Chemical Properties of Human Amniotic Membrane for Potential Opthalmological Use

CLAUDIA FLORIDA COSTEA[1], IOANA SADIYE SCRIPCARIU[2]*, RALUCA DRAGOMIR[3]*, CRISTINA DIMITRIU[4], MIHAELA DANA TURLIUC[5,9], GABRIELA FLORENTA DUMITRESCU[6], NICOLETA DUMITRESCU[7], VALERIANA VORNICU[6], ANCA SAVA[6,8], ANDREI CUCU[9], SERBAN TURLIUC[10], ALEXANDRU CARAULEANU[2,11]

[1] University of Medicine and Pharmacy Grigore T. Popa, Department of Ophthalmology, 16 Universitatii Str.,700115,Iasi, Romania
[2*]Clinic of Gynecology, Cuza Voda Hospital of Obstetrics and Gynecology, Iasi, Romania
[3*]University of Medicine and Pharmacy Grigore T. Popa , Department of Oral and Maxillo-Facial Surgery,16 Universitatii Str.,700115,Iasi, Romania
[4] University of Medicine and Pharmacy Grigore T. Popa, Department of Biochemistry , 16 Universitatii Str.,700115,Iasi, Romania
[5] University of Medicine and Pharmacy Grigore T. Popa, Department of Neurosurgery, 16 Universitatii Str.,700115,Iasi, Romania
[6] Laboratory of Pathology,Prof. Dr. Nicolae Oblu Emergency Clinical Hospital, 2 Ateneului Str.,700309, Iasi, Romania
[7] University of Medicine and Pharmacy Grigore T. Popa , 4rd year student, 16 Universitatii Str.,700115,Iasi, Romania
[8] University of Medicine and Pharmacy Grigore T. Popa, Department of Anatomy, 16 Universitãii Str.,700115, Iasi, Romania
[9] 2nd Neurosurgical Clinic,Prof. Dr. Nicolae Oblu Emergency Clinical Hospital, 2 Ateneului Str.,700309, Iasi, Romania
[10] University of Medicine and Pharmacy Grigore T. Popa, Department of Psychiatry , 16 Universitatii Str.,700115,Iasi, Romania
[11] University of Medicine and Pharmacy Grigore T. Popa, Department of Obstetrics and Gynecology, 16 Universitatii Str.,700115,Iasi, Romania

Human amniotic membrane (hAM) allows the exchange of water and organic and inorganic substances between the amniotic fluid, the fetus, and the maternal circulation. Our paper is aimed at determining the chemical composition of the amniotic membrane and at comparing it with the chemical structure of the tears, in order to identify possible uses of the amniotic membrane in eye surgery. In order to determine the chemical composition of hAM we sampled 7 pieces of fresh amniotic membrane, which were processed to create cell homogenate. The chemical tests run on the amniotic membrane specimens in our research revealed the following values: mean glucose concentration = 3mg/100mL and mean total protein concentration = 0.07g/100mL; the electrolyte concentration was: Na^+ =152 mEq/L; K^+=5.74 mEq/L; Cl^- =131.6 mEq/L, and pH=7.2, whereas the total antioxidant capacity (TAC) = 1.1±0.1 mmol/L. These values are similar to those determined by other authors for the amniotic membrane, which resemble those reported for the chemical composition of tear and aqueous humor. To conclude with, the human amniotic membrane is a useful biological material in ophthalmological transplant.

Keywords: human amniotic membrane, glucose, total proteins, electrolytes, ophthalmology, transplant

The human amniotic membrane (hAM) is the inner avascular membrane that lines the amniotic cavity and protects the developing embryo and the fetus during pregnancy [1,2]. The outer membrane is called the chorion. It contains the amnion and is part of the placenta.

The amniotic membrane (AM) is considered to a substrate which is extremely favorable for the reconstruction of the eye surface in various severe conditions of the anterior eye segment [3-12]. The use of amniotic membrane transplantation (AMT) for corneal repair improves visual acuity, especially in children with corneal ulcer, with the risk of amblyopia and impaired quality of life [13,14].

Conjunctival autografts, conjunctival limbal autografts or allografts, amniotic membrane grafting, and oral mucosa grafting may be used for the reconstruction of the anterior segment of the eyeball [15]. Postoperatively, the patient after AMT in the anterior segment of the eye or in periorbital reconstruction has an excellent cosmetic appearance, as in the case of oral mucosa grafting [16-18]. In the cases of resected tumors of the anterior segment of the eye or orbit, after AMT, other adjuvant treatments such as radiation or chemotherapy are used [17-19].

