

## Use of Umbilical Cord Serum in Ophthalmology

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Among blood preparations, serum has been topically used in the management of various ocular diseases in ophthalmology. Like peripheral blood serum, umbilical cord blood serum contains a high concentration of essential tear components, growth factors, neurotrophic factors, vitamin A, fibronectin, prealbumin, and oil. Umbilical cord serum can provide basic nutrients for epithelial renewal and can facilitate the proliferation, migration, and differentiation of the ocular surface epithelium. Eye drops made from umbilical cord serum have been applied to treat various ocular surface diseases, including severe dry eye with or without Sjögren's syndrome, ocular complications in graft-versus-host disease, persistent epithelial defects, neurotrophic keratopathy, recurrent corneal erosions, ocular chemical burn, and surface problems after corneal refractive surgery. Because mesenchymal stem cells from umbilical cord blood can be used to regenerate corneal tissue and retinal nerve cells, umbilical cord serum might be applied for tissue engineering and regenerative medicine in the future.

**Key Words:** *Ophthalmology; Umbilical cord; Serum; Dry eye*

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### INTRODUCTION

Blood preparations including autologous serum (AS), plasma rich in growth factors, platelets, and umbilical cord serum (UCS) have been introduced to treat many ocular diseases because they contain a high concentration of biologically active components and growth factors. The tear film consists of mucin, aqueous, and lipid layers and contains many growth factors and vitamin A, which are essential for regulating the proliferation, differentiation, and maturation of the ocular surface epithelium. Ocular surface disorders including dry eye disease or keratoconjunctivitis sicca are characterized by a decrease in quality and quantity of the tear film and squamous metaplasia of the conjunctival epithelium. Conventional treatments for ocular surface disorders include the application of artificial tears, topical anti-inflammatory agents, secretagogues, therapeutic contact lenses, and punctal occlusion.<sup>1</sup> Because peripheral blood serum (PBS) harbors essential tear components and growth factors, AS eye drops have been used for the treatment of severe ocular surface diseases. We found that UCS contains a higher level of essential tear components, growth factors, and neurotrophic factors than

AS, and UCS eye drops can be applied in various ocular conditions such as severe dry eye disease, persistent epithelial defects, neurotrophic keratopathy, recurrent corneal erosions, ocular chemical burn, and corneal refractive surgery.<sup>2-8</sup>

### CONVENTIONAL SERUM TREATMENT IN OPHTHALMOLOGY

Serum contains many growth factors [epidermal growth factor (EGF), acidic and basic fibroblast growth factors, platelet-derived growth factor, hepatocyte growth factors, and transforming growth factors (TGF- $\beta$ )], fibronectin, serum antiprotease ( $\alpha_2$ -macroglobulin), vitamin A, neurotrophic factors [substance P, insulin-like growth factor (IGF)-1, and nerve growth factor (NGF)], prealbumin, oil, and antioxidants. Therefore, it can provide the corneal and conjunctival epithelium with basic elements for epithelial regeneration that are lacking in artificial tears.<sup>9</sup>

PBS is known to have higher vitamin A, TGF- $\beta$ 1, IGF-1, NGF, fibronectin, and lysozyme concentrations and lower immunoglobulin A, EGF, and vitamin C concentrations than tears.<sup>10</sup> In an animal model of corneal epithelial de-

fects after refractive surgery, 20% serum eye drops led to faster epithelial healing than artificial tears by decreasing apoptosis of keratocytes, migration of fibroblasts and myofibroblasts, and migration of inflammatory cells.<sup>11</sup> According to controlled studies, AS treatment was shown to provide better improvement in symptoms and signs of ocular surface diseases than artificial tears did.<sup>12,13</sup> Clinically, AS eye drops have been effectively used to treat dry eye associated with Sjögren's syndrome<sup>2</sup> or graft-versus-host disease (GVHD),<sup>3</sup> persistent epithelial defects,<sup>4,5</sup> neurotrophic keratopathy,<sup>6</sup> superior limbic keratoconjunctivitis,<sup>7</sup> and recurrent corneal erosions.<sup>8</sup> AS has also been applied during or after ocular surgeries including macular hole surgery, vitrectomy in patients with diabetes, and trabeculectomy.<sup>14-16</sup>

