Reconstitution of Injectable Poly-d,l-lactic Acid: Efficacy of Different Diluents and a New Accelerating Method

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INTRODUCTION

Over the past decade, injectable fillers have become popular agents for soft tissue contouring and volumizing. Among them, injectable poly-lactic acid is the so-called collagen-stimulating filler. Two injectable poly-lactic acid fillers are available: injectable poly-l-lactic acid (PLLA) and injectable poly-d,l-lactic acid (PDLLA). Injectable PLLA (Sculptra; Galderma, Fort Worth, Tex.) contains PLLA, carboxymethyl cellulose (CMC), and mannitol, while injectable PDLLA (AestheFill; REGEN Biotech, Inc., Seoul, South Korea) contains PDLLA and CMC. Both fillers are available in vials as a lyophilized powder, and reconstitution with a diluent before administration is required. Proper reconstitution of injectable PLLA is a critical factor in reducing complications such as papules and nodules formation after injection. Injectable PDLLA was initially approved by the Korean Food and Drug Administration in 2014. A vial of injectable PDLLA contains 200 mg of lyophilized powder. The PDLLA microparticles are spherical in shape, spongiform in consistency, and 30–70 μm in size. The suggested

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diluent for reconstitution is sterile water for injection (SWFI). However, can we use a diluent other than SWFI for reconstitution? According to the instructions for use, for shallow lines or overall facial wrinkle correction, 8 mL SWFI is used to reconstitute one vial of injectable PDLLA by shaking (“hand-shaking” method) until the SWFI is well mixed with the lyophilized powder; for deep wrinkle correction, 1.4 mL SWFI is used to reconstitute one vial of injectable PDLLA by agitation assisted by the vortex generator (“vortex” method).6,15 However, when 8 mL SWFI is used, more than 30 minutes is usually required for total dissolution by the hand-shaking method, and it is an exhausting process. Agitation assisted by the vortex method is helpful. When 1.4 mL SWFI is used, more than 1 hour is required to achieve full dissolution by the vortex method. Therefore, we developed a novel “back-and-forth” method that can accelerate the reconstitution procedure of injectable PDLLA.

The objectives of this study were to investigate the efficacy of different diluents and this new back-and-forth method in the reconstitution of injectable PDLLA. The critical step in the reconstitution of injectable PDLLA is to dissolve all the CMC particles and prevent CMC particle aggregation. Some dissolution properties of CMC relevant to this study are discussed in this article.

MATERIALS AND METHODS

Materials

The injectable PDLLA used in this study was AestheFill-V200, which comprises 13–15 acorn-shaped lyophilized powder beads of total weight 200 mg stored in a vial. Six different diluents, namely SWFI, normal saline (NS), lidocaine, lidocaine with epinephrine (lidocaine + E), sodium bicarbonate (NaHCO₃), and mannitol, were chosen for reconstitution tests.

Methods

The pH value of each diluent was measured using a pH meter. As recommended by the manufacturer, 2 suspension “concentrations” can be prepared by reconstitution: 200 mg injectable PDLLA per 8 mL (a “thin” suspension) or 1.4 mL (a “thick” suspension) diluent.15 The weight of each lyophilized powder bead of injectable PDLLA was measured and then the corresponding volume needed to prepare a thin or thick suspension was calculated. Using the 6 aforementioned diluents, thin suspensions were prepared by the vortex method, whereas both thin and thick suspensions were prepared by the back-and-forth method.

Vortex Method

One acorn-shaped bead of injectable PDLLA was placed in a 2-mL Eppendorf tube followed by the volume of diluent needed to prepare a thin suspension. The dissolution pattern over the first 3 minutes after immersion of the PDLLA bead was recorded for each diluent. With these tubes holding on fingers, they were agitated by touching on the vibrated platform of a vortex generator at 2,700 rpm. We inspected the suspension every 5 minutes and stopped the agitation process after the suspension became grossly homogenous or after a maximum agitation time of 30 minutes. We then observed the distribution of PDLLA microspheres microscopically. The tubes were then allowed to stand for 30 minutes before 0.1 mL lidocaine solution (both lidocaine and lidocaine + E were used in different experiment groups) was added to the SWFI and mannitol tubes. The resultant mixtures were then agitated by hand-shaking followed by gross and microscopic observations. The 6 suspensions were examined again after 24 and 48 hours. All these experiments were repeated 5 times.

