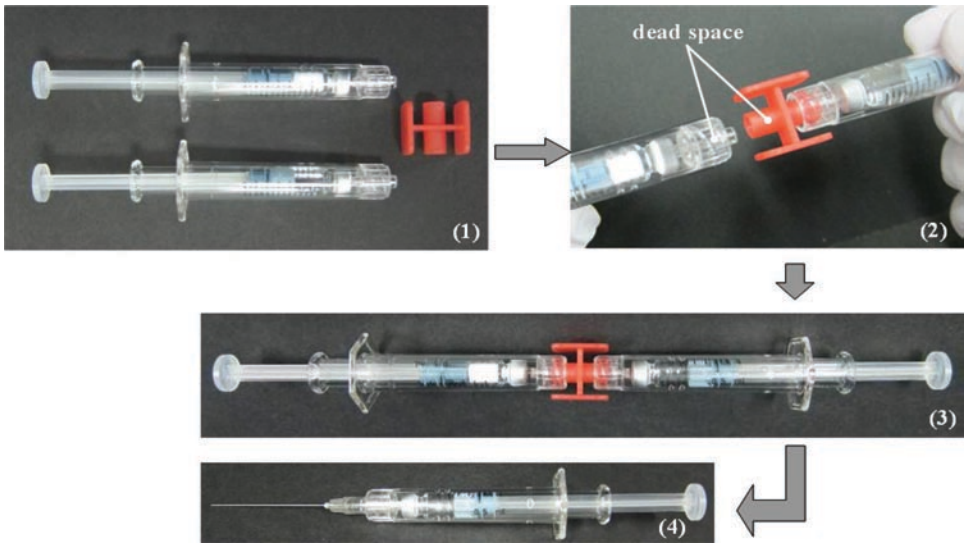

Figure 4. Preparation of formulation using prototype device (Procedure 1).

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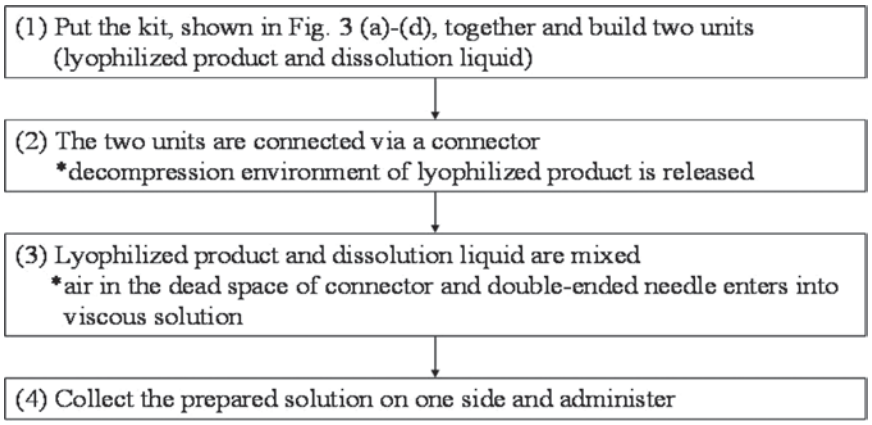

Figure 5. Process flowchart of formulation preparation using prototype device (Procedure 1).