Although the excellent properties of the hAM (anti-scarring, anti-inflammatory, immune-regulatory and anti-fibrotic activities and antimicrobial activity) [20-24] were not known, its clinical use as biomaterial was reported as early as a century ago [25].

In 1910, the American surgeon J.W. Davis, who at the time worked at the Johns Hopkins Hospital in Baltimore, used the hAM for the first time for therapeutic purposes as surgery material for skin burn transplants [26]. The first documented ophthalmic application for conjunctival surface reconstruction was in 1940 by De Rotth. He used AM to repair eye wounds after conjunctival necrosis due to chemical and thermal burns or trachoma [27].

Further clinical research was conducted by Sorsby and Symons (1946) who, in 1946, used dry hAM, which has been chemically processed and used as a patch for eye burns [28]. Eye surface reconstruction by hAM transplant (hAMT) has been recently reintroduced for eye condition therapy by Kim and Tseng in 1995 [29,30].

Our paper is aimed at determining the chemical composition of the AM and at comparing it with the chemical structure of the tears, in order to identify possible uses of the AM in eye surgery.

Experimental part

Pieces of human amnion were sampled in strictly aseptic conditions from donor mothers who chose to have Caesarian section and who had been previously screened serologically for potentially communicable diseases

* email: isscripcariu@gmail.com; Phone +40741112884; drraluca@gmail.com ; Phone +40723315796

including human immunodeficiency virus, hepatitis B and C viruses and syphilis.Placental amnion was obtained using blunt dissection.

In order to determine the chemical composition of hAM we sampled 7 pieces of fresh AM, which were processed to create cell homogenate. The homogenization ratio was 1/8 (g/v), and the device used was a blade homogenizer (speed 10000-40000 rpm). The homogenate was filtered and the supernatant centrifuged for 10 min (3500 rpm). In the solution obtained we determined the protein and glucose concentration by means of an automatic wet biochemistry analyzer, RX-Imola (using control serums and calibrators). Glucose concentration was determined by means of the enzymatic glucose oxidase method, whereas total proteins were determined by means of the copper salt coupling method. The ionogram was performed using a Diestro 103AP (direct ISE potentiometry) electrolyte analyzer. Total Antioxidant Capacity (TAC) was determined using the ABTS® colorimetric reagent method (2, 2'-Azino-di-[3-ethylbenzthiazoline sulphonate]).

Results and discussions

Human amniotic membrane (hAM) allows the exchange of water, organic and inorganic substances between the amniotic fluid, the fetus, and the maternal circulation. The biochemical composition of amniotic fluid started to be investigated as early as 1933, but the findings remained inconclusive for decades.

Some authors highlight the fact that amniotic fluid has a composition similar to that of maternal plasma, but its chemical composition changes when fetal urine production begins. The concentrations of glucose and protein decrease, but the concentrations of creatinine, urea, and uric acid increase. Concentrations of electrolytes, enzymes, hormones and metabolic end products also vary [31].

Garby (1957) demonstrated that there is a similar concentration for sodium, potassium, chloride, creatinine, and glucose in amnion, amniotic membrane and plasma as the amnion functions as a molecular sieve [32].

Mellor and Slater (1971) showed that the amnion as a whole hinders considerably the passage of solute particles and there could be large concentration differences between solutes in amniotic fluid and those in allantoic fluid and maternal and fetal plasma. Amnion presents a relative impermeability to water and ions in order to balance with fetal plasma and not with maternal plasma [33].

In 1961, Sozanskii reported that, at birth, fluid amniotic glucose is 23.4±1.27 mg/100mL, as compared to 86.03±2.18mg/100mL in the mother's blood and 63.5±3.14 mg/100mL in the fetus' blood [34].

In our research (tables 1 and 2), the mean glucose concentration determined in the chemically analyzed AM specimens (3mg/100mL) was comparable to that in tears, reported by Balasubramanyam [35] as 3-10 mg/100mL, as well as that in aqueous humor, reported by Giardini and Roberts as 6.06mg/100m\L [36], which recommends it for use in eye transplantation. However, compared to serum [37], this mean concentration was approximately 20 times lower. In fact, Schmidt (1992) reported similar values for glucose concentration in amniotic fluid and membrane (5-20mg/100mL) with those obtained by us [38].

However, there are studies, like the one published by Assali et al. (1972) who found slightly higher values in the amniotic fluid (29.8mg/100mL) than those obtained by us, but lower than its serum concentration (80-110mg/100mL) [39].

