The composition of the lachrymal film is of great importance for the stability and feasibility of the corneal and conjunctival epithelium. The cornea acquires the main nutrients from the aqueous humor and the growth factors (GF), vitamins and neuropeptides from the lachrymal gland. The latter are very important as they account for the proliferation, migration and differentiation of the corneal and conjunctival epithelium cells, all of which are found in adequate proportions in the tear to ensure the good performance of the ocular surface.

In the dry eye syndrome, the composition of the tear is altered and when the inflammatory process begins new elements appear such as the factors associated to the inflammatory response. In this way the lachrymal film loses its properties and, in the case of a severe dry eye, treatment with artificial tears is clearly insufficient. In these cases ophthalmologists use autologous serum (AS). Serum is the result of the centrifugation of blood. The advantages in its use as a treatment for severe dry eye syndrome are based on the fact that said fluid includes a number of factors which are associated to beneficial effects, particularly those related to the growth and maintenance of epithelial cells (1,2). Said factors include a number of growth factors such as the Epithelial Growth Factor (EGF), the b-transforming growth factor b (TGF-b), acid and basic fibroblast growth factor (aFGF and bFGF), the platelet-derived growth factor (PDGF) and the insulin-1 type growth factor. In addition, other components such as vitamin A, fibronectin, albumin, a-2 macroglobulin are worthy of note, together with neuropeptides such as the P substance.

Growth factors play a crucial role in corneal cicatrization. The epithelial growth factor stimulates the proliferation and differentiation of cells. Anti-apoptotic properties have also been attributed to it. Together with the b-transforming growth factor, it produces migration in epithelial corneal cell populations, in association with epithelial and stromal repair processes.

The acid and basic fibroblast growth factors have demonstrated their ability to accelerate the corneal cicatrization process in rabbits. FibronectinFibronectin is related to re-epithelization, while alpha-2-macroglobulin has exhibited beneficial effects in burns with alkaline substances. Likewise, neural factors such as the P-substance are important for corneal epithelial migration. In addition, serum contains proteins such as albumin which can improve the stability of the afore-mentioned factors and immunoglobulins like IgG as well as supplement factors.

When the serum to be administered belongs to the same individual, it is called autologous serum. The use of autologous serum in severe dry eye treatment has gained widespread acceptance in the past decade. However, it continues to be a restricted area because it must be prepared by experts. In some cases, bovine foetal serum has been utilized, as well as umbilical cord serum, although these fluids do not have the advantage of lacking antigenicity as is the case of the autologous serum.
Autologous serum is considered to be a master formula and must be prepared according to strict protocols. In addition, it must be stored in specific conditions because of its low stability. Accordingly, it is highly recommendable to develop adequate production standards which, in turn, must be adapted to the regulation of blood preparations. In this area it is crucial to take into account the technological factors which may bear on the product quality and properties. Some studies considered the effect of centrifuging autologous serum on its overall quality (3). Blood centrifugation is regarded as a critical factor because, if done inadequately, hemolysis can occur. In addition, the platelet membranes which may remain in the serum can induce apoptosis, thus reducing the beneficial effects of its application.

Once extracted, the serum is diluted with isotonic solutions (sodium chloride or buffered saline solutions) in different concentrations, with 20% being most widely used. The final formulation is placed in flasks requiring special storage conditions (refrigerator and protection against light) due to its instability and light-sensitive components such as Vitamin A. As it is free of preservatives, AS is at high risk of contamination. Although this can occur during the production process, contamination mostly occurs due to faulty manipulation by users. For this reason, the patient must be instructed to make proper use of the serum in order to avoid risks. Contamination may also occur during storage, although information on this is contradictory because, while some authors describe contamination after 30 days of use, others maintain that the autologous serum at a 20% dilution can be used for 12 weeks (4,5). Therefore, when issuing AS eye drop flasks, it is recommended to maintain them in the freezer except the one in use. In any case, it is not recommended to utilize the same flask over one week.

The efficiency of AS is related to the stability of its essential components. The refrigerator storage period (at 4°C) is usually one month, and in the freezer (-20°C) several months. Some authors have demonstrated the stability of specific autologous serum components after one months at 4°C and three months at -20°C.

Taking into account all the above, a strategy for improving the stability of autologous serum would be to use technological resources such as lyophilization of the product. This process eliminates solvent from the product (in this case water) and thus enhances its stability. If the process is carried out correctly, the components will maintain their properties and, when reconstructing the preparation with the removed solvent, it will maintain its initial characteristics. This process is applied in vaccines and preparations similar to AS with excellent results. In addition, lyophilization allows for the inclusion of elements which can enhance the stability of critical AS factors. Also, substances with beneficial properties for corneal regeneration could be added, such as some sugars or bio-polymers.

If the objective is to improve the characteristics of the formulation, it would also be possible to add specific components with the ability to increase the time of contact between the product and the corneal surface. For this, mucous-adhesive polymers could be used. These are currently used for dry eye treatment and have exhibited excellent results.

Obviously, the formulation of autologous serum can be improved with existing technological resources. In the near future we may be able to utilize customized autologous serum solutions adapted to the specific needs of each patient.

REFERENCES