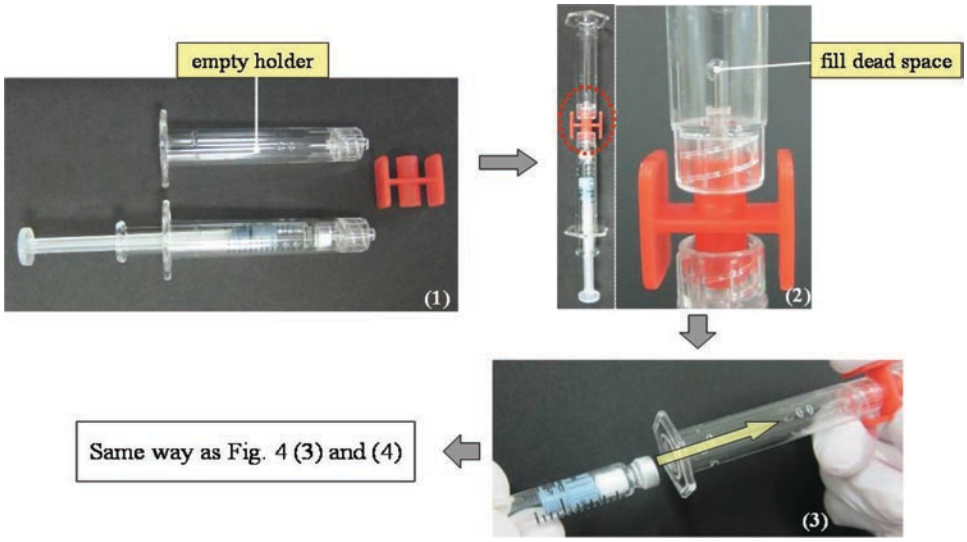

Figure 6. Preparation of formulation using prototype device (Procedure 2).

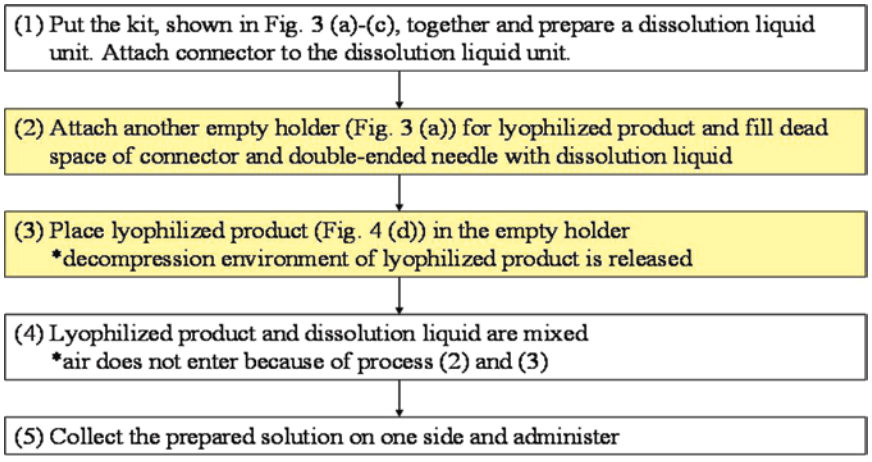

Figure 7. Process flowchart of formulation preparation using prototype device (Procedure 2).

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Results and discussion

Evaluation of vial and ampoule formulation

Evaluation of preparation time

The time required to prepare the formulation is shown in Table 1. It took about 10 min to prepare a nonviscous formulation, though the handling of conventional containers and syringe was complex. In contrast, it took 4 to 5 h to prepare a viscous formulation. Moreover, unlike the case of the nonviscous formulation, it was necessary to agitate the formulation with a needle, which is used to add the dissolution liquid to dissolve the lyophilized product uniformly because the dissolution liquid was sticky.

The medicine used at flap operation should be viscous enough, in order not to flow out of the application site. We had already confirmed that the flow property correlated with the viscosity and the viscosity about 1×10^4 mPa ▪ s was found to be adequate for a proper flow property[7]. A 3% HPC solution used in this study had been found to have preferable physical properties. In addition, proteins such as bFGF must be kept stably in a formulation. Cellulose derivatives, such as HPC, were also found to be inert to bFGF because of their structure, without strong ionic dissociable groups and neutral pH[7]. However, with the viscous formulation there was a problem in that it was very difficult to handle with syringe and needle; thus, it was necessary to leave it standing for about 4 h so that air bubbles that entered while agitating the formulation may disappear completely. The operation was also very complex because it was necessary to open the ampoule and replace the injection needle. In addition, these conventional containers have been reported to have issues such as contamination by rubber and glass chips and microbial pollution[8,9].

It was possible to prepare the nonviscous formulation within a relatively short time, in spite of using conventional containers, because air bubbles disappeared quickly and even if there were some risks of contamination. When a formulation was sticky, it is also preferable to complete the preparation quickly considering the time required for flap operation, 2 to 3 h; therefore, it is necessary to develop a device that makes possible for medical staff to prepare a viscous formulation in a few minutes before or during the flap operation.

Evaluation of air bubbles entering the formulation

The appearance of the viscous formulation just after preparation is shown in Figure 8. The appearance of the formulation prepared with water was clear and colorless. On the other hand, the formulation prepared with viscous dissolution liquid appeared cloudy because of air bubbles. Since the desirable formulation for periodontal disease was viscous and hard to flow, air bubbles entering the formulation did not disappear in a short time; it took more than 4 h for this to happen. Air bubbles in the formulation may influence the administration amount and contact with the diseased site; therefore, it was thought that the device should be developed to stop air bubbles from entering while preparing the formulation.