The total protein concentration found by us in the amniotic membranes (0.07g/100mL) (tables 1 and 2) is 100 times lower than that in the serum (6-8g/100mL), but comparable to that of the humor aqueous (0.004mg/100mL), as reported by Albert et al. (2008) [40]. Some other authors noted that amniotic fluid proteins are principally of maternal origin, but have concentrations lower than in maternal serum [41].

Venkata et al. (2009) achieved a total protein value in tears of healthy individuals of 0.12 +/- 0.047g/100mL [42], close to that obtained by us in amniotic membranes. Johnson (2018) reported a slightly lower value (0.026g/100mL) than that obtained by us [43].

However, Assali (1972) reported a much higher concentration of proteins in the amniotic fluid (2.5g/100ml), although less than in serum [39]. Schmidt also obtained a 10 times higher value (0.28-0.78g/100mL) than that obtained by us, yet 10 times less than that in the serum [38].

Both Balasubramanyam [34] and Gerard and Josset (2011) [44] have analyzed tear biochemistry by showing that the total protein concentration (0.5-2.0g/100mL) is lower than in serum (6-8g/100mL), yet the reported values are 10 times higher than those we obtained in the homogenate and amniotic membrane fluid.

Berman (1991) also mentioned that tears have a different composition than serum. The concentrations of glucose and protein are about 10 and 30 times lower, respectively, in tear fluid than in serum [45].

Chemical composition of hAM	Authors			**Serum** (Estridge, Reynolds & Walters, 2000) [37]
	Costea *et al.*, 2018	Schmidt [38] 1992	Assali *et al.* [39] 1972	
Glucose (mg/100ml)	3	5-20	29.8	70-110
Total Proteins (g/100ml)	0.07	0.28-0.78	2.5	6.0-8.0
Na^+ (mEq/L)	155.4	-	133	135-148
K^+ (mEq/L)	5.74	-	4.9	3.5-5.4
Cl^- (mEq/L)	120.3	-	102.0	98-108
TAC (mmol/L)	1,1±0.1	-	-	-
pH	7.2	-	-	7.35-7.45

Table 1
COMPARISON BETWEEN CHEMICAL COMPOSITION OF hAM AND SERUM

Table 2
COMPARISON BETWEEN CHEMICAL COMPOSITION OF hAM, TEARS, AND SERUM

Chemical substance	Human Amniotic Membrane	Tears			Serum
	Costea *et al.* 2018	Balasubramanyam [35] 2016	Venkata *et al.* [42] 2009	Berman [45] 1991	Estridge, Reynolds& Walters 2000 [37]
Glucose (mg/100ml)	3	3-10	-	-	70-110
Total proteins (g/100ml)	0.07	0.6-2	0.12± 0.047	-	6.0-8.0
Na^+ (mEq/L)	155.4	142	-	120-165	135-148
K^+ (mEq/L)	5.74	15-30	-	20-42	3.5-5.4
Cl^- (mEq/L)	120.3	120-135	-	118-135	98-108
TAC (mmol/L)	1.1±0.1	-	0.7 ± 0.18	-	-
pH	7.2	-	7.4	-	7.35-7.45

The chemical tests run by us on the fresh amniotic membrane specimens revealed a slightly higher Na^+, K+ and Cl^-, ion concentration (Na^+ =152 mEq/L; K^+=5.74 mEq/L;

Cl^- =131.6 mEq/L) than in serum [32], yet comparable to that of ions in the aqueous humor (Na^+=152 mEq/L; K^+=3.9 mEq/L; Cl^-=131.6mEq/L), as reported by Schrage [46].

However, Assali et al. (1972) reported concentrations of the three ions in the amniotic fluid in full-term pregnancy (Na^+ = 133mEq/L; K^+ = 4.9mEq/L; Cl^- = 102.0mEq/L) similar to serum [39].

When analyzing the chemical composition of tears in healthy individuals, Balasubramanyam reported a higher concentration of all three ions analyzed in tears (Na^+ = 142 mEq/L; K^+ = 15-30 mEq/L; Cl^- = 120-135 mEq/L) than in serum, but similar to those obtained by us in the amniotic membranes [35].

The *p*H value obtained in our study was 7.2, comparable to tear secretion, reported to be 6.5 to 7.6 [47].

We found a total antioxidant capacity (TAC) in hAM of 1.1±0.1 mmol/L (tables 1 and 2), lower than that in serum [46]. However, a team of authors from India reported that tears from healthy people have a total antioxidant capacity (TAC) of 0.7 ± 0.18 mmol/L, with a range of 0.41-1.03mmol/L [42], i.e. similar values with those obtained by us for hAM.