Back-and-forth Method

One injectable PDLLA bead was placed in a 3-mL syringe. Another 3-mL syringe was used to retrieve the required volume of diluent to prepare a thin or thick suspension. The 2 syringes were tightly connected with a 3-way stopcock by Luer-lock (Fig. 1). The diluent and injectable PDLLA were then pushed back-and-forth between these 2 syringes for 1 minute, and the resultant suspensions were injected into Eppendorf tubes. Gross and microscopic observations were performed immediately and again after 30 minutes, 24 hours, and 48 hours. All these experiments were repeated 5 times.

RESULTS

Diluents Characteristics

NaHCO₃ is a weakly alkaline diluent; SWFI, lidocaine, and mannitol are neutral diluents; and NS and lidocaine + E are acidic diluents. All 6 diluents contain SWFI as their excipient. NaHCO₃, NS, lidocaine, and lidocaine + E contain dissolved electrolytes in their ingredients, but SWFI and mannitol do not. Therefore, the ionic strength of NaHCO₃, NS, lidocaine, and lidocaine + E is high, whereas that of SWFI and mannitol is low. The characteristics of these diluents are listed in Table 1.

Vortex Method

After immersing the PDLLA beads into each of the diluents for 3 minutes, only the beads in SWFI and mannitol showed signs of dispersion, with mannitol effecting slightly a better dispersion than SWFI. The beads in the
other 4 diluents showed no signs of dispersion after this time period (Fig. 2).

After observation, the tubes were then agitated by a vortex generator at 2,700 rpm. After 5 and 10 minutes, the mannitol and SWFI tubes contained homogenous suspensions, respectively. After 30 minutes, the agitation process was stopped for the other 4 tubes. Although they showed a good suspension, the suspended particles in these tubes were larger than those in the mannitol and SWFI tubes (Fig. 3).

The suspensions in the 6 tubes were then examined microscopically. The mannitol and SWFI tubes contained well-separated microspheres, whereas the other 4 tubes contained aggregates comprising tens to hundreds of microspheres (Fig. 4). Gross and microscopic images did not change significantly after adding lidocaine (or lidocaine + E) solution to the SWFI and mannitol tubes. After standing for 30 minutes, only the SWFI and mannitol tubes retained homogenous suspensions; the other 4 tubes contained 2 distinct layers with particles floating on the diluent (Fig. 5). No further gross or microscopic changes were observed in any of the 6 tubes after 24 and 48 hours.

Of the 6 diluents tested here, only 2 diluents with low ionic strength, SWFI and mannitol, were found to be effective diluents for reconstitution of injectable PDLLA using the vortex method. The reconstitution time was shorter for mannitol than that for SWFI. No correlation was found between the pH values of these diluents and their effectiveness at reconstituting injectable PDLLA. No further changes were found after adding lidocaine (or lidocaine + E) into reconstituted suspension and after 24–48 hours.

### Back-and-forth Method

#### Thin Suspension

All diluents resulted in homogenous suspensions after using the back-and-forth method (Fig. 6). The prepared suspensions remained homogenous without floating particles or precipitation even after standing for 30 minutes (Fig. 7), 24 hours, and 48 hours. The microspheres of injectable PDLLA were well separated in the 6 different diluents both immediately and at 30 minutes after preparation using the back-and-forth method (Fig. 8).

#### Thick Suspension

All suspensions exhibited a paste-like appearance after using the back-and-forth method, which remained for 30 minutes, 24 hours, and 48 hours. Microscopic examination of the suspensions revealed that, despite being close together due to the high concentration, the microspheres were well separated in all 6 different diluents both immediately and 30 minutes after preparation using the back-and-forth method (Fig. 9).

By using the back-and-forth method, all 6 diluents can be used for reconstitution of injectable PDLLA quickly.
and effectively, regardless of the thickness of the desired suspensions.