Evaluation of administration quantity

After measurement, variability was observed in the extruded amount of the viscous formulation (3% HPC solution: 191.3 mg ± 11.5, Water: 198.4 mg ± 3.0). The preparation of a viscous formulation using conventional containers complicated processes, which in turn made it necessary to add and suck out viscous solution by syringe and needle manually. This procedure let air bubbles enter the viscous formulation and caused the variability of the extruded amount of the formulation. Since the viscous formulation had low flowability, much loss of available formulation occurred through this whole preparation process; thus, an excess preparation of the formulation was needed. Most protein-like growth factors are expensive and highly active in a small amount, so the excess preparation of a formulation was not preferable in terms of cost and disposal. Furthermore, there was one incidence in which the administration needle fell out during the extrusion process because of the high resistance of the viscous formulation. Therefore, the administration

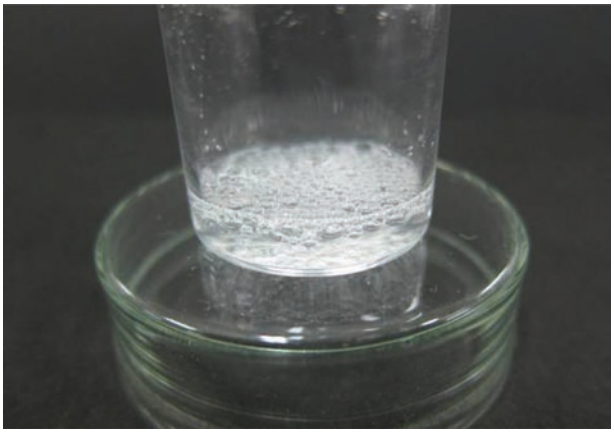

Figure 8. Images of lyophilized product dissolved in viscous liquid (3% HPC solution) in vial container.

Table 1. Time required for preparation of the formulation using an ampoule and a vial as containers.

Dissolution liquid	Time required for each step (min)		Total time (min)
3% HPC solution	T1 (preparation)	5.7 ± 0.5	280.9 ± 13.7
	T2 (dissolution)	269.0 ± 14.3	
	T3 (administration)	6.2 ± 0.3	
Control (water)	T1 (preparation)	4.3 ± 0.4	9.7 ± 0.9
	T2 (dissolution)	0.6 ± 0.1	
	T3 (administration)	4.8 ± 0.4	

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device should be designed in such a way that it can administer medication quantitatively by a simple operation; it should also have a system to stop the administration needle from falling out.

As a result, it was necessary to prepare excess formulation to collect enough volume for the administration amount; this, in turn, became a potential cause for needle injury and microbial contamination because the procedure to detach the injection needle after preparing the formulation and attach the administration needle was very complex.

From these evaluations, the development of an administration device that addressed the problems identified above was thought to be necessary.

2. Development of prototype administration device

The prototype administration device was designed taking into consideration the risk of entry of foreign substances into vial and ampoule formulations, in addition to the problem identified above. Thus, to prepare the formulation in a short time, a system to mix the lyophilized product and dissolution liquid physically became indispensable. A fixed quantity of the lyophilized product and dissolution liquid was filled to two glass cartridges that were loaded into separate holders. Then they were mixed with an 18-gauge double-ended needle via a connector. Moreover, the administration device was designed to administer the whole quantity by using the device as a preparation and administration container. This system led to a decrease in wastage and variability of the administration amount by an operator. In addition, to stop the administration needle from falling out during the extruding procedure, as observed above, the Luer lock system was used for the holder (Figure 9).

Evaluation of prototype administration device

Evaluation of preparation time

The time required to prepare the formulation using prototype administration device is shown in Table 2. The time required to dissolve the lyophilized product when the formulation was prepared according to procedure 1 with the prototype administration device was greatly shortened compared with the vial and ampoule formulation, and total preparation process became possible in about 4 to 5 min, regardless of the formulation type, viscous or not. With viscous formulation, this was because some steps that required a lot of time, such as collecting and adding the low-flowable dissolution liquid by using conventional containers, became unnecessary. In addition, the device that could be used as a preparation and administration container made it possible to mix viscous formulation physically and quickly without preparing an excess formulation, and these advantages contributed greatly to shortening the operation time.