Conclusions

All these biochemical data suggest that the chemical composition of the AM obtained by us is similar to that reported by other authors, being different from serum, but similar to that of tears and aqueous humor, which makes it a biologically useful material in eye transplantation.

References

1.NIKNEJAD,H.,PEIROVI,H.,JORJANI,M.,AHMADIANI,A.,GHANAVI,J.,SEIFALIAN, A.M., Eur. Cell. Mater.,**15**, 2008, p.88-99.

2.PAROLINI,O.,SONCINI,M.,EVANGELISTA, M.,SCHMIDT,D.,Regen. Med, **4**,no.2, 2009, p.275-291.

3.TSUBOTA,K.,SATAKE,Y.,OHYAMA,M., TODA,I., ONO,M., SHINOZAKI, N., SHIMAZAKI,J., Am. J. Ophthalmol.,**122**, no.1,1996,p.38-52.

4.LEE,S.H.,TSENG,S.C.G.,Am. J. Ophthalmol.,**123**,1997,p.303-312.

5.TSENG,S.C.G.,PRABHASAWAT,P.,LEE,S.H., Am. J. Ophthalmol.,**124**, no.6, 1997,p.765-774.

6.PRABHASAWAT,P.,BARTON,K.,BURKETT,G., TSENG,S.C., Ophthalmology, **104**,no.6,1997, p.974-985.

7.SHIMAZAKI,J.,YANG,H.,TSUBOTA,K.,Ophthalmology,**104**, no.12,1997,p.2068-2076.

8.TSENG,S.C.,PRABHASAWAT,P.,BARTON,K, GRAY,T.,MELLER,D.,Arch. Ophthalmol., **116**,no.4,1998,p.431-441.

9.AZUARA BLANCO,A.,PILLAI,C.T.,DUA,H.S.,Br. J. Ophthalmol.,**83**, no.4, 999,p.399-402.

10.HONAVAR,S.G.,BANSAL,A.K.,SANGWAN,V.S.,RAO,G.N., Ophthalmology, **107**,no.5,2000,p.975-979.

11.TSAI,R.J.F.,LI,L.M.,CHEN,J.K.,N.Engl.J. Med., **343**,no.2,2000,p.86-93.

12.SAPTE, E.,Rom. J. Morphol. Embryol., **58**, no.3, 2017, p.731–738.

13.BOGDÃNICI,S.T., MARTINESCU.G.,SANDULACHE,C.B.,BOGDÃNICI, C.M., RCIS, 52, 2016,p.187-194.

14.BOGDANICI,S.T., COSTIN,D., BOGDANICI,C., Rev Med Chir Soc Med Nat,**119**,no.1, 2015,p.214-220.

15.CHAUDHURI, Z., VANATHI,M., J. P. Medical. Ltd, 6.10.3, 2012, p.700-705.

16.PARIDAENS,D.,BEEKHUIS,H.,VAN DEN BOSCH,W., REMEYER,L.,MELLES,G., Br J Ophthalmol,**85**, no.6,2001,p.658-661.

17.ESPANA,E.M.,PRABHASAWAT,P.,GRUETERICH,M.,SOLOMON,A., TSENG, C.S., Br J Ophthalmol, **86**, no.6, 2002,p.640-645.

18.TIUTIUCA,C.,VOICU,D.,BRUJBU,I.,MACOVEI,L.,CIUPILAN,C., BOGDANICI,C., BULIMAR,V., Rev.Chim.(Bucharest), **67**, no.8, 2016, p. 1641

19.PAROLINI,O.,ALVIANO,F.,BAGNARA,G.P.,BILIC,G.,BUHRING,H.J., EVANGELISTA,M., HENNERBICHLER,S., LIUC,B. ,MAGATTI,M., MAO,N., MIKI,T., MARONGIU,F.,NAKAJIMA,H., NIKAIDO,T.,PORTMANN-LANZ,C.B., SANKAR,V.,SONCINI,M., STANDLER,G., SURBEK,D., TAKASHI, T.A., REDL,H., SAKURAGAWA,N., WOLBANK,S., ZEISBERGER,S., ZISCH,A., STROM,S.C., Stem Cells, **26**,no.2 ,2008,p.300-311.

20.FAULK,W.P., MATTHEWS,R., STEVENS,P.J., BENNETT,J.P.,BURGOS,H., HIS,B.L.,Lancet,**1**, no.8179, p.1156-1158.

21.HAO,Y.,MA,D.H.,HWANG,D.G.,KIM,W.S.,ZHANG,F., Cornea,19, no.3, 2000,p.348-352.

