

## Two-fold Serial Dilution: A Simple Method to Adjust Thickness of Injectable Poly-D,L-lactic Acid

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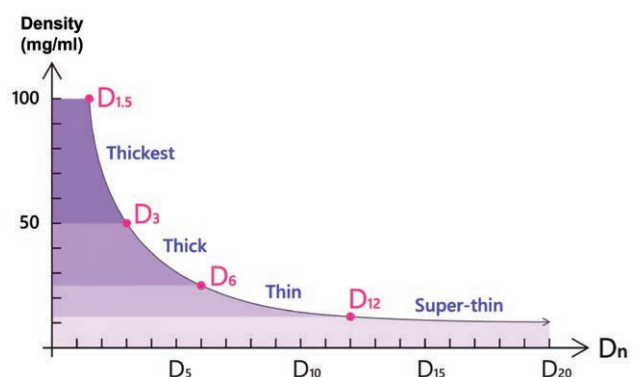
Injectable poly-D,L-lactic acid (PDLLA) is a relatively new subdermal collagen-stimulating filler (AestheFill; REGEN Biotech, Seoul, South Korea) containing PDLLA microspheres and carboxymethyl cellulose. It is supplied as lumps of lyophilized powder in vials. Reconstituting injectable PDLLA with sterile water for injection (SWFI) to form a homogeneous suspension is needed before injection.<sup>1-5</sup> In our past experience, we often use SWFI from 1.5 to 24 ml to reconstitute a vial of injectable PDLLA for a wide range of applications such as from deep injection for volume augmentation to superficial injection for skin quality improvement.

In this article, “Dn” means the density of a suspension when a vial of injectable PDLLA is “diluted” with “n” ml of SWFI. Because one vial contains 154 mg PDLLA, Dn represents there are 154/n mg PDLLA contained per 1 ml of suspension.<sup>2</sup> The suspensions are divided into four groups of density for different purposes of injection: thickest (D<sub>1.5</sub>-D<sub>3</sub>), thick (D<sub>3</sub>-D<sub>6</sub>), thin (D<sub>6</sub>-D<sub>12</sub>), and super-thin (D<sub>12</sub>-D<sub>24</sub>). We use thickest suspension for augmentation purposes like an implant for chin or nose; thick suspension for deep wrinkle correction or for large amount of volume augmentation; thin suspension for shallow wrinkle correction; and super-thin suspension for facial skin rejuvenation (Fig. 1).

The question is, how to adjust density of injectable PDLLA? We conduct a simple “two-fold serial dilution” method. This method is based on the fact that when the amount of the suspension is diluted by SWFI to its double fold, the density of this suspension halves. First, we use vacuum-assisted hydration<sup>5</sup> and the back-and-forth method<sup>3,4</sup> to reconstitute injectable PDLLA into a thickest suspension. After taking the required amount of this thickest suspension for injection, we dilute the residual

amount of this suspension by the same amount of SWFI. The density halves and it becomes a thick suspension. Then we repeat the same procedures described above. We can obtain thin and finally super-thin suspensions. “D<sub>1.5</sub>-D<sub>3</sub>-D<sub>6</sub>-D<sub>12</sub>” is the most common density combination we have been using for 2 years (Fig. 2). There are many other density combinations such as “D<sub>1.7</sub>-D<sub>3.4</sub>-D<sub>6.8</sub>-D<sub>13.6</sub>,” “D<sub>2</sub>-D<sub>4</sub>-D<sub>8</sub>-D<sub>16</sub>,” “D<sub>2.2</sub>-D<sub>4.4</sub>-D<sub>8.8</sub>-D<sub>17.6</sub>,” and so on. It depends on personal preferences and experiences to choose appropriate density combination. Usually, we use 2–5 vials of injectable PDLLA for one session’s injection according to the severity of patient’s tissue deficiency. One important note is not to overcorrect any regions of the face. Treatment can be repeated more than 1 month apart if needed.

This two-fold serial dilution method must be combined with the back-and-forth method for reconstituting injectable PDLLA. It is easier to reconstitute multiple vials into a thickest suspension, and much easier to check how much of the residual amount is left. The only disadvantage of using the back-and-forth method is the possibility of increased contamination rate when transferring PDLLA lumps from the vial into the syringe.<sup>3</sup> Thus, this procedure should be performed by a well-trained professional under strict aseptic conditions. Otherwise, two-fold serial dilution is a simple method to prepare different density suspensions of injectable PDLLA.

