Understanding the Structure and Function of Platelet-Poor Plasma Biofiller, an Electron Microscopic and Fourier Transform Infrared Spectroscopy (FTIR)-Based Analysis

Dear Editor,

Biofiller is a new autologous filler material that is becoming popular in recent times. Various studies have been performed on it. From initial proof of concept, to now prospective non-randomized controlled studies, the biofiller has come a long way.[1,2] It is essential to ascertain the generic structure of biofiller, to standardize the method of preparation, and determine the safety of the procedure.

The first documented evidence of biofiller was given by animal studies and phase I human trials by Woo *et al*.[3] The longevity of the products and their use in aesthetics has been discussed in detail in recommendations for the use of PRP (Platelet-rich plasma) in aesthetics by the IADVL (Indian Association of Dermatologists, Venereologists and Leprologists).[4]

Process of Preparation

Different studies have shown different methods of preparing biofillers. The principle remains the same. It involves heating blood plasma (platelet-rich plasma or platelet-poor plasma) to form a semisolid amorphous gel-like substance. Different temperatures (60°C to 100°C) and times of heating (1 to 10 minutes) have been recommended[4] (temp and times from different studies). An optional step of cooling is recommended by some authors.[2] The authors concur with the observation of Bhatt *et al*. that a higher temperature produces a firmer filler. Figure 1a shows the consistency of filler produced at different temperatures. At 60°C, the filler has almost a water-like consistency, while at 100°C a solid filler is produced. Most studies mention 100°C as the temperature used, but do not use a water bath (which maintains the given temperature) to prepare the biofiller. Instead, they pour hot water into a container and the liquid cools over the next 10 minutes making the process inconsistent. The ideal consistency is observed when the biofiller is kept at 70°C for 10 minutes or 80°C for 2 minutes. An increase in the duration of heat also hardens the product.[2]

There are some concerns about heating the filler in PET (Polyethylene terephthalate) plastic syringes. PET plastic is a thermolabile plastic that has a low melting point near 130°C. Heating up to 90°C–100°C leads to partial melting or softening of the syringe and contamination by plastic as seen in Figure 1b. The authors suggest the use of glass vials like those used with dental syringes [Figure 1c].

Analyzing the structure of biofiller

The process of biofiller formation is similar to the boiling of an egg. The liquid egg gets converted to a white opaque solid. Another similar phenomenon we see in a bedside test for the detection of proteins in urine is the heat coagulation test. Fourier transform infrared spectroscopy (FTIR) analysis is a technique that can be used to compare two chemical samples, as FTIR can be considered a chemical fingerprint of a sample.[5] We performed a FITR analysis to compare the structure of a boiled egg to biofiller. Figure 2a shows coinciding peaks of absorption at two wavelengths, suggesting a very similar chemical structure. We also performed electron microscopy of both egg white and biofiller and found similar amorphous shapeless structures [Figure 2b], unlike electron microscopy of PRF (Platelet-rich fibrin), which shows distinct fibrin meshwork with different distinct adherent cells on the mesh.[6] This indicates that biofiller is an amorphous aggregate of protein that has lost its three-dimensional quaternary structure. It seems safe to assume that any growth factor that might

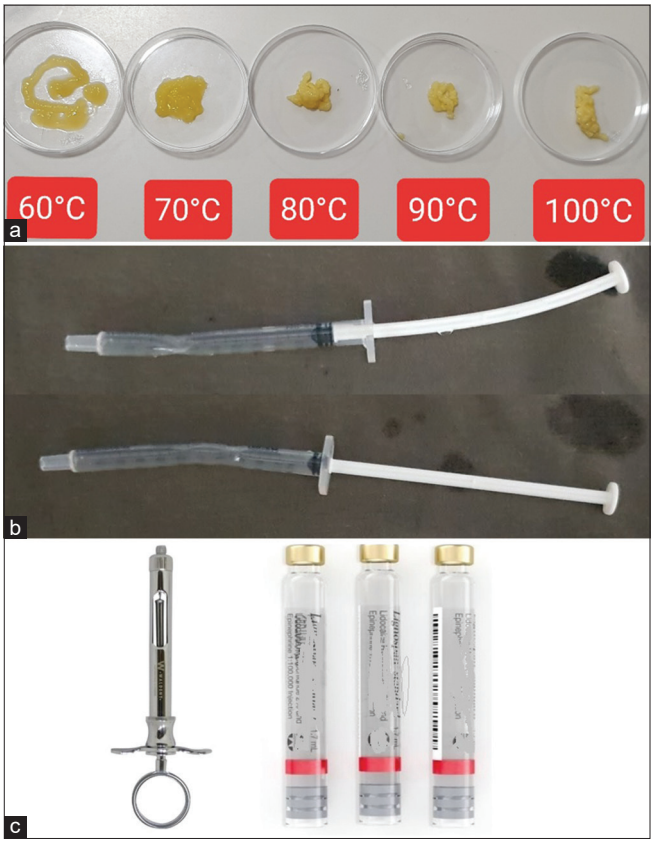

Figure 1: (a) Biofiller consistency at different starting temperatures. Biofiller prepared at lower temperature has more soft consistency as compared to higher temperature (b) Tuberculin syringes that have partially melted and bent due to heat of 90°C (c) Dental syringe and glass vials that are resistant to heat