22.BAILO,M., SONCINI,M.,VERTUA,E., SIGNORONI,P.B., SANZONE, S., LOMBARDI,G.,ARIENTI,D.,CALAMANI,F., ZATTI,D., PAUL,P., ALBERTINI,A., ZORZI,F., CAVAGNINI,A., CANDOTTI,F., WENGLER,G.S., PAROLINI,O., Transplantation, **78**, no.10,2004, p.1439-1448.

23.RICCI,R., GRANATO,A., VASCELLARI,M.M BOSCARATO, M., PALAGIANO,C., ANDRIGHETTO,I., DIEZ,M., MUTINELLI,F., J. Anim. Physiol. Anim. Nutr (Berl),**97**, S1, 2013, p. 32-38.
24.SILINI,A.,R., CARGNONI,A., MAGATTI,M., PIANTA,S., PAROLINI,O., Front Bioeng Biotechnol,3, 2015,p.162.
25.STERN, M.,JAMA, **60**, 1913, p.973-994.
26.DAVIS,J.W.,Johns Hopkins Med. J, **15**, 1910,p.307.
27.DE ROTTH, A., Arch.Ophthalmol., **23**,1940, p.522-525.
28.SORSBY,A.,SYMONS,H.M., Br. J. Ophthalmol., **30**,no.6,1946, p.337-345.
29.KIM,J.C.,TSENG,S.C.G., Cornea,**14**,1995,p.473-484.
30.DUA,H.S.,AZUARA-BLANCO, A., Br. J. Ophthalmol., **83**,no.6,1999,p.748-752.
31.STRASINGER,S.K., DI LORENZO,M.S.,Urinalysis & Body Fluids, 5th Ed., F. A. Davis Company, Philadelphia, 2008, p. 235-236.
32.GARBY, L.,Acta Physiol. Scand. Suppl.,**40**, no.137,1957, p.1-84.
33.MELLOR, D.J., SLATER, J.,J. Physiol.,**217**, no.3, 1971, p.573–604.
34.SOZANSKII, A.M., Bull. Exp. Biol. Med., 51, 1961, p.323.
35.BALASUBRAMANYAM,D., Biochemistry of the eye. In: Talwar GP, Hasnain SE, Sarin SK (ed). Textbook Of Biochemistry, Biotechnology, Allied And Molecular Medicine, 4th Ed., PHI Learning Private Limited, Delhi, 2016, p. 425.
36.GIARDINI, A., ROBERTS, J.R.E., Br. J. Ophthalmol., **34**, 1950, p.737.
37.ESTRIDGE, B.H., REYNOLDS,A.P.,WALTERS,N.J., Basic Medical Laboratory Techniques, 4th Edition, Delmar -Thomson Learning, 2000, p. 440.
38.SCHMIDT, W., Adv. Anat. Embryol. Cell. Biol., **127**, 1992, p.1-100.
39.ASSALI,N.S., DEHAVEN,J.C., BARRETT,C.T., Disorders of water, Electrolyte, and Acid-Base Balance. In: Fetal-Placental Disorders. Vol. III, Academic Press, 1972, p. 175.
40.ALBERT, D.M., MILLER,J.W., AZAR,D.T., BLODI,B.A., Albert & Jakobiec's Principles and Practice of Ophthalmology, 3rd Ed., Saunders Elsevier, 2008, p. 181.
41.JAUNIAUX, E., JURKOVIC,D., GULBIS,B., GERVY,C., OOMS,H.A., CAMPBELL,S., Obstetrics and Gynecology, **78**, no.6, 1991, p.1124-1128.
42.VENKATA,S.J.A., NARAYANASAMY, A., SRINIVASAN, V., IYER, G.K., SIVARAMAKRISHNAN,R., SUBRAMANIAN,M., MAHADEVAN, R., Indian J. Ophthalmol.,**57**, no.4, 2009, p.289-292.
43.JOHNSON, M.H., Essential Reproduction, 8 Ed., John Wiley & Sons Ltd, 2018.
44.GERARD,M., JOSSET, P., Histology and Physiology of the Cornea. In: Chemical Ocular Burns. New Understanding and Treatments. Springer-Verlag, Berlin Heidelberg, 2011, p. 49-50.
45.BERMAN, E.R.,Biochemistry of the Eye, Springer Science+Business Media New York, 1991, p. 69-70.
46.SCHRAGE,N., Physiopathology of the Cornea and Physiopathology of Eye Burns. In: Chemical Ocular Burns. New Understanding and Treatments. Springer-Verlag, Berlin Heidelberg, 2011, p. 61-63.
47.ABELSON, M.B.,UDELL,I.J., WESTON,J.H., Arch. Ophthalmol.,**99**, no.2,1981,p.301.

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