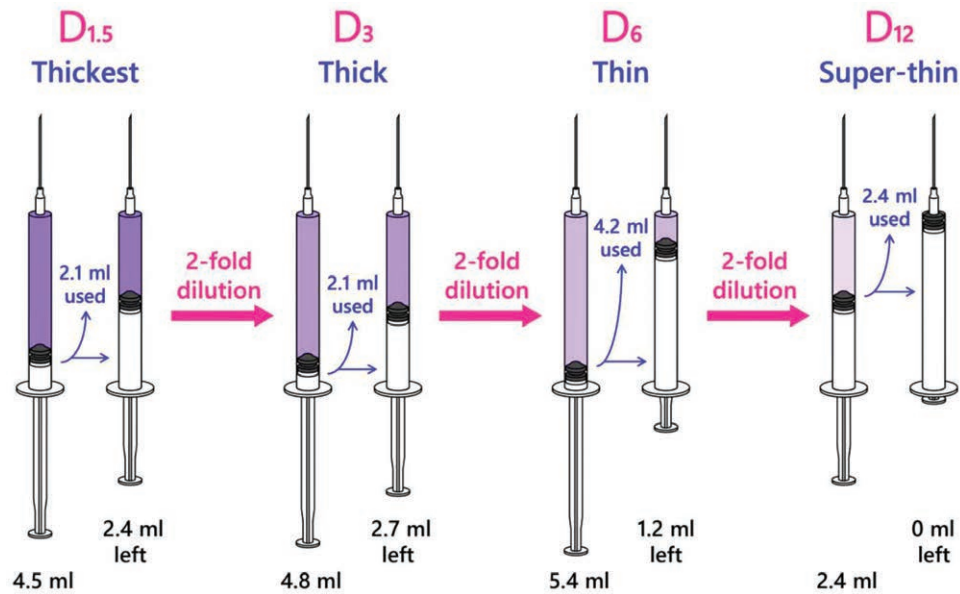


**Fig. 1.** Graph of the density of suspension (mg/ml) vs. suspension when a vial of injectable PDLLA is “diluted” by “n” ml of SWFI (Dn) curve. Because a vial of injectable PDLLA contains 154 mg PDLLA microspheres, the density of the D<sub>1.5</sub>, D<sub>3</sub>, D<sub>6</sub>, D<sub>12</sub>, and D<sub>24</sub> suspensions are 100, 50, 25, 12.5, and 6.25 mg/ml, respectively. We define the density between 100 and 50 mg/ml (D<sub>1.5</sub>-D<sub>3</sub>) as thickest suspension; density between 50 and 25 mg/ml (D<sub>3</sub>-D<sub>6</sub>) as thick suspension; density between 25 and 12.5 mg/ml (D<sub>6</sub>-D<sub>12</sub>) as thin suspension; and density between 12.5 and 6.25 mg/ml (D<sub>12</sub>-D<sub>24</sub>) as super-thin suspension.

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**Fig. 2.** An example of the two-fold serial dilution method. Three vials of injectable PDLLA were reconstituted by 4.5 ml of SWFI. Therefore, 4.5 ml of D<sub>1.5</sub> suspension was obtained. An estimated 2.1 ml of D<sub>1.5</sub> suspension was used to inject patient's chin, then 2.4 ml of D<sub>1.5</sub> suspension was left. A two-fold dilution of the remainder was performed, then 4.8 ml of D<sub>3</sub> suspension was obtained. In total, 2.1 ml of D<sub>3</sub> suspension was used to inject the patient's naso-labial folds, then 2.7 ml of D<sub>3</sub> suspension was left. Again, two-fold dilution of the remainder was performed, and then 5.4 ml of D<sub>6</sub> suspension was obtained. A dose of 4.2 ml of D<sub>6</sub> suspension was used to inject patient's tear trough deformity and marionette lines, and then 1.2 ml of D<sub>6</sub> suspension was left. Finally, a two-fold dilution of the remainder was performed, and then 2.4 ml of D<sub>12</sub> suspension was obtained. This D<sub>12</sub> suspension was used to inject for skin texture improvement of other facial regions.

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