### COMPARISON OF PERIPHERAL BLOOD SERUM AND UMBILICAL CORD SERUM

We have verified that UCS also harbors a high concentration of growth factors, neurotrophic factors, and essential tear components.<sup>17,18</sup> Compared with the levels in PBS, concentrations of EGF and TGF- $\beta$  are 3 and 2 times higher, respectively, in UCS. Although the vitamin A concentration in UCS is lower than that in PBS, it is higher than the concentration in normal tears. UCS has higher NGF and substance P and lower IGF-1 concentrations compared with PBS. UCS-supplemented culture medium supports the proliferation and differentiation of epithelial cells in the conjunctiva and limbus, and UCS contains a higher concentration of growth factors and cytokines than fetal bovine serum and adult serum.<sup>19</sup>

Previous studies have also shown that UCS is superior to AS in the treatment of various ocular surface diseases.<sup>20-23</sup> An initial study showed that UCS could provide faster healing of the corneal epithelium than AS.<sup>20</sup> We reported that compared with AS, UCS was more effective in decreasing symptoms and epithelial staining in severe dry eye and increasing goblet cell density in Sjögren's syndrome.<sup>21</sup> Additionally, UCS eye drops were shown to be more effective in improving corneal wound healing and reducing corneal haze compared with AS eye drops in ocular chemical burn.<sup>22,23</sup>

From a clinical aspect, UCS therapy has several advantages over AS therapy. A larger amount of serum can be collected from the umbilical vein at one time, and many patients obtain benefit from this sampling without waiting for additional preparations. In addition, UCS therapy is feasible in patients who have a poor general condition or blood dyscrasia, especially hematologic malignancy.

### PREPARATION OF UMBILICAL CORD SERUM EYE DROPS

Umbilical cord blood can be obtained from mothers during delivery. From donors, laboratory examination should be performed at 8 and 38 gestational weeks to test for hu-

man immunodeficiency and hepatitis B and C viruses. After fetal delivery, about 60 to 80 ml of umbilical cord blood is sampled from the umbilical cord vein. The blood is kept for 2 hours at room temperature. After 15 minutes of centrifugation at 3,000  $\times$ g, the serum is carefully isolated under sterile conditions. The serum is then diluted to a 20% concentration with balanced salt solution. The aliquots of diluted serum are placed into sterile 5-ml bottles with ultraviolet light protection. Opened bottles are kept in a refrigerator at 4°C for 7 days, and unopened bottles are stored in a freezer at -20°C for 3 to 6 months. UCS eye drops are usually instilled 4 to 6 times per day as required in addition to artificial tears and antibiotics.

### SAFETY AND STABILITY OF UMBILICAL CORD SERUM

Among the components of serum, EGF, vitamin A, and TGF- $\beta$  are well preserved for up to 1 month in a refrigerator at 4°C and up to 3 months in a freezer at -20°C.<sup>4</sup> A strict protocol for preparation and storage is essential for the safety of serum use.

Topical administration of UCS does not cause adverse effects on the eye, because the components of serum include growth factors and tear components, rather than umbilical cord tissue-derived cells, which may result in reduced immunogenicity.<sup>24</sup> The titres of IgM and IgG2 antibodies are low in umbilical cord blood, and anti-A and anti-B antibodies are absent or only weakly detectable in UCS.<sup>25</sup> Serum has a bacteriostatic effect because it contains antibacterial agents such as IgG, lysozyme, and complement. In addition, because serum contains no preservatives, serum therapy can avoid the risk of toxic reaction in the ocular surface.

### CLINICAL APPLICATION OF UMBILICAL CORD SERUM IN OPHTHALMOLOGY

We have safely and effectively applied UCS eye drops for the treatment of intractable ocular conditions including dry eye disease with or without Sjögren's syndrome, GVHD, persistent epithelial defects, neurotrophic keratopathy, recurrent corneal erosions, ocular chemical burn, and keratorefractive surgery.<sup>17,18,21,22,26-29</sup>

The efficacy of 20% UCS eye drops was evaluated in patients with severe dry eye disease with or without Sjögren's syndrome after 2 months of treatment.<sup>17</sup> Tear film breakup time, corneal epithelial staining score, grade of conjunctival squamous metaplasia, goblet cell density, and symptom score significantly improved after UCS use. Our study comparing the therapeutic effect between AS and UCS in the treatment of severe dry eye disease revealed that, although both serum treatments led to improvement of symptoms and signs, symptom and corneal staining scores were lower in the UCS group after 1 and 2 months of treatment.<sup>21</sup> In Sjögren's syndrome patients, goblet cell density was higher in the UCS group than in the AS group

after 2 months of treatment.<sup>21</sup> Recently, it was reported that the use of standardized and quality-controlled UCS eye drops was a promising therapy for the healing of severely injured corneal epithelium as well as the relief of subjective symptoms in severe cases of dry eye.<sup>30</sup>