Meanwhile, according to Procedure 2, the formulation could be prepared in about 5 to 6 min in both types of formulation. Procedure 2 also markedly shortened the preparation time, which avoided some unnecessary steps that form part of Procedure 1; however, two volunteers made errors and the dissolution liquid overflowed. Therefore, it was thought that the device would become more useful if the air-removing process were easy to carry out.

When using conventional containers, poor handling of HPC solution was directly linked to an increase in preparation time. However, it was found that the

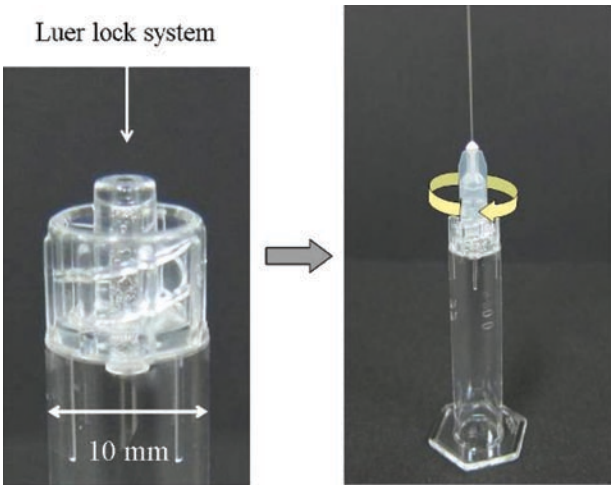

Figure 9. Holder with Luer lock system.

Table 2. Time to prepare formulation using prototype device.

Preparing procedure	Dissolution liquid	Time required for each step (min)		Total time (min)
Procedure 1	3% HPC solution	T1 (preparation)	3.5 ± 0.3	5.1 ± 0.5
		T2 (dissolution)	0	
		T3 (administration)	1.6 ± 0.2	
	Water (control)	T1 (preparation)	3.0 ± 0.2	4.5 ± 0.4
		T2 (dissolution)	0	
		T3 (administration)	1.5 ± 0.2	
Procedure 2	3% HPC solution	T1 (preparation)	4.0 ± 0.3	5.5 ± 0.4
		T2 (dissolution)	0	
		T3 (administration)	1.5 ± 0.2	
	Water (control)	T1 (preparation)	3.3 ± 0.2	4.8 ± 0.3
		T2 (dissolution)	0	
		T3 (administration)	1.5 ± 0.1	

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preparing time involved with using a prototype administration device did increase by the low-flowablility of HPC solution, regardless of which preparing procedure was used.

Evaluation of air bubbles entering the formulation

The appearance of the viscous formulation just after preparation by Procedure 1 is shown in Figure 10A. The appearance of the formulation prepared with water was clear and colorless. By contrast, the formulation prepared with viscous dissolution liquid appeared cloudy because of its low flowability. This was thought to be caused by air bubbles depending on the level of relief of the decompression of lyophilized product and the amount of dead space of the device. When the lyophilized product was placed in a holder (Figure 4A), the decompression was released by a double-ended needle. A certain amount of air equivalent to the volume of lyophilized product and dead space in the device, such as the connector and double-ended needle of the holder, entered the dissolved formulation. It was found that this event was certain to occur if a formulation prepared using Procedure 1 were to be used.

The appearance of the viscous formulation prepared by Procedure 2 is shown in Figure 10B.The amount of air bubbles entering the formulation was significantly reduced by Procedure 2, in which the cartridge of the lyophilized product was placed after the holders for the dissolution liquid and lyophilized product were connected via a connector. The dead space of the device was filled with the dissolution liquid and then the dissolution operation was conducted. The lyophilized product was kept in a vacuum by this process until mixing, and the air in the dead space did not enter the formulation. This process was found to be significantly useful in reducing air bubbles in the formulation.

Therefore, it was thought that a system that could control the operating order, so that the vacuum of the lyophilized product could be maintained up until the point before mixing, and a simple system that could fill the dead space with dissolution liquid were necessary for the administration device.