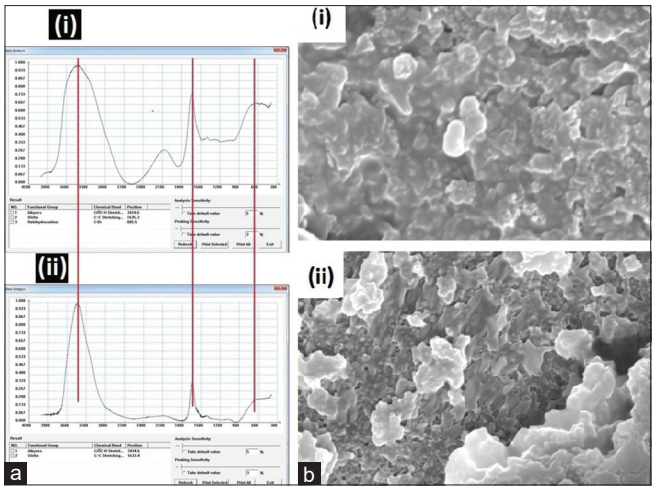

Figure 2: (a) Fourier transform infrared spectroscopy (FTIR) shows overlapping absorption spectra of biofiller and egg white. (i) Absorption spectra of biofiller with peak absorption at 3434.6 nm and 1635 nm. (ii). Absorption spectra of egg white at 3434.6 nm and 1633 nm (b) Scanning electron microscopy (SEM) pictures of egg white and biofiller show amorphous protein aggregates. No three-dimensional fibrin meshwork or cellular structure is seen. (i). SEM of egg white at 20000x, EHT = 20kv. (ii). SEM of biofiller at 20000x, EHT = 20kv

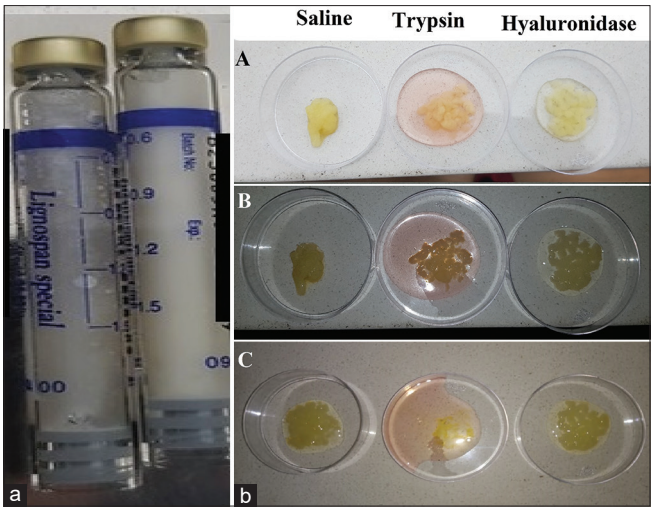

Figure 3: (a) Biofiller-like substance formed by heating injection albumin (b) Digestion of filler using saline, enzyme trypsin 0.025%, and enzyme hyaluronidase at baseline (A), 3 hours (B), and 24 hours (C). Near complete digestion of filler is seen at 24 hours by trypsin, but hyaluronidase does not digest the biofiller

have been present in platelet-poor plasma must have lost its quaternary structure and function. However, there is no study to confirm or refute this finding. The common protein in platelet-poor plasma and egg is albumin, which imparts similar properties to both (human albumin and ovalbumin). Thinking on the same lines, commercially available injection albumin, on being heated, should also produce similar semisolid filler material. Figure 3a shows a heated injection albumin biofiller. This concludes that platelet-poor plasma biofiller is predominantly an albumin filler. However, injection albumin must not be used clinically because of the high risk of antigenicity.

Safety

The major safety concerns with making this filler are infection and vascular occlusion. Strict aseptic techniques must be used to ensure that any sort of infection is not deposited in the skin. Unfortunately, unlike hyaluronidase, there is no enzyme that can digest this filler. In a vascular accident, surgical removal remains the only option. Figure 3b shows the digestion of biofiller with different enzymes. Enzyme trypsin 0.05% can digest the filler in about 3 hours, but injection of the enzyme trypsin in human tissues can be dangerous and ineffective as it will be immediately neutralized by alpha-1-antitrypsin present in the blood.

Conclusion

Biofiller is an excellent tool that can be utilized in various indications, but is off-label. When using off-label or new biologic products, the Center for Biologics Evaluation and Research (CBER) correctly mentions that clinicians "have the responsibility to be well-informed about the product, to base its use on firm scientific rationale and on sound medical evidence, and to maintain records of the product's use and effects."[7]

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Conflicts of interest

There are no conflicts of interest.

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