GVHD, which is one of the major complications after allogeneic hematopoietic stem cell transplantation, can result in many ocular diseases. Among the GVHD-related ocular diseases, dry eye is the most frequent manifestation, leading to serious complications such as punctate keratitis, persistent epithelial defects, and keratinization, ulceration, or perforation of the cornea. The use of UCS can be an effective treatment option for severe ocular surface manifestations associated with GVHD. In patients with GVHD-related dry eye, symptom score, corneal sensitivity, tear film breakup time, and corneal staining score significantly improved after 2 months of UCS treatment, and the improvement was maintained by 6 months after treatment.<sup>27</sup>

Persistent epithelial defects of the cornea are caused by medications, chemical or thermal injury, secondary infection following herpetic keratitis, and autoimmune diseases such as rheumatoid arthritis, ocular cicatricial pemphigoid, and erythema multiforme. The use of UCS could effectively decrease the defect size and shorten the healing time in patients with persistent corneal epithelial defects.<sup>20,26</sup>

Neurotrophic keratitis is characterized by impaired healing of the corneal epithelium due to damage to trigeminal innervations and depletion of trophic mediators. Causative factors include herpes simplex and zoster keratitis; chemical, physical, and surgical injuries; neurosurgical procedures for acoustic neuroma and meningioma; and systemic diseases such as diabetes, multiple sclerosis, and leprosy. We applied UCS eye drops in patients with neurotrophic keratitis who did not respond to conventional treatment. The epithelial defect healed at around 4 weeks, and visual acuity as well as corneal sensitivity also improved after treatment.<sup>18</sup>

Recurrent corneal erosion syndrome is defined as repeated episodes of corneal de-epithelization that result from trauma or dystrophy of the cornea, resulting in pain, tearing, and potential visual loss. We found that the use of 20% UCS eye drops significantly reduced the number of recurrences compared with artificial tears.<sup>28</sup>

In ocular chemical burn, early epithelial wound healing is essential to prevent serious long-term complications, which include ulceration, neovascularization, and opacification. UCS treatment showed better epithelial wound healing and lower corneal haze scores compared with PBS or conventional treatment.<sup>22,31</sup> In addition, stromal inflammation and the interleukin-1 $\beta$  level in the cornea were decreased in the UCS-treated group.<sup>22</sup>

Finally, UCS treatment can be tried for ocular complications associated with Stevens-Johnson syndrome and ocular cicatricial pemphigoid, ocular surface keratinization, Mooren's ulceration, and epithelial maintenance after ocu-

lar surface reconstruction or corneal refractive surgery. Application of 20% UCS eye drops in addition to conventional treatment after laser epithelial keratomileusis could reduce early postoperative corneal haze and improve tear film and ocular surface parameters.<sup>29</sup>

## COMPLICATIONS AND CONSIDERATIONS OF UMBILICAL CORD SERUM

Although no significant complications have been documented, potential adverse effects should always be considered when using UCS. Despite two laboratory examinations in pregnant donors, the possibility of transmission of blood-borne infectious or blood-borne diseases cannot be absolutely excluded. In addition, bacterial contamination and allergy are other possible problems. Legal and regulatory issues as well as additional costs for serum preparation should also be considered before obtaining the serum.

## SUMMARY AND FUTURE APPLICATIONS

Owing to the high concentrations of essential tear components, growth factors, and neurotrophic factors in UCS, UCS eye drops can be safely and effectively applied in intractable ocular conditions such as severe dry eye disease with or without Sjögren's syndrome, ocular GVHD, persistent epithelial defects, neurotrophic keratopathy, recurrent corneal erosions, ocular chemical burn, and surface problems after corneal refractive surgery.

Mesenchymal stromal cells can be obtained from umbilical cord blood, amniotic fluid, and adipose tissue as well as bone marrow. Umbilical cord blood contains not only hematopoietic progenitor cells but also mesenchymal progenitor cells.<sup>32</sup> Corneal stromal cells have mesenchymal stromal cell-like characteristics.<sup>33</sup> Therefore, it is expected that umbilical cord blood could be used in corneal tissue engineering and regeneration. Furthermore, human umbilical cord blood cells can differentiate into retinal nerve cells.<sup>34</sup> In the future, human cord blood cells may be used for the regeneration of retinal nerve cells in retinal degeneration or dystrophy.

## CONFLICT OF INTEREST STATEMENT

None declared.

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