Evaluation of administration quantity

The falling-out of the administration needle, as observed in extrusion of the formulation prepared by using conventional containers, did not occur by adopting the Luer lock system for the holder of a prototype administration device. The amount of extruded formulation prepared using Procedures 1 and 2 is shown in Table 3.

The variability of the extruded quantity of the formulation prepared by Procedure 1 was much improved compared with vial and ampoule formulation. It was thought that a constant amount of the lyophilized product and dissolution liquid filled in glass cartridges contributed to this improvement. However, the results with the viscous formulation varied relatively widely compared with the nonviscous formulation because of air bubbles in the viscous formulation.

In Procedure 2, air bubbles were not observed in the viscous formulation and the variability of the extruded amount showed further improvement. This improvement was thought to be a result of the air-removing operation during Procedure 2, which could stop the air bubbles from entering the formulation.

Ready-to-use formulations, such as prefilled syringe, were credited with having merits in terms of usability and safety compared with vial and ampoule formulations[10,11]. From the results gathered so far, the prototype administration device designed in this study was found to be able to shorten the preparation time greatly, reducing the risk of foreign substances and microbial contamination and enabling administration of the formulation without variability and the needle falling out; however, though Procedure 1 was a straightforward measure, there was a clear possibility

Table 3. Effect of preparation procedure on extruded amount of formulation.

Dissolution liquid		Extruded amount (mg)
Procedure 1	Control (water)	204.6 ± 1.8
	3% HPC solution	209.5 ± 3.5
Procedure 2	Control (water)	199.7 ± 1.1
	3% HPC solution	201.4 ± 0.8

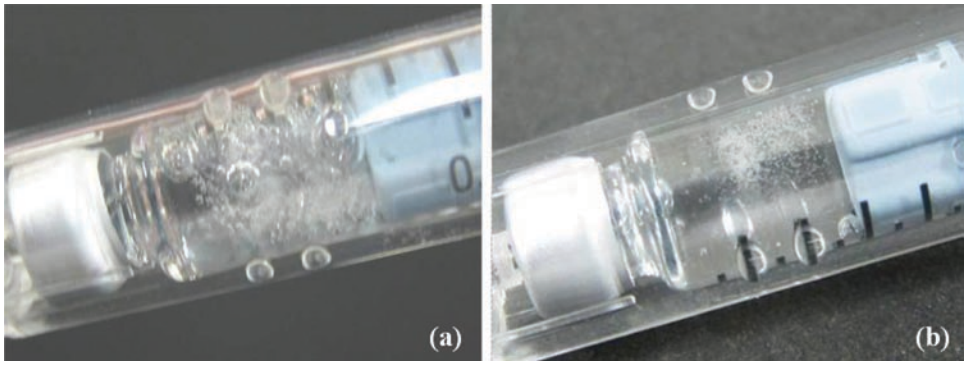

Figure 10. Images of formulations prepared by different procedures with prototype device: (A) formulation prepared using Procedure 1, and (B) formulation prepared using Procedure 2.

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of air bubbles entering the viscous formulation. Thus, it became necessary to enhance the quality of formulation, which became possible with Procedure 2 that showed a considerable reduction in the level of air bubbles entering the formulation.

Conclusions

In this study, to develop a formulation administration device to be used in the flap operation for periodontal disease, we tried to resolve the problems in the preparation of low-flowable formulation by preparing the formulation using conventional containers, a vial and ampoule, and designing and evaluating a prototype. Using this prototype administration device, the time required for preparation was greatly shortened, regardless of the level of viscosity of the formulation. The administration amount of a formulation became constant regardless of the formulation type. In addition, the risk of formulation pollution was reduced because of simple and easy handling of the device.

However, it was suggested that the device should be able to prepare a viscous formulation that restricts entry of air bubbles, making it simple and practical to use. Therefore, it was thought that a device suitable for flap operations and excelling in convenience and patient safety could be developed by further improving the prototype administration device as a preparing system by controlling and manipulating the operation steps in such a way that it becomes possible to fill the dead space with the dissolution liquid.

In future research, we would like to focus on improving the device system in order to achieve ease of use and a decrease in the level of air bubbles entering the formulation. Finally, it could be concluded that the improved device discussed here would not only help with preparing the viscous formulation used in treating periodontal disease that restricts entry of air bubbles but also with administering medicines dissolved at the point of use, thus making it a versatile and an easy-to-use device.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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