Micro-Fisheyes of Carboxymethyl Cellulose: The Cause of Micro-Clumps in the Suspension of Injectable Poly-L-Lactic Acid

Aesthetic Surgery Journal
2020, 1–3

DOI: 10.1093/asj/sjaa040
www.aestheticsurgeryjournal.com
OXFORD
UNIVERSITY PRESS

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Editorial Decision date: February 3, 2020; online publish-ahead-of-print March 16, 2020.

Injectable poly-L-lactic acid (PLLA), a biostimulatory filler (Sculptra; Galderma, Fort Worth, TX), can stimulate a fibroblastic response and collagen neogenesis to achieve volume restoration.[1] This filler is supplied as lyophilized powder. Prior to injection, reconstitution with sterile water for injection (SWFI) to create a homogeneous suspension is required. Subcutaneous papules and nodules may form after injectable PLLA administration. The presence of dry PLLA micro-clumps in the suspension is a risk factor for the formation of subcutaneous papules and nodules.[1-3] However, the literature on the mechanism of PLLA micro-clump formation remains scarce. This mechanism appears to involve an interaction between SWFI and the 3 ingredients of injectable PLLA: PLLA microparticles, carboxymethyl cellulose (CMC), and nonpyrogenic mannitol.

CMC, a type of hydrocolloid, tends to form clumps when added to water.[4,5] The dissolution process of CMC begins with particles swelling, followed by disruption of swollen particles, leading ideally to the formation of a homogeneous dispersion of separated, hydrated CMC molecules. CMC clumps form during the dissolution process when partially hydrated CMC particles collide and adhere to each other. The outer surface of a CMC clump hydrates quickly, producing a sticky, gelatinous coating and leaving a dry interior.[4] These clumps are known as "fisheyes" because of their appearance. The gelatinous coating of a fisheye slows the rate of water diffusion from the periphery to the dry interior.[4] Once fisheyes are formed, it may take a considerable time for the dry interiors to become wet. Some fisheyes may even be strong enough to resist any further hydration.[4,5]

The critical element in the reconstitution of injectable PLLA is the dissolution process of CMC. Two methods are commonly used to prevent fisheye formation when dissolving a hydrocolloid. The first involves high-agitation mixing. Ideally, hydrocolloid powders should be sifted slowly into a vortex of rapidly stirred water, where high shear forces tear away partially hydrated molecules and disperse particles completely without forming clumps.[4,5] However, in clinical practice, it is not feasible to add injectable PLLA powders into a vortex of rapidly stirred water. The second method to prevent fisheye formation is to preblend the hydrocolloid with another dry ingredient such as sugar. This technique enhances the probability that hydrocolloid particles are separated from each other before significant hydration occurs.[4,5] Mannitol, one of the ingredients of injectable PLLA, is a kind of sugar alcohol. To the best of our knowledge, there is no medical literature detailing the role of mannitol in injectable PLLA. We know that mannitol can be used to increase the low osmotic pressure of injectable PLLA when reconstituted with SWFI. We believe

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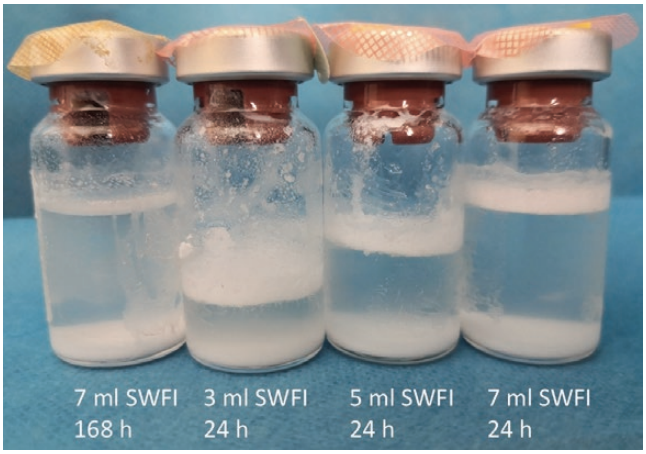