**DISCUSSION**

Injectable PDLLA must be stored in powdered form because PDLLA gradually decomposes into lactic acids via hydrolysis, and the CMC hydrogel undergoes hydrolytic degradation. The PDLLA microspheres are prepared by a new solvent spray technique, suspended in CMC solution, and then lyophilized into powdered form. The powdered form needs to be reconstituted with a liquid, called the diluent, before it can be administered. In this experiment, we chose SWFI, NS, lidocaine, lidocaine + E, NaHCO₃, and mannitol as diluents for the reconstitution test. The reasons why we choose these diluents are described below. SWFI is the diluent recommended by the manufacturer. NS is readily available and is the diluent used for the reconstitution of botulinum toxin. It is the diluent that is most often used to replace SWFI for the reconstitution of injectable PLLA. Lidocaine and lidocaine + E solutions are the diluents used for the reconstitution of the acellular dermal matrix. They are also often added to the suspension after reconstitution of injectable PDLLA with SWFI for anesthesia. NaHCO₃ can be added to the lidocaine solutions to adjust their pH to reduce
pain on injection. It is the only alkaline diluent used in this test. It was included in this study not for the purpose of clinical use but to check whether the pH value of the diluent exhibits any correlation with the ease of reconstitution. Mannitol, a naturally occurring sugar alcohol used clinically for its osmotic diuretic properties, is one of the ingredients of injectable PLLA. It was included in this study not for the purpose of clinical use but to check whether mannitol has any effect on the reconstitution of injectable PDLLA.

The goal of reconstitution is to obtain a homogenous suspension without any PDLLA microsphere aggregates. There are 3 steps in the reconstitution of injectable PDLLA. The first is to dissolve all the solid particles of CMC into a solution without CMC particle aggregation, the second is to separate all the PDLLA microspheres, and the third is to disperse the PDLLA microspheres homogeneously in the solution. Because PDLLA microspheres are spherical in shape and spongiform in consistency, they can be separated and dispersed easily in a solution. Therefore, the critical step in the reconstitution of injectable PDLLA is to dissolve all the CMC particles and prevent CMC particle aggregation.

CMC was first prepared in 1918 and was produced commercially in the early 1920s. It readily dissolves in hot or cold water to form viscous, transparent solutions with a range of thickening, dispersing, gelling, stabilizing and film-forming properties, with many applications in the food, cosmetics, pharmaceutical, and detergents industries. CMC gel is present in several commercially available subdermal fillers such as Larese (FzioMed, Inc., San Luis Obispo, Calif.), Radiesse (Bioform Medical, Inc., San Mateo, Calif.), and Ellanse (AQTIS Medical BV, Utrecht, The Netherlands) as filling or carrier. These products containing CMC gel were supplied as prefilled syringes that were ready for immediate use. For injectable PDLLA that requires reconstitution before administration, the dissolution properties of CMC play an important role in the reconstitution process. CMC is produced by partially substituting the hydroxyl groups on the cellulose backbone with carboxymethyl groups, and this carboxymethylation is what makes CMC water-soluble. The dissolution process comprising mechanical stirring, agitating, and pumping or shearing is a necessary step to create CMC solutions. When it dissolves in water, the nonsubstituted

![Fig. 7. All suspensions prepared by the back-and-forth method remained homogenous without floating particles or precipitation after standing for 30 minutes.](image)

![Fig. 8. The PDLLA microspheres in the thin suspension prepared by the back-and-forth method were fully separated in each of the 6 different diluents immediately after reconstitution. A, Sodium bicarbonate; B, sterile water for injection; C, normal saline; D, lidocaine; E, lidocaine + E; F, mannitol (original magnification ×50).](image)
hydroxyl groups remaining in the CMC molecules may interact in a very specific manner by intramolecular and/or intermolecular hydrogen bonding, and this interaction leads to the formation of aggregates or associates that can be significantly influenced by the solvent. \(^{35,36}\) CMC solutions are sensitive to variations in pH and ionic strength, but the ionic strength of the solution has more influence on the conformation of CMC than the pH. \(^{37–40}\)

With the 3 methods described in this test, the shearing force generated from the back-and-forth method is the strongest, whereas that of the vortex and hand-shaking methods is moderate. Of the 6 diluents studied here, NaHCO\(_3\) was the strongest, whereas that of the vortex and hand-shaking force generated from the back-and-forth method is around 2 millimeters in diameter. When we performed the back-and-forth reconstitution process, the flow velocity of the solution when it passed through this small through hole was extremely high. This induced a strong solution jet, which resulted in swirls and turbulence inside one syringe. This situation occurred inside both syringes alternatively throughout the back-and-forth process. As a result, the CMC particles could dissolve and disaggregate in a very short time, regardless of the ionic strength of the diluents. This process also led to completely separated and homogenously dispersed PDLLA microspheres.