Figure 1. Comparison of 4 vials of injectable PLLA reconstituted with different volumes of SWFI and for different hydration times. From left to right: 7 mL, 168 h; 3 mL, 24 h; 5 mL, 24 h; 7 mL, 24 h. The floating layers of these 4 vials were CMC micro-fisheyes which contained PLLA micro-clumps. The thickness of the floating layer decreased with increased hydration time. Some micro-fisheyes adhered to the glass wall. The micro-fisheyes appeared to be stickier when smaller volumes of SWFI were used for reconstitution. The precipitates were PLLA microparticles without micro-fisheyes. PLLA, poly-L-lactic acid; SWFI, sterile water for injection.

that mannitol also preblends with CMC particles to prevent fisheye formation during the dissolution of CMC. In general, prevention of fisheye formation requires the ratio of the other ingredient to the hydrocolloid to be at least 5:1 by weight.[4] However, a vial of injectable PLLA comprises 90 mg CMC, but only 127.5 mg mannitol.[1] This ratio is probably the reason why CMC particles in PLLA cannot be completely separated from each other and form micro-clumps, known as "micro-fisheyes." Some PLLA microparticles become entrapped inside these micro-fisheyes, thus creating dry PLLA micro-clumps which are difficult to eliminate.

In our experience, no matter how much SWFI we added to reconstitute injectable PLLA, when the vial was allowed to stand for at least 30 minutes, the suspension would eventually divide into 3 layers, namely an upper floating layer, a middle transparent layer, and a lower precipitating layer. Inititally, the upper floating layer was thick and the lower precipitating layer was thin. As time progressed, the upper floating layer became thinner while the lower precipitating layer became thicker (Figure 1). To perform a microscopic examination, we agitated the vial to create a homogeneous suspension and then extracted a drop from the middle part of the suspension via a syringe with an 18G needle. The sample was then placed onto a microscopic glass slide without a cover glass, where it formed a droplet. Tiny particles could be seen floating on the upper part of this droplet. By focusing on these particles under the microscope, we were able to obtain photographs of the micro-fisheyes (Figure 2). Placing a cover glass on the droplet causes most of the micro-fisheyes to be broken by compression due to the cover glass, leading to difficulty in identifying micro-fisheyes. We observed suspensions from more than 10 different vials of injectable PLLA by this method. All the samples retrieved from these different vials contained micro-fisheyes. The longest hydration time of injectable PLLA we ever tested was more than 2 months. Although the upper floating layer was very thin, we could still observe some micro-fisheyes from this layer under the microscope. Because some air was contained inside the micro-fisheyes, they eventually floated on the upper part of the suspension. If the vial was allowed to stand for a considerable time (more than 2 hours), we found that the upper floating layer comprised mostly micro-fisheyes, while the precipitating layer essentially comprised PLLA microparticles without micro-fisheyes. Due to the stickiness of their gelatinous coatings, micro-fisheyes readily adhere to the glass wall or rubber lid of the vial when agitated, and are difficult to rinse off for further hydration (Figure 1).[2] Various methods, such as lengthening the reconstitution time,[2] sonication,[1] and a lukewarm water bath,[6] have been used to eradicate micro-fisheyes, but cannot totally eliminate them. Because micro-fisheyes will eventually float on the suspension, discarding the floating layer before agitating the vial is the best method to prevent injection of micro-fisheyes (Figure 3). Larger micro-fisheyes may clog injection needles. Once micro-fisheyes have been injected into human tissue, massaging the injection site might prove helpful in breaking them up and hydrating their dry interiors.

In conclusion, the formation of micro-clumps in injectable PLLA is due to entrapment of PLLA microparticles inside CMC micro-fisheyes. These micro-fisheyes are difficult to eliminate entirely once they have formed. The concept of CMC micro-fisheye formation and the roles played by mannitol have not been discussed before. Further studies should be designed to investigate how to decrease formation or increase elimination of micro-fisheyes based on their properties.

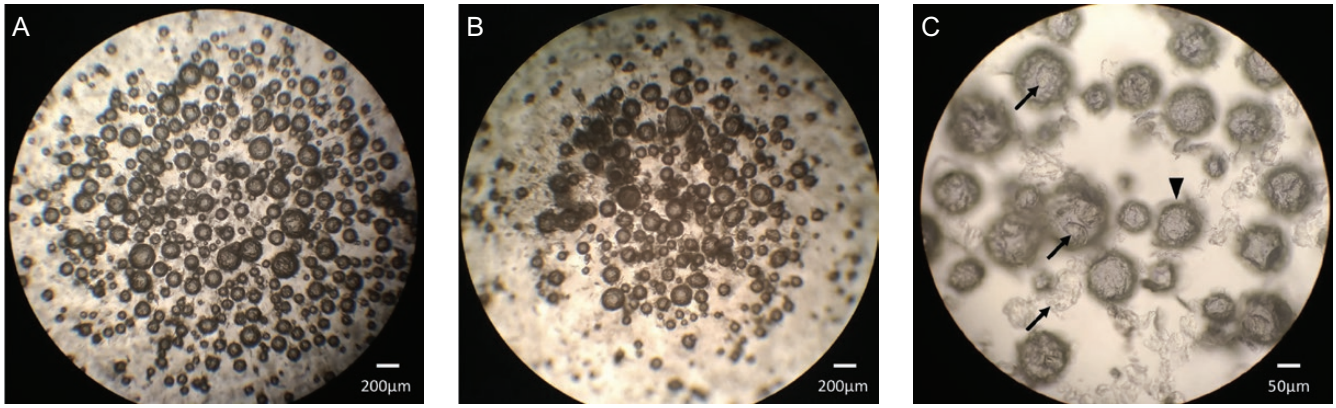

Figure 2. Microscopic views of micro-fisheyes in a suspension of injectable PLLA. A vial of injectable PLLA was reconstituted with 7 mL of SWFI for (A) 2 h, 50×; (B) 48 h, 50×; (C) 48 h, 200×. All these micro-fisheyes contained PLLA micro-clumps. Arrows, PLLA microparticles; arrowhead, micro-fisheye. PLLA, poly-L-lactic acid; SWFI, sterile water for injection.

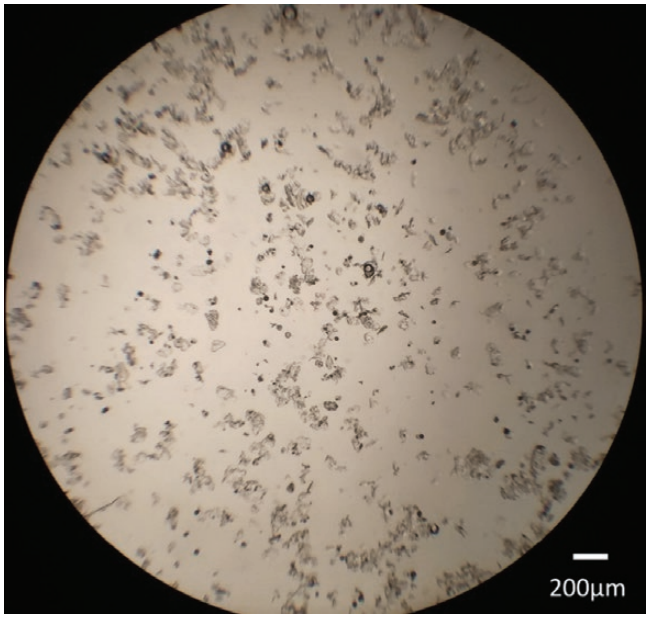

Figure 3. Microscopic view of PLLA microparticles in a suspension of injectable PLLA. A vial of injectable PLLA was reconstituted with 7 mL of SWFI for 2 hours; then the floating layer was discarded before agitating the vial. Well-dispersed PLLA microparticles without micro-fisheyes were found. PLLA, poly-L-lactic acid; SWFI, sterile water for injection.

Acknowledgments

The authors thank Sha-Sha Hang for her advice on the properties of CMC, and Xin-Feng Zhang for his assistance with preparing injectable PLLA.

Disclosures

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Funding

The authors received no financial support for the research, authorship, and publication of this article.

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