Interestingly, the time required for dispersion of the PDLLA microspheres was shorter for mannitol than for SWFI tube by the vortex method. This result implies that mannitol is a solubilizer of CMC. Besides, NS cannot be used as a diluent for injectable PDLLA by the vortex method but can be used for injectable PLLA. \(^{35}\) This may be due to the pre-blending effect of CMC and mannitol. By pre-blending, the CMC particles are separated from each other by mannitol before entering the liquid, thereby minimizing aggregation. \(^{41}\) However, there is a scarcity of published information detailing the role of NS and mannitol in injectable PLLA. Further study on the interaction among NS, mannitol, and injectable PLLA is needed. Clinically, it is not practical for us to choose mannitol as a reconstitution diluent for injectable PDLLA. Therefore, only SWFI can be effectively used as a reconstitution diluent for injectable PDLLA by the vortex or hand-shaking method.

The back-and-forth method has been used to mix some fillers, \(^{35,45}\) with lidocaine for anesthesia or as a carrier. In these previous methods, only simple mixing of drugs was required, and 2 syringes were connected by a coupler or connector with a large through hole. The purpose of the back-and-forth method used here was to completely dissolve and disaggregate the CMC particles. Because CMC exhibits shear-thinning, a dissolution process involving mechanical stirring, agitation, pumping, or shearing is necessary. The 3-way stopcock has a small through hole, around 2 millimeters in diameter. When we performed the back-and-forth reconstitution process, the flow velocity of the solution when it passed through this small through hole was extremely high. This induced a strong solution jet, which resulted in swirls and turbulence inside one syringe. This situation occurred inside both syringes alternatively throughout the back-and-forth process. As a result, the CMC particles could dissolve and disaggregate in a very short time, regardless of the ionic strength of the diluents. This process also led to completely separated and homogenously dispersed PDLLA microspheres.

The advantages of this method are not only that it is quick and can be used for reconstitution with various diluents, but it is also easier to obtain a thick suspension that is difficult to achieve using the vortex method. Clinically, when we perform reconstitution of one vial of injectable PDLLA (13–15 acorn shape beads), the syringes used were 10 mL instead of 3 mL. The time needed for the back-and-forth process is about 5 minutes, regardless of whether 8 mL or 1.4 mL SWFI is used. However, a disadvantage of this method is the need to transfer PDLLA beads from the vial into the syringe, during which the chances of contamination increase and accidental spilling of the PDLLA beads may happen. Therefore, this procedure should be performed by a well-trained professional under strict aseptic conditions. Although we did not encounter complications such as infection, nodule, or granuloma formation using this back-and-forth reconstitution method in our 2 years’ clinical experience, we seldom used diluents other than SWFI for the reconstitution of injectable PDLLA. One of the limitations of this study is that the experiments performed were in vitro experiments. Understanding whether using this new back-and-forth reconstitution method with diluents other than SWFI can lead to an effect that is comparable to the effect achieved using the traditional vortex or hand-shaking reconstitution method with SWFI after injectable PDLLA administration still requires further controlled studies in vivo.
CONCLUSIONS

CMC possesses interesting dissolution properties. A dissolution process of CMC that involves shearing force is necessary. The shearing force generated by different methods from high to low is back-and-forth, vortex, and hand-shaking method, respectively. The conformation of CMC particles will also be affected by the ionic strength of the diluent. The ionic strength of NS, lidocaine, lidocaine + E, and NaHCO₃ is larger than that of SWFI and mannitol. When high ionic strength solutions (NS, lidocaine, lidocaine + E, and NaHCO₃) were used, CMC particles easily aggregate. These CMC particle aggregations cannot be separated totally by less powerful shearing force vortex method. Therefore, only SWFI and mannitol can be used in this method. On the contrary, when using the back-and-forth method, all these diluents can be used for the reconstitution of injectable PDLLA because the CMC particle aggregations can be separated totally by a more powerful shearing force.

Clinically, only SWFI can be used by the hand-shaking or vortex reconstituted method. The back-and-forth method is a good choice for quick reconstitution of injectable PDLLA. Moreover, when SWFI is not available, we can use NS, lidocaine, or lidocaine + E as a diluent for reconstitution of injectable PDLLA by this novel back-and-forth method. However, further studies are needed to understand more about the clinical outcomes after the administration of injectable PDLLA reconstituted using nonstandard diluents and the back-and-forth method proposed here.

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